ONCOGENES: 20 YEARS LATER

Organizers: Inder M. Verma and Peter Vogt

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SRC: Key to Kinases and Receptors

A1-001 REGULATION AND FUNCTIONS OF SRC FAMILY TYROSINE KINASES. Sara A. Courtneidge[1,2], Gema Alonso[1], Thorsten Erpel[1], Stefano Fumagalli[1], Manfred Kögl[1], Serge Roche[1] & Giulio Superti-Furga[1]. [1]European Molecular Biology Laboratory, Postfach 10.2209, 69012 Heidelberg, Germany, [2]SUGEN Inc., Redwood City, Ca 94063, USA.

The kinase activity of cSrc is negatively regulated by phosphorylation of tyr 527, by an enzyme called Csk. Both the SH2 domain and the SH3 domain of Src participate in intramolecular interactions that are required for the correct regulation of Src by Csk. The conserved sequences in the SH3 domain are required for this regulation. However, chimeric molecules in which the SH3 domain of Src is replaced by those of either spectrin or Lck fail to be regulated by Csk, suggesting that other residues dicate the specificity of the intramolecular interaction. We have recently isolated a protein, Sam68, that associates with the SH3 domain of Src during mitosis, and is a mitotic substrate of Src. Sam68 is related to the GAP-associated protein, p62. Mutational analysis has shown that highly conserved residues in the SH3 domain are required both for intramolecular interaction, and association with Sam68. Our current analyses on the function of Sam68 will be presented.

Stimulation of quiescent cells with PDGF or CSF-1, causes the Src family tyrosine kinases (Src, Fyn and Yes) to become activated, and transiently associate with the cognate receptors. Using a microinjection system to introduce dominant negative constructs and neutralising antibodies into cells, we have shown that Src, Fyn and Yes are required in order for PDGF, CSF-1 and EGF, but not bombesin or LPA, to transmit a mitogenic signal. We are now investigating the function of the Src family kinases in the PDGF response.

A1-002 TRANSFORMATION BY V-SRC, Dana Aftab, Laura England, Maoxin Tian, James Trager and G. Steven Martin, Department of Molecular and Cell Biology, 401 Barker Hall #3204, University of California, Berkeley, CA 94720-3204.

pp60$^{v\text{-}src}$, like its progenitor, pp60$^{c\text{-}src}$, contains N-terminal non-catalytic domains, the myristoylation, unique, SH3 and SH2 domains, and a C-terminal tyrosine kinase domain. To define the structural features of v-*src* that are necessary for transformation and their roles in targeting v-*src* to specific substrates, we have characterized a variety of deletion, linker insertion, translocation and substitution mutants. Mutations in the N-terminal domains can lead to transformation-defective, morph^{f} (fusiform) or host cell-dependent (host range) phenotypes. The membrane-targeting sequence of H-*ras* can functionally substitute for the myristoylation sequence of v-*src*, implying that a specific mode of attachment of p60$^{v\text{-}src}$ to cellular membranes is not necessary for transformation. The unique, SH2 and SH3 domains can permit wild-type transformation even when translocated to the C-terminus of v-*src*, but do not function when expressed in *trans*. Thus the N-terminal domains of *src* must be present in *cis* to target pp60 to substrates necessary for wild-type (morph^{r}) transformation. The host range phenotype can result from mutations in the SH3, SH2 or catalytic domains. The host range phenotype is associated with alterations in the regulation and targeting of the *src* kinase and with alterations in signaling through the SHC/Ras/MAP kinase and PI 3'kinase pathways. The properties of these mutants and the information they provide about the mechanisms of transformation by v-*src* will be discussed.

Cytoplasm: The Connectors

A1-003 **REGULATION OF TYROSINE PHOSPHORYLATION BY THE PLATELET INTEGRIN, $a_{IIb}b_3$,** J.S. Brugge[1], E.A. Clark[1], M.H. Ginsberg[2], S.S Shattil[3], [1]ARIAD Pharmaceuticals, Inc., Cambridge, MA 02139. [2]Scripps Research Institute, La Jolla, CA. [3]Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

We have previously shown that fibrinogen binding to its integrin receptor, $a_{IIb}b_3$, is required for thrombin-induced tyrosine phosphorylation of multiple platelet proteins. The platelet system has been valuable in dissecting the intracellular signaling events that transduce integrin-regulated changes in cell behavior. We have found that $a_{IIb}b_3$ regulated tyrosine phosphorylation could be separated into two distinct events: 1) the phosphorylation of p140 and several proteins of M_r 50-72 Kd (p50-72), which is induced by either dimerization of $a_{IIb}b_3$ with fibrinogen, crosslinking $a_{IIb}b_3$ monoclonal antibodies, or by treating with the dimeric disintegrin, contortrostatin, and 2) the phosphorylation of p95/97 and the protein tyrosine kinase, p125^{FAK}, which is dependent on fibrinogen-induced platelet aggregation and a second costimulatory event that requires protein kinase C and calcium. The induction of tyrosine phosphorylation of all of these proteins was inhibited in cytochalasin D treated platelets, suggesting that actin-dependent cytoskeletal complexes may couple $a_{IIb}b_3$ with tyrosine kinases and their substrates. At least three classes of tyrosine protein kinases appear to participate in these events: p125^{FAK}, which is activated following platelet aggregation; pp60^{src}, which is activated independent of $a_{IIb}b_3$ but redistributes to integrin-regulated cytoskeletal complexes after platelet aggregation, and Syk, which is phosphorylated on tyrosine following dimerization of $a_{IIb}b_3$ by fibrinogen. The mechanisms involved in the activation of these kinases and the roles of these kinases in integrin mediated signal transduction will be discussed.

Cytoplasm: The Connectors II

A1-004 THE JAK:STAT PATHWAY--SIGNALLING FROM THE CELL SURFACE TO THE NUCLEUS, James E. Darnell, Jr.,The Rockefeller University, New York, N.Y. 10021

Binding of IFN-α (interferon alpha) and IFN-γ (interferon gamma) to their cell surface receptors promptly induces tyrosine phosphorylation of latent cytoplasmic transcriptional activators, which we designate STATs (signal transducers and activators of transcription). IFN-α activates both Stat1 [a 91 kD protein] and Stat2 [a 113 kD protein] while IFN-γ activates only Stat1. After phosphorylation the proteins form homo- or heterodimers and then move into the nucleus and directly activate genes induced by IFN-α or IFN-γ. Somatic cell genetics experiments have demonstrated a requirement for tyrosine kinase 2 (Tyk2) in the IFN-α response pathway and Jak2, a kinase with similar sequence, in the IFN-γ response pathway. We have investigated the tyrosine phosphorylation events on STAT proteins after treatment of cells with IFN-α, γ, and EGF. Stat1 is phosphorylated on tyrosine^{701} after cells are treated with IFN-α and EGF, just as we had earlier found after IFN-γ treatment. Details in the steps of interaction of the STAT proteins with receptor kinase complexes are under study and will be described.

In addition to activation by the IFNs it has now been found by us and other investigators that EGF, PDGR, IL-6 and many other cytokines also lead to tyrosine phosphorylation of JAK and of STAT proteins and that a new protein, Stat3, is the major protein that carries these signals to DNA sites in the nucleus. In addition, a fourth family member, Stat4, is present in high concentration in the testes and is localized to early cells in the pathway to spermatocyte formation. These results imply that many other ligands may use similar direct nuclear signalling mechanisms for a variety of regulatory purposes. In support of the generality of this pathway a newly isolated Drosophila STAT protein will be described.

A1-005 POLYOMA T ANTIGENS: OLD ONCOGENES--NEW TRICKS Thomas M. Roberts[1], Kathryn Campbell[1], Wen Su[1], Kai Xia[1], Kurt Auger[1], Jing Wang[1], Sadhana Agarwal[1], Toni Jun[1], Radha Narsimhan[1], Wayne Haser[1], Fred King[1], Wei Liu[1], Akio Yamakawa[1], Haian Fu[2], John Collier[2], Egon Ogris[1], David Pallas[1], and Brian Schaffhausen[3], [1]Dana Farber Cancer Institute, Boston, MA02115, [2] Harvard Medical School, Boston, [3] Tufts Medical School, Boston

Polyoma middle T antigen(MT) has been an important player in the short history of signal transduction. The first tyrosine kinase assays were carried out on MT immunopreciptiates, as were the first definitive studies on the PI 3' kinase. A model has emerged of MT in which the viral oncogene forms a complex with cellular proteins which mimics an activated receptor tyrosine kinase. The availability of thousands of mutants of MT allows study of the MT complex at a high level of refinement. We now know that the MT complex consists of at least a dozen cellular proteins including tyrosine kinases of the src family, two subunits of protein phosphatase 2A, the hsp70 family of proteins, and a variety of SH2 containing proteins including SHC, PI3 kinase and PLCγ. The most recent addition to this list is the 14-3-3 family of proteins. These proteins are conserved from plants to man, and share the interesting biochemical property of being the obligate cellular cofactors for the ADP-ribosylation of the ras family of small G proteins by a bacterial "toxin". The 14-3-3 proteins have recently been found in complex with other signaling molecules including Raf-1 and bcr-abl. Results of studies of the MT complex and the Raf-1 signaling pathway will be presented.

Nucleus: The Responder

A1-006 TRANSCRIPTIONAL ACTIVATION AND REPRESSION MEDIATED THROUGH THE MYC TRANSCRIPTION FACTOR NETWORK Don E. Ayer, Peter Hurlin, Christophe Queva, Quentin Lawrence, and Robert N. Eisenman. Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle WA.

Myc proteins are members of the bHLHZip class of transcription factors and are important in proliferation, apoptosis and differentiation. Max is a bHLHZip protein which is an obligate partner for Myc function. Myc and Max form sequence-specific DNA binding heterocomplexes. Mad is another bHLHZip protein which can compete with Myc by forming heterocomplexes with Max. Mad:Max complexes repress, while Myc:Max complexes activate, transcription from promoter constructs. We have shown that the Mad protein is rapidly induced upon differentiation of several myeloid cell lines. During differentiation a switch from Myc:Max heterocomplexes to Mad:Max heterocomplexes is observed. Similar results have been obtained for differentiation of primary human keratinocytes. HPV immortalized keratinocytes also induce Mad upon dfifferentiation, but rare, non-differentiating variants, fail to express Mad. Thus induction of Mad is closely linked to differentiation in at least two distinct cell lineages. We have also detected Mad expression in the dorsal root ganglia and restricted regions of the neural tube in early (E10) mouse embryos. These expression patterns are also suggestive of a role in diffrentiation.

The switch from Myc:Max to Mad:Max complexes may reflect the repression of transcription of Myc-regulated genes by Mad. To understand how Mad functions as a repressor we searched for additional protein-protein interactions. We have found that Mad and Mxi1, but not Myc or Max, specifically interact with two homologues (mSin3A and B) of the *S. cerevisiae* repressor protein Sin3. The mSin:Mad association is mediated through one of four paired amphipathic helix domains in mSin3 and a potential amphipathic helix located in the N-terminal portion of Mad. Furthermore the mSin:Mad complex can interact with Max, through the Mad bHLHZip region, and bind DNA as a ternary complex. Point mutations within the Mad N-terminal helix dsirupt interactions with mSin3 and inhibit Mad mediated repression in vivo. We speculate that Mad may function as a negative regulator of gene expression in association with Max by tethering a novel mammalian repressor to specific DNA binding sites.

Because Max is expressed constitutively and capable of interacting with several key regulatory proteins we explored the possibility that Max might interact with additional, as yet uncharacterized, proteins. We therefore employed Max as "bait" in a yeast two hybrid screen of a mouse embryonic cDNA library. Four unique Max-interacting bHLHZip proteins were identified as well as each of the known Max partners. Additional cloning and sequence analysis revealed that these constituted four novel bHLHZip proteins. Two of the proteins appear to be related to Mad primarily in their b-HLHZip domains. The other two proteins are not members of either the Mad or Myc families but possess proline- and glutamine-rich segments reminiscent of transcriptional activation regions. These clones have tissue-specific expression patterns and appear to be differentially expressed during embryonic development and cell differentiation.

Max appears to be at the center of a network of transcription factors whose activites and levels may be key determinants of cell behavior.

A1-007 ONCOGENES CODING FOR TRANSCRIPTION FACTORS, Peter K. Vogt[1], Hwai Wen Chang[1], Meihua Gao[1], Martin Goller[1], Jian Li[1], Chen Liu[1], and Iain Morgan[2], Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, California 92037[1] and Celltech Group, Slough, Berkshire, UK[2].

Jun is the oncogene of avian sarcoma virus 17 (ASV 17). Its cellular counterpart c-*jun* codes for a protein that functions as a major component of the transcription factor complex known as AP-1. The mechanism by which viral *jun* (v-*jun*) induces tumors is not known. A popular hypothesis states that the oncogenicity of *jun* is related to an increase of its transactivation potential. We have tested this hypothesis by comparing v-*jun* with c-*jun* and by characterizing numerous *jun* mutants. Overexpression of c-*jun* in chicken embryo fibroblasts promotes growth, but does not make the cells tumorigenic. In contrast, v-*jun* not only transforms cells in culture but is also highly oncogenic *in vivo*. V-*jun* carries several mutations, all of which make an incremental contribution to oncogenic activity. *Jun* mutants and chimeras between Jun and other transcription factors have been compared with respect to oncogenicity and transactivation potential. Contrary to hypothesis, AP-1 activity is not correlated with oncogenicity. Although the transactivation domain of Jun is essential for oncogenicity, mutations that make *jun* oncogenic do not result in a consistent gain of AP-1 activity. The effects of oncogenic mutations are more subtle, probably altering the interaction of Jun with a few specific target promoters. Discovering Jun-interacting proteins and Jun target genes that are relevant in oncogenesis are important current goals.

The *qin* oncogene was isolated from avian retrovirus ASV 31 which induces transformation in cell culture and sarcomas in the animal. Qin is a phosphoprotein that belongs to the large HNF3/fkh transcription factor family defined by a highly conserved DNA binding domain that contacts specific sequences as a monomer. Members of the HNF3/fkh family control development and differentiation in vertebrates and invertebrates. Qin is closely related to and may be the homolog of rat brain factor 1. We have cloned and sequenced the human and chicken cellular counterparts of v-*qin*. The human *qin* gene is a single locus situated on chromosome 14q13, a region frequently rearranged in cancer, especially in leukemias. Expression of *qin* is highly tissue specific. In developing chick embryos, the *qin* message is found in the telencephalon only. We are now investigating the functions of *qin* in normal development by defining upstream regulators and downstream targets. We are also relating ectopic expression of *qin* to tumorigenesis and examining the role of HNF3/fkh proteins in human cancer.

Cancer: Chromosomes and Translocation

A1-008 DISTURBANCE OF CELL SURVIVAL AND PROLIFERATION IN LYMPHOID NEOPLASIA. Cory, S. Strasser, A., Bodrug, S., Warner, B., Metz, T., Harris, A.W. and Adams, J.M. *The Walter and Eliza Hall Institute of Medical Research, P.O. Royal Melbourne Hospital, Victoria 3050, Australia.

While the oncogenic contribution of increased cell proliferation is well accepted, only recently has the importance of cell survival been appreciated. We are using transgenesis to explore the tumorigenic impact of altered proliferation and cell survival, focussing on the lymphoid system, where cellular attrition plays a dominant role. Studies involving *myc*, *bcl*-2, cyclin D1 transgenic mice and p53$^{-/-}$ mice will be discussed.

1. Strasser, A., Harris, A.W., Bath M.L. and Cory, S. 1990. Novel primitive lymphoid tumours induced in transgenic mice by cooperation between *myc* and *bcl*-2. Nature **348**, 331-333.
2. Strasser, A., Harris, A.W. and Cory, S. 1991. *bcl*-2 transgene inhibits T cell death and perturbs thymic self-censorship. Cell, **67**, 889-899.
3. Strasser, A., Whittingham, S., Vaux, D.L., Bath, M.L., Adams, J.M., Cory, S. and Harris, A.W. 1991. Enforced *bcl*-2 expression in B lymphoid cells prolongs antibody response and elicits autoimmune disease. Proc. Natl. Acad. Sci. USA **88**, 8661-8665.
4. Strasser, A., Harris, A.W. and Cory, S. 1993. Eμ-*bcl*-2 transgene facilitates transformation of early pre-B and immunoglobulin-secreting cells but not T cells. Oncogene **8**, 1-9.
5. Bodrug, S.E., Warner, B.J., Bath, M.L., Lindeman, G.J., Harris, A.W. and Adams, J.M. 1994. Cyclin D1 transgene impedes lymphocyte maturation and collaborates in lymphomagenesis with the *myc* gene. EMBO J. **13**, 2124-2130.
6. Strasser, A., Harris, A.W., Jacks, T. and Cory, S. 1994. DNA damage can induce apoptosis in proliferating lymphoid cells via p53-independent mechanisms inhibitable by Bcl-2. Cell. in press.

A1-009 RETINOID RECEPTORS IN DEVELOPMENT AND DISEASE, Dr. Ronald M. Evans, Howard Hughes Medical Institute, The Salk Institue, La Jolla, California, USA.

Nuclear receptors comprise a large family of ligand-dependent transcription factors that display considerable specificity and selectivity in regulating the genetic programs that they ultimately influence. The retinoic acid (RA) are mediated by two families of transcription factors which include the RA receptors (RARs) and the retinoid X receptors (RXRs). The RARs bind all-trans retinoic acid (atRA) and RXR binds the novel RA stereo isomer 9-cis RA. In addition to its role as a 9-cis RA receptor, a non-liganded RXR plays a central role in a variety of other signaling pathways. In this alternate context, RXR functions to form a heterodimer with at least four other receptors including those for vitamin D3, thyroid hormone, all-trans-RA and peroxisome proliferation. In this context, RXR may be seen as a master regulator integrating diverse physiologic responses by establishing a common mechanism basis for signal transduction. We will describe recent structural studies that include NMR analysis as well as X-ray crystallographic studies of RXR DNA. We will also discuss homologous recombination studies to knock out the function of RXRa in mice. In human acute promyelocytic leukemia, the RAR becomes an activated oncogene as a consequence of its fusion to the PML locus. We demonstrate that PML is part of a novel nuclear structure that is the target of RA chemotherapy.

A1-010 CHROMOSOMAL TRANSLOCATIONS: GOOD GENES GONE WRONG, Janet D. Rowley, Departments of Medicine and of Molecular Genetics and Cell Biology, University of Chicago, Chicago, Illinois.

Chromosome translocations have been most closely associated with human leukemias and lymphomas. Recently, they have been shown to occur in sarcomas as well. In each type of tumor, the translocations are relatively specifically associated with particular subtypes of these tumors.

Cloning the translocation junctions has identified the genes affected by the breakpoints in the diseases. Well over two dozen new genes have been identified in this process. Some of these genes are not normally active in hematopoietic cells. Although the genes that are involved participate in a number of steps in the complex pathway of transmitting growth regulatory signals from the cell surface to the nucleus, most of those identified in the acute leukemias and lymphomas act as transcriptional activators. That is they are DNA binding proteins that directly regulate the level of transcription of the target genes. All types of activators are involved including zinc fingers, leucine zippers, homeobox domains, LIM motifs and helix-loop-helix motifs. Some of the genes recently cloned from breakpoint junctions in sarcomas, also act to regulate transcription. Although the genes involved in leukemia and lymphoma differ from those in sarcomas there are a few exceptions.

Part of the challenge for the future is to understand the mechanisms leading to recurring translocations and also to understand the tumor-specificity of the translocations. It is hoped that this increasing understanding of the biology of these malignant diseases will lead to more accurate diagnosis, to improved therapy, and possibly to more effective prevention.

A1-011 GENETIC ANALYSIS OF CANCER, Michael H. Wigler, Michael White, Linda Van Aelst, Peter Barker, Nikolai Lisitsyn. Cold Spring Harbor Laboratory, P.O. Box 100, Cold Spring Harbor, NY 11724.

Mutational activation of the *RAS* oncogene occurs in a large proportion of human cancers. The *RAF* oncogene encodes a serine/threonine protein kinase that is a leading candidate target for RAS. RAF phosphorylates and activates MEK, a protein kinase that activates MAP kinase. Using the two hybrid system of Fields and Song, we have demonstrated the existence of physical complexes between RAS and RAF when both are expressed in the yeast Saccharomyces cerevisiae. RAS also interacts with byr2, a MEK kinase from the yeast Schizzosaccharomyces pombe that is not a RAF homolog. We used the two hybrid system to derive a mutant H-ras protein that fails to interact with wild type RAF but still interacts with byr2, and a mutant H-ras protein that interacts with RAF but not byr2. Each mutant has greatly attenuated ability to transform NIH3T3 cells in culture, but they act synergistically to transform NIH3T3 cells. We next derived mutant RAF proteins capable of interacting with the mutant H-ras protein that does not bind wild type RAF. The pair of mutant H-ras and RAF proteins were capable of efficiently transforming NIH3T3 cells. These results indicate that the direct physical interaction of RAS and RAF is required for transformation, but that at least one other pathway, yet undefined, is also required. We have utilized the two hybrid system to screen cDNAs that encode additional candidate targets for RAS, and the identity of these will be discussed.

Genetic abnormalities accumulate in tumor cells, and are potential clues to their evolution. To identify such abnormalities, we have developed a powerful approach based on subtractive hybridization and kinetic enrichment that we call RDA (representational difference analysis). The method effectively selects for small PCR-amplifiable restriction fragments present in only one of two genomic DNAs, or present in one of the genomes in higher copy number. With this method we have isolated probes for chromosomal regions undergoing deletions or amplifications in tumor cells. When pure tumor DNA (driver) is used in excess to subtract normal DNA from the same individual (tester), RDA yields collections of fragments deleted in the tumor. Using 16 matched pairs of normal and tumor DNA's derived from renal cell carcinoma and colon cancer patients we were able to identify 2 regions which were homozygously deleted in renal cell carcinoma cells, and 2 homozygous losses in colon cancer cells. RDA was also performed on DNA derived from nuclei, taken from biopsies of patients with Barrett's esophagus, that had been sorted into diploid and aneuploid fractions by flow cytometry. Analysis of the DNAs derived from these fractions yielded a probe that detects frequent homozygous loss in colon cancer cell lines. When RDA was performed using normal DNA as driver and matched DNAs from cultured tumor cells as tester, we obtained collections of probes from genomic regions found to be amplified in tumor DNAs. RDA can, in principle, detect other other types of genetic abnormalities in cancers.

The Suppressors

A1-012 THE FUNCTIONS OF THE P53 TUMOR SUPPRESSOR GENE, Arnold J. Levine, The Harry C. Wiess Professor in the Life Sciences, Department of Molecular Biology, Princeton University, Princeton, NJ 08544-1014.

Mutations at the p53 locus are the single most common genetic alteration in the cancers of humans. The p53 gene product is a transcription factor which acts as a checkpoint, recognizing DNA damage, and responding by inducing pathways for cell division arrest or apoptosis. The cell cycle regulatory events are mediated by two p53 responsive genes; pic-1 (p21) and mdm-2. Pic-1 binds to a cyclin dependent kinase and blocks its function while mdm-2 protein binds to p53 and prevents it from transcribing p53 responsive genes. Mdm-2 is the product of an oncogene when this gene is amplified. P53 mediated apoptosis is initiated in response to the overexpression of the transcription factors myc or E2F-1 in the presence of high levels of wild-type p53 protein. Thus, p53 also acts as a checkpoint for the functions of the retinoblastoma susceptibility gene product, Rb, as it in turn regulates E2F-1 functions.

Mutant p53 proteins fail to transcriptionally activate the pic-1 or mdm-2 genes, but gain a new transcriptional specificity; the multi-drug resistance-1 (mdr-1) gene. This "gain of a new function" no longer requires p53 DNA binding directly but does require the transactivation domain of the p53 protein. These results suggest a model where mutant p53 protein, interacting with another transcription factor, gains a new specificity and a new set of functions.

A1-013 THE RB PROTEIN AND CELL CYCLE CONTROL, Tomi Mäkelä, Masanori Hatakeyama, Raphael Herrera, Robert A. Weinberg, Whitehead Institute for Biomedical Research and the Department of Biology, M.I.T. Cambridge MA 02142

The regulation of cell proliferation is achieved during discrete time windows in the cell cycle. The most critical of these periods appears to be the time in mid/late G1 prior to S phase entrance which is termed the restriction point. Cells passing through early G1 will reach the restriction point gate and decide whether they should continue in the cell cycle, remain in G1 or return to G_0. The phosphorylation of pRB, the retinoblastoma protein, occurs concomitantly with this transition and may represent the molecular mechanism that effects this decision. We have developed evidence that at least two distinct G1 cyclin/kinase complexes are responsible for this phosphorylation. In yeast cells this appears to require CLNs 1 or 2 plus CLN3; complementation of yeast mutants indicates that cyclins E and D(1/2/3) play this role in mammalian cells. Loss of the Rb gene, as is seen in Rb-/- mouse embryo cells, results in abrogation of the restriction point by at least one operational definition. A variety of afferent signals appear to impinge on the decision to pass through the restriction point. At least one of them is the mediated by the cyclin activating kinase (CAK) which, by phosphorylating cdc2, the cyclin E-associated kinase, causes its functional activation. The kinase subunit of CAK (MO15) is associated with and controlled by its own cyclin termed, cyclin H. The role of cyclin H in conveying signals regulating cdc2 will be described.

The Cell Cycle

A1-014 CONTROL OF THE G1/S TRANSITION, Steven I. Reed, Eric Bailly, Vjeko Dulic, Ludger Hengst, Dalia Resnitzky, Kevin Sato, Joyce Slingerland, and Kwang-Ai Won, Department of Molecular Biology, MB-7, The Scripps Research Institute, La Jolla, CA 92037 USA.

Cyclin dependent kinases (Cdks) have been implicated in regulation of the G1/S phase and G2/M phase transitions of eukaryotic cells ranging from yeast to human. In budding yeast, control of the G1/S phase transition has been shown to be based on the regulation of G1 cyclins or Clns, a class of regulatory proteins that activate the endogenous Cdk, Cdc28, for G1 functions. For example, mating pheromones that arrest the cell cycle in G1, block G1 cyclin transcription and regulate the function and stability of G1 cyclins post-translationally. We sought to determine if signals that block the mammalian cell cycle in G1 do so in an analogous fashion, by control the accumulation and/or function of cyclins and Cdks. In this context, we have investigated the effects of gamma-irradiation of fibroblasts in early G1, treatment of epithelial cells with TGF-β, and treatment of HeLa cells with the drug lovastatin all of which confer cell cycle arrest late in G1. Candidates for targets of these negative regulators are the cyclin E/Cdk2 and cyclin D1/Cdk4 kinases, which accumulate late in G1 and the cyclin A/Cdk2 kinase, which accumulates concomitantly with initiation of S phase and which has shown to be required for DNA replication. We find that all treatments block activation of the cyclin E and cyclin A associated kinases. However, whereas cyclin A does not accumulate, cyclin E accumulates and binds to Cdk2 but these complexes remain inactive. In each case Cdk inhibitor proteins were found to be responsible for cyclin E/Cdk2 and probably cyclin D1/Cdk4 kinase inactivity. However, whereas arrest in response to ionizing radiation depended on accumulation of the p53 dependent inhibitor $p21^{Cip1/Waf1}$, TGF-β and lovastatin mediated arrest were associated with a 28 kD inhibitor, $p28^{Ick}$. $p28^{Ick}$, also known as $p27^{Kip1}$, has primary structure and, most likely, functional homology to $p21^{Cip1/Waf1}$, and appears to be regulated at multiple levels in response to antiproliferative signals. These inhibitors, although differentially regulated, establish a new paradigm for cell cycle control in mammalian cells.

We have also sought to determine if G1 cyclins are rate-limiting for the G1/S phase transition. Both cyclin D1 and cyclin E could accelerate entry into S phase when expressed ectopically. However, analysis of possible downstream targets of the respective cyclin associated kinases suggests that the two cyclins have differential functions. This idea is supported by the observation that co-expression of cyclin D1 and cyclin E is synergistic in terms of acceleration of S phase.

A1-015 POSITIVE AND NEGATIVE REGULATION OF D-TYPE CYCLIN-DEPENDENT KINASES DURING G1 PROGRESSION, Charles J. Sherr,[1,2] Masaaki Matsuoka,[1,2] Jun-ya Kato,[2], Martine F. Roussel,[2] and Dawn E. Quelle,[1,2]
Howard Hughes Medical Institute,[1] Department of Tumor Cell Biology,[2] St. Jude Children's Research Hospital, Memphis TN 38105.

Rate limiting steps in progression through the G1 phase of the cell cycle are regulated by G1 cyclins and their associated catalytic subunits, the cyclin-dependent kinases (cdks). D-type cyclins assemble preferentially with two catalytic partners, cdk4 and cdk6, to form holoenzymes whose activities are first detected in mid-G1 phase and increase as cells approach the G1/S transition. D-type cyclin genes are induced during the delayed early response to mitogens, resulting in the progressive accumulation of cyclin D-cdk complexes which execute their rate limiting function(s) later in G1 phase. Ectopic overexpression of D-type cyclins bypasses the inductive effects of mitogens on their synthesis, shortening G1 phase and reducing the cell's dependency on growth factors. However, both the assembly and activation of the cyclin D-dependent kinases are subject to additional post-translational controls, at least some of which are growth factor dependent. One regulator of cdk activity is a cdk-activating kinase (CAK), which phosphorylates cyclin D-bound cdk4 on Threonine-172, a modification required for cyclin D-cdk4 kinase activity. CAK is itself composed of a cdk-like catalytic subunit (MO15 or cdk7) and cyclin H, but its activity is detected throughout the cell cycle, suggesting that it is not usually rate-limiting nor subject to positive regulation.

D-type cyclins are degraded when growth factors are withdrawn during G1 phase, leading to a collapse of the cyclin D-cdk4 complex and the failure of cells to enter S phase. Even in the face of mitogenic stimulation, however, cdk inhibitors can negatively regulate G1 progression in response to anti-proliferative signals. The levels of a stoichiometric cdk inhibitor, $p27^{Kip1}$, are relatively high in quiescent cells, but decline after mitogenic stimulation. In proliferating cells, $p27^{Kip1}$ is expressed in threshold amount, much of it bound to cyclin D-cdk4. Accumulation of cyclin D-cdk4 titrates $p27^{Kip1}$, countering its effects and enabling cyclin D-dependent kinase activity to become manifest as the quantity of holoenzyme exceeds that of Kip1. By inhibiting cdk4 synthesis in mink lung cells, TGF-β increases the amount of unbound $p27^{Kip1}$, facilitating its association with cyclin E-cdk2 and leading to the inhibition of both cyclin D- and E-dependent cdks. In contrast, cAMP blocks the mitogenic effects of CSF-1 in macrophages by increasing $p27^{Kip1}$ synthesis, thereby inhibiting the cyclin D-dependent kinase and inducing mid-G1 arrest. Under these conditions, the binding of $p27^{Kip1}$ to cyclin D-bound cdk4 blocks CAK from phosphorylating and activating the holoenzyme. Thus, $p27^{Kip1}$ is a dually acting inhibitor, which can both interfere with CAK-mediated activation of cdk4 as well as directly inhibit cdk activity.

The Whole Animal

A1-016 IDENTIFICATION AND CHARACTERIZATION OF COLLABORATING ONCOGENES IN GENETICALLY MANIPULATED MICE. A. Berns, Jos Jonkers, Blanca Scheyen, Nathalie van der Lugt, Mark Alkema, Dennis Acton, Maarten van Lohuizen, Els Robanus Maandag, Marian van Roon, and Jos Domen. Division of Molecular Genetics of the Netherlands Cancer Institute, Amsterdam.

Transgenic mice overexpressing oncogenes are predisposed to tumorigenesis. However, the tumors that arise spontaneously are usually of monoclonal origin and appear after a variable latency period indicating that additional stochastically occurring event are required. Proviral tagging was applied to get access to these collaborating oncogenes as proviruses can activate genes and simultaneously mark them to facilitate their subsequent cloning. We have applied this methodology to search for collaborating oncogenes in Eμ-myc transgenic mice. Eμ-myc transgenic mice are predisposed to B cell lymphomas. Infection of newborn mice with Murine Leukemia Virus infection accelerates lymphomagenesis. By identifying common insertion sites in a lymphoma tumor panel we have found a number of genes that can collaborate with myc in transformation: pim-1, bmi-1, pal-1, and fat-1. Some of these genes, e.g. pim-1, bmi-1, pal-1 are involved in early stages of the tumorigenic process whereas others, E.G. fat-1 are involved in tumor progression and are found after selecting for tumor subclones that have acquired the capacity to propagate upon transplantation in syngeneic hosts. Up to four consecutive events could be identified in this fashion. The role of of some of these genes in tumorigenesis and in normal development will be discussed. Utilizing compound transgenic/knock-out mice we have shown that the proviral tagging system can also give access to genes acting in a defined signal transduction pathway and elucidate the epistasic relationship between the distinct oncogenes that collaborate with *myc* in lymphomagenesis.

A1-017 GENETIC COMPLEMENTATION: SEEKING ONCOGENIC PARTNERS USING TRANSGENIC MICE. Philip Leder, Timothy Lane, Fred Lee, Ari Elson, David Seldin and Chuxia Deng. Department of Genetics, Harvard Medical School, HHMI, 200 Longwood Avenue, Boston, MA

Tumor progression is generally held to depend upon genetic mechanisms that recruit cumulative and complementary mutations, each of which contributes to a more aggressive malignant phenotype. This is perhaps best illustrated by classical genetic complementation assays in which transgenic mice bearing different oncogenes are combined in a single mouse, dramatically accelerating the rate and the extent of tumor formation. Such an approach has allowed us to assess transforming relationships in specific tissue contexts. Thus, c-myc and Ha-v-RAS can collaborate to accelerate tumor formation in the mammary gland, but not in the salivary epithelium. We have used this approach to search for genes that can cooperate with specific, dominantly acting oncogenes in mammary carcinoma. For example, a transgenic mouse bearing the int-2 gene (an FGF-family member) driven by the mouse mammary tumor virus promoter induces mammary hyperplasia and, over time, the stochastic occurrence of mammary carcinomas. Infecting these mice with MMTV accelerates tumor formation by virtue of the insertion of MMTV as an activating mutagen in the vicinity of genes that can collaborate with int-2 to induce tumor formation. These studies (carried out in collaboration with G. Shackelford) have allowed us to identify a spectrum of genes that can collaborate with int-2 to bring about this phenotype. One of these genes is particularly interesting as it appears to be a new member of the Wnt family of genes known to play a role in carcinogenesis and early development. Further studies have explored the ability of p53 deficiency mutations to complement the action of dominant oncogenes. Interestingly, while rapidly accelerating several other tumor types, the p53 deficiency mutation has no effect on the kinetics of mammary carcinoma in mice bearing MMTV-MYC and -NEU oncogenes. Such observations suggest that, despite its presumed role in a ubiquitous cell cycle, p53-induced transformation is strongly influenced by cell context, an issue that is likely to be of great importance in human breast cancer.

The New Frontiers

A1-018 BCL-2 GENE FAMILY AND THE REGULATION OF PROGRAMMED CELL DEATH, Stanley J. Korsmeyer, Xiao-Ming Yin, Elizabeth Yang, Jiping Zha, Thomas Sedlakand Zoltán Oltvai, Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, MO.

The maintenance of homeostasis in normal tissues reflects a balance between cell proliferation and cell death. The importance of both positive and negative regulators of cell growth has been well documented in neoplasia. Bcl-2 argues for the existence of a new category of oncogenes, regulators of cell death. The Bcl-2 gene was identified at the chromosomal breakpoint of t(14;18) bearing B cell lymphomas. Bcl-2 has proved to be unique among proto-oncogenes in blocking programmed cell death rather than promoting proliferation. In adults, Bcl-2 is topographically restricted to progenitor cells and longlived cells but is much more widespread in the developing embryo. Transgenic mice that overexpress Bcl-2 in the B cell lineage demonstrate extended cell survival, and progress to high grade lymphomas. Bcl-2 has been localized to mitochondria, endoplasmic reticulum, and nuclear membranes, also the sites of reactive oxygen species generation. Bcl-2 does not appear to influence the generation of oxygen free radicals but does prevent oxidative damage to cellular constituents including lipid membranes. Bcl-2 deficient mice complete embryonic development and display relatively normal hematopoietic differentiation but undergo fulminant lymphoid apoptosis of thymus and spleen. Moreover, they demonstrate profound renal cell death and develop polycystic kidney disease and also hair hypopigmentation. A family of Bcl-2 related proteins regulate cell death and share highly conserved BH1 and BH2 domains. BH1 and BH2 domains of Bcl-2 were required for it to heterodimerize with Bax and to repress apoptosis. A yeast-two-hybrid assay accurately reproduced this interaction and defined a selectivity and hierarchy of further dimerizations. Bax also heterodimerizes with Bcl-x_L, Mcl-1, and A1. Substitutions in BH1 of Bcl-x_L disrupted its heterodimerization with Bax and also abrogated its inhibition of apoptosis in mammalian cells. To further investigate the molecular mechanism of Bcl-2 function, we searched for additional interactive targets of Bcl-2. Both the yeast-two-hybrid system and lambda expression cloning identified a new protein, Bad, which has homology clustered in BH1 and BH2 domains. Bad heterodimerizes with either Bcl-2 or Bcl-x_L. Bad reverses the protective effect of Bcl-x_L, restoring apoptosis, but did not reverse the death inhibition by Bcl-2. Bad appears to counter the function of Bcl-x_L by preferentially dimerizing with Bcl-x_L and decreasing the amount of Bcl-x_L/Bax complexes. The competition of Bad with Bax for Bcl-x_L results in more unbound Bax, leading to unopposed cell death. Thus, the susceptibility to a death stimulus in a given cell is dictated by a complex set point determined by the relative levels and interactions amongst these family members.

Late Abstracts

EXAMINATION OF TUMOR SUPPRESSOR GENE FUNCTION USING GENE TARGETING IN THE MOUSE, Tyler Jacks, Howard Hughes Medical Institute, Center for Cancer Research, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

Using the technique of gene targeting in embryonic stem (ES) cells, we have created a series of mouse strains carrying germline mutations in the murine homologues of several human tumor suppressor genes. Like their human counterparts, mice heterozygous for mutations in *Rb*, *Nf1* or *p53* are highly cancer prone, although the particular tumor types to which the two species are predisposed differ somewhat. Consistent with Knudson's two-hit model, most of the tumors arising in mice heterozygous for a tumor suppressor gene mutation show loss of the wild-type allele. We have also bred these mutations to homozygosity in order to determine what role, if any, the different tumor suppressor genes play in normal development. Absence of function for *Rb*, *Nf1*, or *Nf2* causes embryonic lethality, with specific developmental defects. The majority of *p53*-deficient embryos survive gestation normally, although a fraction exhibit abnormal neural tube closure and exencephaly. Those *p53* homozygotes which are born succumb to tumors within the first several weeks of postnatal life.

We have exploited this system in several different ways to continue to probe the tumorigenic and developmental effects of inactivation of individual or multiple tumor suppressor genes. For example, we have constructed ES cells which are homozygous the *Nf1* mutation and used them to create adult chimeric animals partially composed of *Nf1*-deficient cells. These chimeras develop hyperplastic lesions around nerves which are very reminiscent of human neurofibromas. Also, we have intercrossed the different tumor suppressor gene mutant strains to examine possible cooperative tumorigenic effects of inactivation of multiple members of this class. In the most well-studied cross, we have observed novel tumor phenotypes in animals doubly mutant for *Rb* and *p53*, suggesting that the loss of function of both of these genes can contibute to the transformation of certain cell types in the mouse.

Primary cells and cell lines isolated from the different mutant strains have proven very useful in the analysis of tumor suppressor gene function. For example, cells lacking *p53* are defective in G1 arrest or apoptosis in response to DNA damage or other adverse conditions. The role of *p53* in apoptosis appears to be critical in the function of this gene in tumor suppression as well as in the resposiveness of tumor cells to anti-cancer treatment.

STRUCTURE AND FUNCTION OF SH2 SIGNALLING PROTEINS

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The mechanisms by which SH2 domains recognize distinct phosphotyrosine-containing sites, and the relevance of this selectivity for in vivo signalling, have been investigated. The structure of the C-terminal PLC-γ1 SH2 domain has been solved in association with a phosphotyrosine-containing peptide representing the Tyr1021 autophosphorylation site in the tail of the βPDGF-receptor. The PLC-γ SH2-C domain has an extended hydrophobic cleft that binds the +1 to +6 peptide residues C-terminal to the phosphotyrosine. This is quite distinct from the Src SH2 binding pocket, and starts to address the mechanisms by which SH2 binding specificity is achieved.

Site-directed mutagenesis has been used to investigate the binding specificity of the Src SH2 domain. By changing a single residue in the EF loop (EF1) from a Thr to a Trp, the binding specificity of the Src SH2 domain has been altered to resemble that of Sem-5/drk/Grb2, an adaptor protein that couples tyrosine kinases to the Ras pathway. This mutant Src SH2 domain effectively substitutes for the Sem-5 SH2 domain in C. elegans in activation of the Ras pathway in vivo. Hence the binding specificity of an SH2 domain correlates with its biological activity.

We have begun to address the functions of SH2 signalling proteins by disrupting the relevant genes in the mouse germline. The effects of targeted mutagenesis of the gene for Ras GTPase activating protein will be described.

SRC: Key to Kinases and Receptors; Cytoplasm: The Connectors

A1-100 *Abstract Withdrawn*

A1-101 ANALYSIS OF INTERACTIONS BETWEEN THE ABELSON TYROSINE KINASE AND ITS PUTATIVE SUBSTRATE ENABLED.

S. M. Ahern, F. Gertler, A. Comer and F. Michael Hoffmann. McArdle Laboratory, University of Wisconsin, Madison, WI 53706, (608)262-8854.

The Abl protein tyrosine kinase has been implicated in human chronic myelogenous leukemia and acute lymphoblastic leukemia. The *Drosophila Abl* gene is required for adult viability and proper development of the embryonic nervous system. Heterozygous mutations in *enabled* (*ena*)suppress the lethality associated with mutations in the *Abl* locus. Sequence analysis of the *ena* gene identified a proline rich domain which was subsequently shown to interact with the Abl SH3 domain on filter binding assays. The ena protein is expressed as a phosphorylated protein in *Drosophila* embryos, pupae, and cultured cells, is hyperphosphorylated when co-expressed with Abl in cultured cells and is hyperphosphorylated in Abl mutant pupae. Taken together, these genetic and biochemical data are consistent with a model in which the critical function of the Abl tyrosine kinase may be to negatively regulate ena activity, perhaps by phosphorylating the ena protein.

A1-102 THE 16K VACUOLAR PROTON PUMP SUBUNIT ASSOCIATES WITH TYROSINE KINASE GROWTH FACTOR RECEPTORS AND THREE ADDITIONAL CELLULAR PROTEINS.

Thorkell Andresson and Richard Schlegel. Department of Pathology, Georgetown University, Washington, DC, USA.

The E5 gene encodes the main transforming protein of BPV-1. The transformation ability of E5 has been linked to ligand independent activation of tyrosine kinase (TK) growth factor receptors. This event is accompanied by formation of a stable complex between E5 and TK receptors such as PDGF-R. Furthermore, E5 has been shown to associate with the 16 kDa (16K) pore forming component of the vacuolar proton ATPase. The binding site for E5 on 16K has been mapped to the highly conserved fourth transmembrane (TM) domain suggesting that E5 is targeting a critical domain of 16K. Even more suggestive of the importance of 16K for E5 transformation is the notion that mutants altered in this fourth TM domain of 16K are transforming in NIH 3T3 cells. While the interaction between E5 and the PDGF-R has been established in NIH 3T3, C127, 32D and COS cells, we have shown, in COS cells, that 16K has the ability to associate with the PDGF receptor without the presence of E5.

To further evaluate the importance of 16K for cell growth regulation we have studied, in more detail, the interaction between 16K and the TK growth factor receptors. We have also looked for additional cellular factors associated with 16K.

We demonstrate here by using co-transfection and immunoprecipitation experiments in COS cells that, in addition to interacting with PDGF-R, 16K is capable of associating with least two other TK growth factor receptors: the EGF- and erbB2-receptors. In contrast, neither the Tac-alpha antigen and the MHC I receptors, which are highly expressed, co-precipitate with 16K, suggesting that 16K specifically interacts with the aforementioned TK growth factor receptors. The binding site for the receptor on 16K was also mapped to the fourth TM domain on 16K. These data indicate that 16K is a potential component of TK receptor complexes and we have initiated studies to determine its role in the E5/16K/PDGF-R complex. In addition, we have identified 3 cellular proteins (60K, 35K, and 31) which are also associated with 16K and which may represent component of the vacuolar proton pump.

A1-103 THE PDGFβ RECEPTOR AND SRC ACTIVATE PLCγ2 UPON CO-EXPRESSION IN FISSION YEAST.

Steve Arkinstall, Mark Payton and Kinsey Maundrell, Glaxo Institute for Molecular Biology, 1228 Plan-les-Ouates, Geneva, Switzerland.

The fission yeast *S. pombe* has no detectable *endogenous* receptor tyrosine kinases or associated signalling apparatus and we have used this cell system to reconstitute mammalian PDGFβ receptor-linked activation of phospholipase C γ2 (PLCγ2). The PDGFβ receptor migrates as a glycosylated protein of 165kDa associated exclusively with membrane fractions. No tyrosine autophosphorylation was detected when PDGFβ was expressed alone. PLCγ2 appears as a 140kDa protein distributed between particulate and soluble fractions which exhibits characteristic selectivity for PIP_2 and is sensitive to powerful activation by Ca^{2+}. When co-expressed, both PDGFβ and PLCγ2 undergo tyrosine phosphorylation and this is accompanied by a >26-fold increase in $[^3H]IP_2$ and $[^3H]IP_3$ production. Treatment with the tyrosine phosphatase inhibitor pervanadate further increased PLCγ2 tyrosine phosphorylation as well as $[^3H]IP_2$ and $[^3H]IP_3$ generation. Phosphorylated PLCγ2 was found predominantly in membrane fractions. To test a non-receptor tyrosine kinase we expressed the human proto-oncogene c-Src together with its negative regulator Csk. These were immunodetectable as bands at 60kda (c-Src) and 50kDa (Csk) and distributed between membrane and cytosolic fractions. When yeast co-expressing c-Src, Csk and PLCγ2 were incubated with pervanadate, PLCγ2 was tyrosine phosphorylated and $[^3H]IP_2$ and $[^3H]IP_3$ production increased by 11.0- and 7.0-fold, respectively. Csk expressed alone with PLCγ2 was ineffective. In summary, this is the first report of a reconstitution of mammalian tyrosine kinase-linked effector activation in yeast and also the first demonstration of direct PLCγ2 activation by the proto-oncogene c-Src. These observations indicate that *S. pombe* provides a powerful cell system in which to study critical molecular interactions and activities underlying receptor and non-receptor tyrosine kinase-dependent cell signaling.

A1-104 THE MAP KINASE PATHWAY IN HUMAN LUNG CANCERS, Anu Bansal^, Melanie H. Cobb* and John D. Minna^, Simmons Cancer Center^, and Department of Pharmacology*, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX75235-8593.
The MAP kinase pathway is involved in cellular proliferation and differentiation. This pathway is activated by several hormones and growth factors and involves Ras, Raf, MEK and MAP kinase (also known as ERK). c-ras is mutated in some human cancers. In addition, recently it was shown that constitutively active MEK leads to cellular transformation and tumor growth in nude mice (1-2). Thus, it is possible that components of this pathway (in lieu of *ras* mutations) could be deregulated leading to malignant transformation. To investigate the involvement of this pathway in human cancer we determined ERK activities under serum starved and serum and growth factor stimulated conditions in three lung cancer cell lines (one with and two without K-*ras* mutations) and in normal human bronchial epithelial cells growing in cell culture. The basal ERK activities (approx. 120 pmol/min/mg), under serum starved conditions, in all these cell lines and epithelial cell cultures are higher than those reported for Rat-1, Swiss-3T3 or PC-12 cells (20-50 pmol/min/mg) which have been used for many other MAP kinase studies. However, there was no marked difference between normal and malignant cells under serum starved conditions, even for the cancer line with a *ras* mutation. Interestingly, with serum or 10 nM insulin stimulation (5 min.) cancer cells showed 2-10 fold activation in their ERK activities, while the normal bronchial epithelial cells did not although all had equivalent levels of MAP kinase protein as detected by immunoblotting. We conclude there are differences between lung cancer and normal epithelial cells in the response of the MAP kinase pathway to growth stimulatory signals.

1. Cowley, S., Paterson, H., Kemp, P., and Marshall, C.J. (1994) Cell *77*, 841-852.
2. Mansour, S.J., Matten, W.T., Hermann, A.S., Candia, J.M., Rong, S., Fukasawa, K., Vande Woude, G.F., and Ahn, N.G. (1994) Science *265*, 966-970.

A1-105 IDENTIFICATION OF SEQUENCES IN TRK RESPONSIBLE FOR THE ACTIVATION OF PHOSPHOINOSITIDE 3-KINASE. Ruth M.Baxter and C.Peter Downes, Dept of Biochemistry, Medical Sciences Institute, University of Dundee, Dundee, DD1 4HN, UK.

The mechanism by which NGF leads to the activation of phosphoinositide 3-kinase has yet to be fully understood. The receptor for NGF, the trk tyrosine kinase, contains potential binding sites for PI 3-kinase, phospholipase C-γ and SHC. PC12 cells transfected with chimera receptors of the trk signalling domain with mutations in the potential binding sites, fused to the PDGF receptor ligand binding domain, were supplied by A. Ullrich and colleagues. We have used these cells to assess the effects on signalling pathways of the different mutations. We will present data to show that the activation of PI 3-kinase does not require the 'high affinity' p85 binding site but appears to be dependent on the binding of SHC to the receptor. We will also show that the activation of PLC-γ is prevented when its binding site has been mutated. These results will help to further elucidate the mechanisms by which NGF causes PC12 cells to differentiate.

A1-106 TYROSINES 720, AND 731/742 ARE PHOSPHORYLATION SITES IN THE PDGF α RECEPTOR, AND ARE REQUIRED FOR THE BINDING OF Syp AND PI3K RESPECTIVELY, Chantal E. Bazenet and Andrius Kazlauskas, National Jewish Center, Dept.of Pediatrics, 1400 Jackson Street, Denver, CO 80206.
The human platelet-derived growth factor α receptor (αPDGFR) was expressed in PhB cells, fibroblasts derived from *Ph/Ph* mouse embryos, which express the βPDGFR, but do not express the αPDGFR. In response to PDGF binding, the αPDGFR autophosphorylates on tyrosine residues which become binding sites for signal transduction molecules that include phospholipase Cγ1 (PLC-γ1), phosphatidylinositol 3-kinase (PI3K), the phosphotyrosine phosphatase Syp, Grb2, $pp60^{src}$ and a 120 kd protein. Unlike the βPDGFR, the activated αPDGFR does not stably associate with the GTPase activating protein of ras (rasGAP), nor does it mediate tyrosine phosphorylation of rasGAP. Despite its apparent inability to interact with rasGAP, the αPDGFR is fully able to trigger PDGF-dependent $p21^{ras}$ activation and DNA synthesis (1).

In order to assess which proteins or events are necessary for activation of $p21^{ras}$ and DNA synthesis, we started to identify the tyrosine phosphorylation sites of the αPDGFR. To date we have identified three in vivo, and in vitro tyrosine phosphorylation sites which are located in the kinase insert domain of the receptor at the positions 720, 731, and 742. Mutation at Y742 significantly decreases the amount of PI3K that associates with kinase insert-containing fusion proteins, whereas mutation at Y731 reduces PI3K binding to an undetectable level. Y720 does not have any homologous tyrosine in the β PDGFR subunit and is found to be fully responsible for the binding of Syp. We are currently looking at the biological response triggered by these mutated receptors.

1- C.E. Bazenet and A. Kazlauskas (1994) Oncogene 9, 517-525.

A1-107 MAPPING OF AN INTERACTION BETWEEN THE CYTOPLASMIC DOMAINS OF THE TGF-ß RECEPTOR TYPE II AND TYPE III USING AN ANTIPEPTIDE ANTIBODY. Michel Beauchemin, Marie-Claude Pepin, Aristidis Moustakas, Josée Plamondon, Harvey F. Lodish and Maureen D. O'Connor-McCourt. Cell Surface Recognition Group, Biotechnology Research Institute, National Research Council Canada, Montréal, Québec, Canada.

TGF-ß modulates many cellular events such as proliferation, differentiation and extracellular matrix production. It is involved in many physiological or pathological events such as cancer, fibrosis, wound healing and immunosuppression. Most cells express mainly three types of membrane receptors for TGF-ß. Type I and type II receptors are similar, being composed of an extracellular domain, a single transmembrane domain and a serine-threonine kinase cytoplasmic domain which is related between the two receptors. Type III receptor has a large extracellular domain, a single transmembrane domain and a very short cytoplasmic domain which shows no evident signaling motif. Signal transduction following TGF-ß binding involves an interplay between these receptors. Binding of TGF-ß to type II receptor stimulates heteromerization with type I receptor and signal transduction. Type III receptor can interact with type II receptor and this appears to result in the delivery (i.e. increased binding) of TGF-ß to type II receptor. Current evidence suggests that this interaction occurs between the extracellular domain of both receptors. Using an antipeptide antibody against residues 833 to 847 of the cytoplasmic domain of the type III receptor and co-transfection studies of type III and type II receptors, we show here that the amino acid sequence recognized by this antibody becomes shielded (as shown by inhibition of immunoprecipitation) upon interaction with the type II receptor. Control immunoprecipitation with antibodies against the type II receptor or epitope-tagged receptors showed that this effect was specific to the anti-type III antibody. Results obtained with a truncated type II receptor indicate that the cytoplasmic domain of type II receptor is required to shield the cytoplasmic domain of receptor type III. The shielded sequence corresponds to a region that has high homology with endoglin, a TGF-ß binding membrane protein which is also able to interact with type II receptor. Our results strongly suggest that the cytoplasmic domains of type II and type III receptors interact upon TGF-ß binding and we suggest that this may modulate the response to TGF-ß.

A1-108 SHC IS COSTITUTIVELY ACTIVATED BY A TRANSFORMING EGF RECEPTOR MUTANT WITH A POINT MUTATION IN THE TRANSMEMBRANE DOMAIN.

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A point mutation was inserted in the transmembrane domain of the human EGF Receptor (EGF-R), glu-> val(aa 627), mimicking the activating mutation in the neu oncogene. This glu^{627}EGF-R was transfected in NIH3T3 cells and gave rise to focal transformation and growth in agar in the absence and presence of EGF. The transforming ability of glu^{627}EGF-R, without the ligand, was 20% of that of wild type EGF-R stimulated by EGF, while in the presence of the ligand it was similar to that of wild type receptor. The sensitivity to EGF for transformation was higher in glu^{627}EGF-R. NIH 3T3 cells expressing glu^{627}EGF-R showed a transformed phenotype and were not arrested in G_0 upon serum deprivation.

The glu^{627}EGF-R was costitutively autophosphorylated and several other cellular proteins were phosphorylated on tyrosine in the absence of the ligand. Among these, the SHC adaptor protein was phosphorylated in the absence of EGF and was costitutively associated with glu^{627}EGF-R. In contrast, other EGF-R substrates, phospholipase Cγ and GAP, were not found costitutively phosphorylated. The glu^{627}EGF-R showed a higher sensitivity to cleavage by calpain in the absence or in the presence of EGF and appeared as a 170 and 150 Kd bands in cell extracts. A specific calpain inhibitor blocked the appearance of the 150 kd form, indicating that this was a proteolytic fragment of the intact EGF-R. Since the calpain cleavage site is located in the cytoplasmic tail of the EGF-R (aa 1037), this finding suggests that the glu^{627} mutation induces a slightly different conformation in the intracellular domain of the receptor. In conclusion our data show that a point mutation in the transmembrane domain of the EGF-R is able to costitutively activate the receptor and induce transformation probably via a costitutive activation of the ras pathway.

A1-109 REGULATION OF KIT/STEM CELL FACTOR RECEPTOR SIGNALING BY PROTEIN KINASE C,

Peter Blume-Jensen, Lars Ronnstrand, Agnetha Siegbahn[1] and Carl-Henrik Heldin
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The Kit/stem cell factor receptor is encoded by the proto-oncogene c-*kit*. It is a transmembrane tyrosine kinase receptor of importance for the normal development of hemopoietic cells, melanoblasts and germ cells. We have earlier shown that protein kinase C (PKC) is involved in a negative feedback loop regulating the Kit/SCF-R by direct phosphorylation on serine residues in the receptor. Inhibition of PKC led to increased SCF-induced tyrosine kinase activity and mitogenicity, but PKC was necessary for SCF-induced cell motility. Of several signaling pathways examined only SCF-induced phosphatidyl inositol-3'-kinase activation paralleled the increased SCF-induced mitogenicity after inhibition of PKC.

We have mapped the serine phosphorylation sites for PKC as well as several tyrosine autophosphorylation sites in the receptor, and stable cell lines of the mutated receptors have been established. The phosphorylation sites in the receptor and the signaling properties of the mutated receptors stably expressed in porcine aortic endothelial cells will be presented.

A1-110 NEGATIVE REGULATION OF p21ras SIGNALLING.

Johannes L. Bos, Boudewijn M.Th. Burgering. Alida M. M. de Vries-Smits, D. Henk J. van Weering, Maikel P. Peppelenbosch and Jan-Paul Medema. Laboratory for Physiological Chemistry, Utrecht University, 3508 TA Utrecht, The Netherlands

We have identified cAMP and calcium as negative regulators of p21ras signalling. (i) In most fibroblasts, the cAMP analogue 8-bromo-cAMP, as well as activators of adenylate cyclase, inhibit p21ras signalling. This inhibition occurs at the level of raf1 kinase, a direct effector of p21ras. This inhibition may explain, at least in part, the observed inhibition of mitogenesis by elevated levels of cAMP in these cells. (ii) In keratinocytes we observed that calcium inhibits p21ras signalling at the level of p21ras. This calcium signal functions in the induction of differentiation and is presumably mediated by a calcium receptor. The steps between this putative receptor and p21ras is currently under investigation, but may involve p120GAP and the GAP-associating protein p62.

A1-111 DOWN-REGULATION OF RAS SIGNALLING PATHWAY INVOLVES PHOSPHORYLATION OF SOS EXCHANGE PROTEIN,

László Buday and Julian Downward, I. Institute of Biochemistry, Semmelweis University School of Medicine, Budapest, Hungary and Imperial Cancer Research Fund, London, U.K.

We have recently shown that upon T cell receptor/CD3 cross-linking the complex of Sos guanine nucleotide exchange factor and Grb2 associates with a 36 kDa tyrosine phosphoprotein. Here we report that both T cell receptor/CD3 triggering and phorbol ester-treatment of T lymphocytes induce Sos phosphorylation on serine/treonine residues. The phosphorylation does not affect the association of Grb2-Sos complex. However, a phosphopeptide (Y-1068) derived from the EGF receptor that can precipitate the Grb2-Sos complex fails to precipitate the phosphorylated Grb2-Sos from T cells stimulated with phorbol ester for more than 5 mins. In addition, phorbol ester pretreatment of T cells for 10 mins completely inhibits the UCHT1 antibody-induced p36-Grb2-Sos complex formation in vivo. We show that purified and activated MAP kinase can in vitro phosphorylate Grb2-Sos complex bound to the tyrosine phosphopeptide. These data suggest a MAP kinase-dependent feedback mechanism for the termination of ras signalling pathway in which phosphorylated Sos is released from the plasma membrane.

A1-112 ACTIVATION OF AN INTRACELLULAR PROTEIN TYROSINE KINASE CASCADE IN NIH 3T3 CELLS OVEREXPRESSING WILD-TYPE FIBROBLAST GROWTH FACTOR-1,

Wilson H. Burgess and Anne Shaheen, Department of Molecular Biology, American Red Cross, Rockville, MD 20855

NIH 3T3 cells were transfected with expression vectors encoding either wild-type or a lysine$_{132}$ to glutamic acid mutant of fibroblast growth factor-1 (acidic fibroblast growth factor). The mutant growth factor is a relatively poor mitogen when added exogenously to cells. Cells expressing the wild-type protein exhibited a transformed phenotype and a loss of contact inhibition of growth. Cells expressing similar levels of the mutant protein displayed a phenotype characteristic of untransfected cells. Examination of lysates of the transfected cells by Western blot analysis revealed the presence of a variety of phosphotyrosine-containing proteins in the wild-type transfectants that were not seen in cells overexpressing the mutant protein. These phosphotyrosine-containing proteins were, in general, distinct from those seen when the wild-type growth factor is added exogenously to cells. The phosphotyrosine content of some, but not all, of the proteins was reduced following exposure of the cells to suramin. There was no apparent down-regulation of cell surface receptors in the wild-type transfected cells when compared to the mutant transfectants implying that the recombinant growth factor was not released. Together these data indicate that multiple protein tyrosine kinase cascades are activated by high levels of intracellular fibroblast growth factor-1. The results support an intracrine role for the growth factor. Preliminary studies indicate that the src or a related tyrosine kinase pathway is activated in the wild-type transfected cells. This work was supported, in part, by National Institutes of Health grant HL35762 and American Heart Association grant 92011200.

A1-113 STUDIES OF THE FUNCTION OF ECK; A RECEPTOR PROTEIN TYROSINE KINASE OF THE EPITHELIA .

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ECK is a receptor protein tyrosine kinase originally cloned from a HeLa cell library, and found to be preferentially expressed in epithelial tissues and epithelially derived cell lines. ECK cDNA clones are deduced to encode a protein of 976 amino acids which belongs to the *eph/elk* family of receptor tyrosine kinases. This family contains at least 12 members, yet little or no information exists linking any family member to a specific cellular function. Very recently B61 has been identified as a ligand for ECK. B61 was originally identified as the product of an immediate/early response gene in TNFα stimulated human umbilical vein endothelial cells.

We are currently studying the signal transduction mechanisms activated by B61 binding to ECK. On binding B61 ECK is phosphorylated on multiple tyrosine residues and we are identifying these autophosphorylation sites and looking for GST-SH2 domain fusion protein binding as the first steps in defining downstream effectors utilised by B61/ECK. Another approach we are taking is to look at the effect of ECK activation on the decision to proliferate or differentiate in the human keratinocyte line, HaCaT, stably overexpressing human ECK. We have constructed a C-terminally truncated kinase negative form of ECK and are studying the effects of this putative dominant negative receptor in HaCaT cells and in transgenic mice. The results of these investigations will be presented.

A1-114 BIOCHEMICAL MECHANISMS OF SIGNAL TRANSDUCTION THROUGH TGF-ß RECEPTOR KINASE COMPLEXES,

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Transforming growth factor-beta (TGF-ß) signals through a receptor complex containing the type I (TGF-ßRI) and type II (TGF-ßRII) receptors. We describe here biochemical studies on early events in the TGF-ßR signaling pathway. TGF-ßRII is highly phosphorylated when expressed alone in COS1 cells; its autophosphorylation occurs via an intramolecular (cis-) mechanism that is independent of ligand binding. TGF-ßRI is also highly phosphorylated when expressed alone in COS1 cells. Both wild type TGF-ßRI and a kinase-deficient mutant thereof are transphosphorylated by the co-expressed TGF-ßRII kinase in a ligand-independent fashion in these cells. We propose that the association of TGF-ß type I and II receptors, induced either by ligand binding or overexpression, leads to transphosphorylation of the TGF-ßRI by the TGF-ßRII kinase, which in turn triggers the receptor kinase activities towards the downstream substrates. This represents a novel mechanism distinct from that of tyrosine kinase receptors and may well apply to other serine/threonine kinase receptors.

A1-115 UV ACTIVATION OF RECEPTOR TYROSINE KINASE ACTIVITY

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The exposure of mammalian cells to ultraviolet radiation may lead to DNA damage resulting in mutation and possibly cancer. In bacterial and yeast systems exposure to UV sources leads to the induction of genes involved in DNA repair, however transcriptional responses in mammalian cells do not appear to involve genes directly regulating DNA repair itself. More than a decade ago it has been demonstrated that that UV-treatment of eukaryotic cells leads to the induction of immediate early genes such as c-*fos*. More recently the mammalian UV response has been linked to activation of the Ras-signalling pathway. The nature of the most upstream target(s) of UV action, however, remained undefined.

We have been investigating the identity of these UV activated signalling molecules focussing on the role of growth-factor receptors. We have determined that UV stimulation of mammalian cells leads to tyrosine phosphorylation and activation of both the insulin and EGF receptors. This activation results in the propagation of many of the intracellular signalling pathways utilised by these receptors including/involving : IRS-1 and SHC phosphorylation, PI-3 kinase activation, leukotriene synthesis, MAP kinase activation and gene induction. We further show that "kinase-dead" receptor mutants act in a dominant-negative manner blocking leukotriene synthesis, MAP kinase activation and gene transactivation in response to UV stimulation. These data point towards a critical role for receptor mediated events in regulating the response of mammalian cells to UV exposure.

A1-116 *Ron* IS A HETERODIMERIC TYROSINE KINASE RECEPTOR ACTIVATED BY THE HGF HOMOLOGUE MSP, Paolo M. Comoglio*, Antonia Follenzi*, Luigi Naldini*, Chiara Collesi*, Massimo Santoro*, Kathleen A. Gallo§, Paul J. Godowski§ and Giovanni Gaudino*, *Department of Biomedical Sciences and Oncology, University of Torino School of Medicine, Torino, Italy §Department of Cell Genetics, Genentech, San Francisco, U.S.A.

RON, a cDNA homologous of the Hepatocyte Growth Factor receptor gene (*MET*), encodes a putative tyrosine kinase. Here we show that the *RON* gene is expressed in several epithelial tissues as well as in granulocytes and monocytes. The major *RON* transcript is translated into a glycosylated single-chain precursor, cleaved into a 185 kDa heterodimer ($p185^{RON}$) of 35 kDa (α) and 150 kDa (β) disulfide-linked chains, before exposure at the cell surface. The *Ron* β chain displays intrinsic tyrosine kinase activity *in vitro*, after immunoprecipitation by specific antibodies. *In vivo*, tyrosine phosphorylation of $p185^{RON}$ is induced by stimulation with MSP (Macrophage Stimulating Protein), a protease-like factor containing four "kringle" domains, homologous to Hepatocyte Growth Factor (HGF). In epithelial cells, MSP-induced tyrosine phosphorylation of $p185^{RON}$ is followed by DNA synthesis. $P185^{RON}$ is not activated by HGF, nor the HGF receptor is activated by MSP, in biochemical and biological assays. $P185^{RON}$ is also activated by a pure recombinant protein containing only the N-terminal two kringles of MSP. These data show that $p185^{RON}$ is a tyrosine kinase activated by MSP and that it is member of a family of growth factor receptors with distinct specificities for structurally-related ligands.

A1-117 IDENTIFICATION AND CHARACTERIZATION OF HUMAN ENDOMETRIAL PROTEIN TYROSINE KINASES: EXPRESSION OF lyn, A src-FAMILY TYROSINE KINASE, G. COUCHMAN, D. WALMER, J.McLACHLAN, K.NELSON. Dept. of Obstet. and Gyn. Duke University Medical Center, Durham, N.C.,Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences,RTP,N.C.

The human endometrium is a dynamic reproductive tissue involved in the complex, cyclic changes from menstruation to implantation or regeneration and repair. Signal transduction pathways involved in these processes are of particular interest in attempting to understand the nature of endometrial function. In particular, protein tyrosine kinases (PTKs), a family of signaling peptides, are believed to play a major role in growth and differentiation of many cells. The identification and characterization of novel protein tyrosine kinases will likely offer new insights into the regulation and differentiation of the endometrium, not only in the regulation of normal cells but also in disease states such as cancer. We developed a screening and cloning approach to identify novel PTK-related genes from human cDNA libraries constructed from proliferative and secretory endometrium. Cloning strategies used to select for PTK genes were the use of antibodies to phosphotryosine and the use of degenerate oligos against the highly conserved catalytic domains of phosphotyrosine kinases. By these methods, we have successfully identified and partially sequenced numerous PTK genes that are likely involved in endometrial functioning. Of particular interest was one kinase clone, lyn, a member of the src family. To date, neither lyn nor any other src family tyrosine kinase has been described in the human reproductive tract but lyn has been implicated in hepatocytes to be critical in cell-cell adherens junctions as signal mediators between cells. Our lyn clone is used to investigate its expression in normal cycling endometrium and in proliferative disorders such as hyperplasia and adenocarcinoma. Protein expression in the female reproductive tract by immunohistochemistry and Western blotting reveals an abundance of protein expression in glandular epithelium in the secretory phase of the menstrual cycle, in certain hyperplastic glands and in some higher grade endometrial adenocarcinomas. Probing Northern blots for endometrial RNA demonstrates lyn expression in normal endometrium and carcinomas with over-expression of adenocarcinomas compared to normal glandular endometrium. We conclude that the abundant expression of lyn in the secretory phase of the menstrual cycle, its variable protein expression in abnormal tissue and over-expression in certain adenocarcinomas suggests and important role in reproductive function. In progress are studies to further elucidate the physiologic role of lyn and other tyrosine kinases in the growth and differentiation of the endometrium by comparing tyrosine kinase expression to other known physiologic parameters such as steroid hormone receptor status and their proliferative index.

A1-118 MAP KINASE ACTIVATION BY β-ADRENERGIC RECEPTORS AND BY LPA INVOLVES $\beta\gamma$ SUBUNITS OF HETEROTRIMERIC G PROTEINS ACTING ON $p21^{ras}$
Piero Crespo, Ningzhi Xu, William F. Simonds[1] & J. Silvio Gutkind, Molecular Signalling Unit, Laboratory of Cellular Development and Oncology, NIDR, and [1]Metabolic Diseases Branch, NIDDK, NIH, Bethesda, MD 20892.

In previous studies we have shown that activation of MAP kinase mediated by muscarinic acetylcholine receptors m1 and m2 ectopically expressed in COS-7 cells is mediated by $\beta\gamma$ subunits of heterotrimeric G proteins (Crespo *et al.*, Nature 369: 418, 1994). Activation of MAP kinase by these receptors is prevented by *ras*-inhibitory constructs. We now report that stimulation of endogenously expressed receptors for the mitogenic phospholipid LPA and for β-adrenergic agonists can also trigger MAP kinase activation in a $\beta\gamma$ and *ras*-dependent fashion. Furthermore, both receptors induce an increase in GTP-bound *ras* through $\beta\gamma$. However, signaling to the MAP kinase cascade from β-adrenergic receptors appears to be complex: two counteracting pathways are simultaneously induced upon agonist addition. Whereas an activating signal emanates from $\beta\gamma$, the GTP-bound $G\alpha s$ induces an inhibitory pathway mediated by cAMP and PKA. Work in progress will be discussed in view of *ras*-exchange factors involved.

A1-119 ACTIVATORS OF PROTEIN KINASE C STIMULATE ASSOCIATION OF SHC AND THE PEST TYROSINE PHOSPHATASE, Stuart J. Decker, Roman Herrera, and Tania Habib, Parke-Davis Pharmaceuticals, 2800 Plymouth Road, Ann Arbor, MI 48106 and The Department of Microbiology, University of Michigan, Ann Arbor, MI 48104
Using the yeast two-hybrid system, complementary DNA clones were isolated from a Hela cell library encoding proteins which interacted with $p52^{shc}$. One of these clones encoded the non-catalytic, C-terminal half of the cytosolic protein tyrosine phosphatase PTP-PEST. Expression of truncated forms of $p52^{shc}$ in the two-hybrid system revealed that the amino terminal half of $p52^{shc}$ was sufficient for interaction with PTP-PEST. The p52 and p66 forms of SHC, but not the p46 form, bound to a glutathione transferase fusion protein containing the region of PTP-PEST isolated from the two-hybrid screen. Similarly, when Hela cell lysates were immunoprecipitated with PTP-PEST antiserum, $p52^{shc}$ and $p66^{shc}$ proteins, but not $p46^{shc}$ co-precipitated. SHC-PTP-PEST complex formation was stimulated 6-8 fold by the protein kinase C activator phorbol 12-myristate 13-acetate (PMA), while EGF and serum had no effect. The muscarinic agonist carbachol (also an activator of protein kinase C) stimulated complex formation 3-5 fold in SH-SY5Y neuroblastoma cells. These results suggest a role for PTP-PEST in G protein receptor signaling and in cross-talk between G protein receptor and tyrosine kinase receptor pathways.

A1-120 IDENTIFICATION OF PROTEIN TYROSINE PHOSPHATASES EXPRESSED IN HEMATOPOIETIC STEM CELLS, Mercedes Dosil, Neville Leibman and Ihor R. Lemischka, Department of Molecular Biology, Princeton University, Princeton, New Jersey, NJ 08544-1014
The hallmark of the hematopoietic system is a precisely controlled production of at least eight cell lineages. At the center of this process lies the hematopoietic stem cell, which possesses both an ability to self-renew and to produce committed progenitors for all hematopoietic lineages. As an approach to understanding the function of hematopoietic stem cells, we have used enriched stem-cell populations to identify molecules that may be important in regulating developmental decisions and cell proliferation. One class of molecules that may be involved in these processes are protein tyrosine phosphatases (PTPs). Several studies have shown that some PTPs, such as CD45 and HCP, can play critical roles in signal transduction pathways in the hematopoietic system. However, very little is known about the importance of these and/or other PTPs in primitive hematopoietic cells.

We have used a PCR cloning approach to isolate PTPs expressed in highly purified fetal liver stem cells. Analysis of 1300 independent clones revealed sequences derived from 13 different PTPs. Eleven cDNAs were identical to known PTP genes (LRP, PTPε-1, PTPβ, HCP, CD45, PTPT-9, PTPε-2, PTP-1, MEG2, PTP-BAS, and LPTPase), and two cDNAs corresponded to unreported genes. The sequences of these two novel PTPs, named Flp-1 and Flp-2, show high homology with the intracellular PEST-containing PTPs and the receptor PTP-ζ, respectively. We have investigated the expression of each PTP in hematopoietic cell populations isolated from yolk sac, fetal liver and bone marrow, and found that PTP-β, MEG-2 and Flp-1 are preferentially expressed in stem/progenitor cell populations.

A1-121 *IN VITRO* RECONSTITUTION OF THE LCK/TCR-ζ/ZAP-70 SIGNAL TRANSDUCTION AXIS FOR CRYSTALLOGRAPHIC STUDIES Michael J. Eck, Winfried Weissenhorn, Don Wiley and Stephen Harrison, Department of Molecular Medicine and the Howard Hughes Medical Institute, Children's Hospital, 300 Longwood Ave, Boston, MA 02115

The protein $p56^{lck}$ (Lck) is a lymphocyte-specific Src-family tyrosine kinase. It transduces signals required for T-cell development and antigen dependent T-cell activation, and is physically associated with the cytoplasmic domains of CD4 and CD8. Lck, like all Src-family tyrosine kinases, has a unique amino-terminal domain, followed by SH3, SH2, and catalytic domains. The N-terminal domain of Lck mediates its association with the cytoplasmic tail of CD4 or CD8. The SH2 and SH3 regions are homologous to domains found in many other proteins involved in signal transduction, and mediate protein-protein interactions. We have previously reported structures of fragments of Lck containing the SH2 domain, and both the SH3 and SH2 domains.

We have expressed and purified a fragment of Lck containing both the regulatory and catalytic domains in quantities suitable for crystallographic studies using a baculovirus/insect cell system. The purified kinase is catalytically active and phosphorylates the zeta chain of the T-cell receptor (TCR-ζ), a likely cellular substrate. Phosphorylation of pairs of tyrosine-containing motifs in the cytoplasmic region of TCR-ζ (called tyrosine activation motifs, or TAM's) creates binding sites for the downstream kinase Zap-70, which is composed of two SH2 domains followed by a tyrosine kinase domain. We have in vitro phosphorylated TCR-ζ (produced in a bacterial expression system) with the Lck kinase described above and reconstituted its interaction with an N-terminal fragment of Zap-70 containing both SH2 domains. The stoichiometry of phosphorylation of TCR-ζ by Lck, and the stoichiometry of binding of a "tandem" SH2 fragment of Zap -70 to TCR-ζ will be discussed.

A1-122 THE IGF-II/MANNOSE 6-PHOSPHATE RECEPTOR NEGATIVELY REGULATES IGF-II SIGNALLING IN BREAST CANCER CELLS Matthew J. Ellis, Brett Leav, Jia Yang, Marc Lippman and Kevin Cullen. Lombardi Cancer Research Centre, 3800 Reservoir Rd, Washington DC 20007 USA.

The insulin-like growth factors, IGF-I and II, have distinct roles in normal breast physiology and disease. IGF-I is critical for embryonic development as IGF-I -/- mice have hypoplastic reproductive organs and are sterile. Furthermore IGF-I is expressed in the stroma of normal breast and stimulates the growth and development of breast ducts at puberty. In contrast, IGF-II is not significantly growth-hormone regulated and IGF-II "knock out" mice are fertile. A further distinction between IGF-I and II is that the stomal cells in invasive breast tumors predominantly express IGF-II, not IGF-I, a reversal of the normal situation. These results imply that IGF-I is associated with normal breast physiology and IGF-II with carcinogenesis.

To contrast the action of IGF-I and II on breast epithelial cells, retroviruses were used to drive the expression of IGF-I, IGF-II or a series a mutant IGF's that have selective alterations in affinity for either the IGF-I receptor (IGF-IR) or the IGF-II/Mannose 6-Phosphate receptor (IGF-II/M6PR). Experiments in MCF-7 breast cancer cells demonstrate that the cellular response to activation of the IGF-IR is biphasic. Low levels of receptor tyrosine phosphorylation trigger proliferation, higher levels morphological changes, alterations in cellular adhesion, repression of fibronectin mRNA synthesis and anchorage-independent growth. The selective affinity of IGF-II for the IGF-II/M6PR restricts the ability of IGF-II to activate the IGF-IR. Thus the IGF-II/M6PR opposes IGF-II action. These findings suggest that therapeutic strategies that enhance IGF-II/M6PR function might be effective in IGF-II-dependent malignancies, without necessarily disrupting normal reproductive organ function.

A1-123 BACTERIAL LIPOPOLYSACCHARIDE (LPS) AND INTERFERON-GAMMA (IFN-γ) TRIGGER THE PROMPT TYROSINE PHOSPHORYLATION OF *VAV* IN RAW 264.7 MACROPHAGES. B. Keith English, Department of Pediatrics, The University of Tennessee, Memphis 38103

Several *src*-related tyrosine kinases play critical roles in the signaling pathways that control hematopoietic differentiation and activation. Macrophage activation by bacterial lipopolysaccharide (LPS) leads to the rapid phosphorylation of several proteins on tyrosine; inhibitors of tyrosine kinases (e.g., herbimycin A) block these tyrosine phosphorylation events and markedly reduce macrophage production of LPS-induced cytokines, including tumor necrosis factor (TNF). Recently we have provided evidence that the *hck* tyrosine kinase plays a critical role in LPS-mediated TNF production by murine macrophages (English, et. al., *J. Exp. Med.* 178: 1017, 1993).

We hypothesized that *vav*, a recently identified proto-oncogene that is known to be rapidly phosphorylated during the activation of T cells, B cells, and mast cells, would also be a substrate of LPS-mediated tyrosine phosphorylation in macrophages. We examined the phosphorylation status of $p95^{vav}$ in the murine macrophage cell line RAW 264.7. We found that exposure of quiescent RAW264.7 cells to either LPS or interferon-gamma (IFN-γ) led to the prompt tyrosine phosphorylation of $p95^{vav}$, in a dose and time-dependent fashion. Both LPS and IFN-γ provoked rapid increases in the tyrosine kinase activity of $p59^{hck}$. Finally, preincubation of RAW264.7 cells with antisense oligonucleotides specific for murine *hck* inhibited the tyrosine phosphorylation of $p95^{vav}$ after exposure to either LPS or IFN-γ. These observations suggest that the *hck* tyrosine kinase may mediate the tyrosine phosphorylation of *vav* during macrophage activation.

A1-124 The role of Syp phosphatase in signal transduction

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Autophosphorylation of activated receptor tyrosine kinases creates docking sites for immediate downstream signaling proteins including Src family kinases, GAP and PLC-g. These proteins share a so-called Src homology 2 (SH2) domain that mediates their interaction with activated receptor PTKs by directly recognizing specific phosphotyrosine-containing sites. Following the cloning of PTP1C(SH-PTP1, HCP, SHP), we and others identified a ubiquitously expressed PTP with two SH2 domains (Syp, SH-PTP2, PTP1D and PTP2C). Syp is able to physically bind to and tyrosine phosphorylated by a number of activated receptors, including PDGFR, EGFR, neu and c-Kit. Both the N-terminal and C-terminal SH2 domains are involved in the association with the receptors, and share the same binding specificity. In the case of PDGFR, for example, the two SH2 domains recognize specifically the P-Tyr1009. Syp is also a component of insulin signaling system since it binds to phosphorylated insulin receptor substrate 1 (IRS1) upon insulin stimulation. Furthermore, Syp is constitutively phosphorylated in *v-Src* and *Bcr-Abl* transformed cells, suggesting that the phosphatase is a downstream target of these transforming kinases. The mouse *Syp* gene has been mapped to chromosome 5F region. In order to understand the biological functions of Syp, we have disrupted the *Syp* gene in mouse embryonic stem (ES) cells, and obtained chimeric mice partly contributed by the Syp (-/+) ES cells. Heterozygous and homozygous mice mutated at the *Syp* locus are being produced.

A1-125 THE CRYSTAL STRUCTURE OF THE PLECKSTRIN HOMOLOGY DOMAIN OF HUMAN DYNAMIN, Kathryn M. Ferguson*, Mark A. Lemmon†, Joseph Schlessinger† and Paul B Sigler#, Departments of *Chemistry and of #Molecular Biophysics and Biochemistry and the Howard Hughes Medical Institute, Yale University, New Haven, CT 06510, †Department of Pharmacology, New York University Medical Center, New York, New York 10016.

The X-ray crystal structure of the pleckstrin homology (PH) domain from human dynamin has been determined and refined to 2.2 Å resolution. The PH domain is a region of approximately 120 amino-acids present in many proteins involved in intracellular signaling pathways. PH domains have been identified in protein kinases; regulators of small GTP-binding proteins (such as Sos and the Vav oncogene); cytoskeletal proteins; putative signaling adapter molecules; phospholipase C isoforms; as well as proteins involved in cellular membrane transport. The structure consists of a seven-stranded β-sandwich that is formed from two orthogonal antiparallel β-sheets. A C-terminal α-helix closes off one of the splayed corners of the sandwich. Opposite to this helix are three loops that are the most variable amongst different PH domains, and close off the other splayed corner. The interior of the sandwich comprises a very well packed hydrophobic core, containing many of the more conserved hydrophobic residues. Two molecules are present in the asymmetric unit and interact intimately with one another. In spite of the weak sequence similarity, the basic fold of the domain is very similar to those of two other PH domains recently determined by NMR, indicating that PH domains are distinct, characteristic structural modules. Their structure bears some resemblance to the calycin family of proteins, which bind to small lipophilic molecules. However, while all the calycins have a cavity in their interior, in which the hydrophobic ligand binds, the crystal structure of the dynamin PH domain shows no evidence for such a cavity. There is also a topological resemblance to streptavidin, which has no cavity in its hydrophobic core. Each of the PH domains with known structure is electrostatically polarized, with the three most variable loops in a positively charged region on one surface. This surface includes the residue (Y33) that corresponds to the position of the X-linked immunodeficiency mutation in the Btk PH domain, and may serve as a ligand binding surface; possibly for a small negatively charged ligand.

A1-126 RECEPTOR-MEDIATED RECRUITMENT OF p53/56^{LYN} TO A CAVEOLIN-CONTAINING MEMBRANE DOMAIN

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Certain src-family kinases have been recently found to associate with low-density, Triton X-100-insoluble membrane fractions which also contain the v-src substrate, caveolin[1]. We have found that about 30% of the cellular p53/56^{lyn} associates with similar caveolin-containing, low-density membranes following sucrose gradient fractionation of Triton-lysed RBL-2H3 cells. The amount of p53/56^{lyn} in this fraction increases substantially upon aggregation of the high affinity receptor for IgE (FcεRI). The p53/56^{lyn} in this fraction appears to be in a complex with 70-80 kDa and 40 kDa kinase substrates, but <1% of FcεRI is found in this fraction both before and after aggregation. In addition, both Thy-1 (a GPI-linked protein) and a GD_{1b} ganglioside recognized by the AA4 monoclonal antibody are found almost exclusively in this low-density membrane domain. The amount of tyrosine phosphorylation of the 40 and 70-80 kDa proteins in this domain increases significantly upon FcεRI aggregation, suggesting a possible functional role for this membrane structure in receptor-mediated signaling.

[1] Lisanti, M.P. *et al.* (1994) *J. Cell Biol.* **126**, 111-126. Chang, W. *et al.* (1994) *J. Cell Biol.* **126**, 127-138.

A1-127 FUNCTIONAL AND BIOCHEMICAL CHARACTERIZATION OF *XENOPUS* PROTEIN TYROSINE PHOSPHATASE-α, Robert E. Friesel, Claire Yang, Karen Neilson and Sharron A.N. Brown, Department of Molecular Biology, Holland Laboratory, American Red Cross, Rockville, MD 20855

We have cloned the *Xenopus* homolog of protein tyrosine phosphatase-α (XPTPα). *Xenopus* PTPα share 80% amino acid sequence identity with its human counterpart. The homology increases to 92% when the cytoplasmic domains are compared. RNA gel blot analysis demonstrates that XPTPα is expressed throughout early *Xenopus* development, although the highest level of expression is in blastula stage embryos before mid-blastula transition. XPTPα mRNA is also expressed in several regions of tailbud stage embryos as well as most adult tissues. XPTPα was expressed in insect cells following infection with a recombinant baculovirus. Multiple forms of the enzyme with molecular masses from 100-120 kDa were detected by immunoblotting of infected insect cell lysates. Purified recombinant XPTPα displayed phosphotyrosyl phosphatase activity towards the artificial substrate tyrosyl-phosphorylated myelin basic protein. We have also demonstrated that XPTPα is itself phosphorylated on tyrosine residues by the cytoplasmic tyrosine kinases c-src and csk. Microinjection of XPTPα mRNA into *Xenopus* oocytes does not result in detectable activation of endogenous c-src. Microinjection into *Xenopus* oocytes of a constitutively activated src (d-src) results in pigment aggregation at the site of injection, as well as tyrosine phosphorylation of polypeptides of 84 and 100 kDa. Co-injection of XPTPα mRNA with d-src mRNA results in an inhibition of pigment aggregation as well as decreases in the tyrosine phosphorylation of the 84 and 100 kDa polypeptides. The mechanism by which XPTPα inhibits the d-src effects in *Xenopus* oocytes is currently under investigation.

A1-128 ***SPRK*: AN SH3 DOMAIN-CONTAINING PROLINE-RICH KINASE WITH SERINE/THREONINE KINASE ACTIVITY,**

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Protein kinases play important roles in the growth and differentiation of cells. We have recently identified a novel, widely-expressed gene that encodes a protein kinase of 95 kDa, which we call SPRK. SPRK contains an NH_2-terminal glycine-rich region followed by an SH3 domain and a kinase domain that is similar to both tyrosine and serine/threonine kinases. Adjacent to the kinase domain are two closely-spaced leucine/isoleucine "zipper" motifs, and a stretch of basic amino acids that resembles karyophilic nuclear localization signals. The COOH-terminal half of SPRK is basic, and the COOH-terminal 216 amino acids are rich in proline (24%), serine (11%), and threonine (7%).

We have shown that SPRK exhibits serine/threonine kinase activity in an *in vitro* autophosphorylation assay. Thus SPRK is the first demonstrated example of a protein kinase that both contains an SH3 domain and has serine/threonine kinase activity. In order to address the role that SPRK may play in signal transduction we have used retroviral vectors to introduce SPRK stably into rodent fibroblast cell lines. Expression of wildtype SPRK correlates with an apparent morphological transformation of 3T3 cells. We are further characterizing this apparent cellular transformation by determining whether SPRK-expressing cell lines are able to form colonies in soft agar or tumors in nude mice. In addition, upon serum stimulation of these 3T3 cells we observe a mobility shift of SPRK on SDS/polyacrylamide gels, suggesting that SPRK is phosphorylated in response to serum. We are further investigating this observation and how it pertains to SPRK signaling and activation.

A1-129 MOUSE ENABLED (M-ENA): A HOMOLOG TO A GENETIC SUPPRESSOR OF DROSOPHILA ABL MUTATIONS.

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Drosophila enabled (ena) was identified as a dosage-dependent suppressor of lethality and central nervous system defects that occur in Abl⁻ genetic backgrounds (Gertler et al. Science (1990): 248: 857-860). ena has been cloned and encodes a novel protein with a proline-rich core that contains multiple potential SH3-binding sites. ena binds to mammalian abl and src SH3 domains *in vitro*. The cDNA was used to isolate a murine homolog of ena, M-ena. M-ena has strong similarity to the Drosophila protein at the N and C termini and also contains a proline-rich core. Since ena binds to src *in vitro*, we looked to see if altered levels of src family kinase activity affected the phosphorylation state of M-ena *in vivo*. To this end, we used cells derived from embryos that lack Csk, a protein which negatively regulates src family kinase activity. The phosphotyrosine content of M-ena increases severalfold in Csk⁻ cells, indicating that the phosphorylation state of M-ena is in part regulated via a pathway that includes src-family kinases. Experiments to determine the contribution of c-abl activity to ena phosphorylation are in progress. Since ena functions in neuronal development, we looked to at the expression of M-ena during the differentiation of P19 cells into neurons. M-ena message is induced early in the process of P19 neuronal differentiation. Targeted disruption of M-ena will be used to assess the requirement of M-ena for normal development and its role in the signal transduction pathways that are mediated by c-abl and src family kinases. Experiments to investigate the involvement of M-ena in abl and src mediated oncogenic transformation are also in progress.

A1-130 INHIBITION OF PHOSPHATIDYLINOSITOL 3 KINASE (PI 3 KINASE) ACTIVITY BLOCKS PLATELET-DERIVED GROWTH FACTOR (PDGF)-INDUCED DNA SYNTHESIS IN GLOMERULAR MESANGIAL CELLS.

Goutam Ghosh Choudhury, Fabio Marra, Constantinos Karmitsos, Hideyasu Kiyomoto and Hanna E. Abboud, Department of Medicine, The University of Texas Health Science Center at San Antonio, San Antonio Texas 78284.

Mesangial cells are vascular pericytes in glomeruli, the filtering units of the mammalian kidney. PDGF is a potent mitogen for these cells and increased expression of PDGF and PDGFRβ is associated with mesangial cell proliferation in glomerular disease. We have recently shown that PDGF stimulates association of PI 3 kinase with tyrosine phosphorylated and activated PDGFRβ in these cells resulting in its increased enzymatic activity. In PDGFRβ, phosphorylated Tyr 751 (and Tyr 740) is the binding site of PI 3 kinase that is shared by the SH2-SH3 domain containing adaptor protein Nck. Mutagenesis of these tyrosine residues in PDGFRβ inhibits DNA synthesis, PI 3 kinase binding as well as binding of Nck indicating that inhibition of DNA synthesis by this mutant receptor may have been due to the lack of binding of Nck. In the present study, we tested the hypothesis that PI 3 kinase is involved in PDGF-induced DNA synthesis in mesangial cells using a recently identified inhibitor of PI 3 kinase, wortmannin (WMN). Exposure of human mesangial cells to WMN inhibited PDGF-induced PI 3 kinase activity in a dose dependent manner in antiphosphotyrosine and anti-PDGFRβ immunoprecipitates. However WMN did not inhibit the association of PI 3 kinase with tyrosine phosphorylated PDGFRβ as determined by antiphosphotyrosine immunoblotting of anti-PI 3 kinase immunoprecipitates. *In vitro* immunokinase assay of anti-PI 3 kinase immunobeads also showed no effect of WMN on association of this enzyme with PDGFR. ^3H-thymidine incorporation assay showed that WMN inhibited PDGF-induced DNA synthesis in a dose dependent manner. The half maximal inhibition was obtained at a concentration of 100 nM of WMN. Taken together these data indicate a direct role of PI 3 kinase in PDGF-mediated mitogenesis in human mesangial cells.

A1-131 EXPRESSION OF ACTIVATED HUMAN $pp60^{c\text{-}src}$ IN MAMMARY GLANDS OF TRANSGENIC MICE

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Recent reports have indicated that breast carcinomas and cell lines contain functionally activated $pp60^{c\text{-}src}$ suggesting the involvement of c-src in human breast cancer. To analyze the contribution of $pp60^{c\text{-}src}$ in mammary tumorigenesis, we established nine lines of transgenic mice expressing mutationally activated human c-src, which has the regulatory COOH-terminal tyrosine residue at aa 530 replaced with phenylalanine. Transcription of the activated c-src was under the control of the murine whey acidic protein (Wap) promoter, targeting expression of the transgene to lactating mammary glands. The transgene was expressed in eight of nine lines, and six of nine lines were altered in breast alveolar development when biopsied during lactation. One founder female mouse developed a breast ductal adenocarcinoma and expressed activated human c-src, as well as multiple tyrosine phosphorylated proteins in the tumor tissue. Offspring of this founder show multi-focal preneoplastic and neoplastic lesions as early as the first and second litters. In addition, papillary alveolar nodules in the breast tissue were observed in several transgenic lines. This condition is similar to human papillary hyperplasia. These results suggest that activated c-src may be an early defect leading to mammary neoplasia. Analyses of both preneoplastic and neoplastic lesions in this model of breast cancer will be discussed.

A1-132 SPECIFIC TRANSMEMBRANE INTERACTIONS MEDIATE THE ACTIVATION OF THE PDGF RECEPTOR BY THE BPV-1 E5 ONCOPROTEIN

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The E5 gene of bovine papillomavirus type 1 encodes a 44 amino acid, transmembrane polypeptide which induces cell proliferation through the activation of the ß-type platelet-derived growth factor receptor (ßPDGF-R). To further analyze the molecular requirements for E5-mediated activation of signal transduction via the PDGF-R, we utilized a non-tumorigenic, murine myeloid precursor cell line (32D) which is strictly dependent upon interleukin 3 (IL-3) for growth. This IL-3 dependence can be functionally substituted by the ectopic expression of growth factor receptors and the addition of the corresponding ligand. Coexpression of the ßPDGF-R and E5 protein induced sustained proliferation in the absence of IL-3 and PDGF, indicating that E5 activates this receptor complex by a ligand-independent mechanism. Cell proliferation induced by PDGF-R and E5 is also highly specific, since the strongly conserved αPDGFR and other tyrosine kinase receptors cannot functionally interact with E5 in these cells.

To determine the domains of the ßPDGF-R which confer E5-responsiveness, we constructed a series of receptor mutants consisting of varying combinations of extracellular, transmembrane, and intracellular regions of the α and ßPDGF-Rs. Analysis of these chimeric PDGF-Rs revealed that the ßPDGF-R transmembrane domain was required for E5 binding, receptor activation, and cellular transformation. In addition, we showed that a receptor lacking the entire ßPDGFR extracellular domain was capable of binding E5. When coupled with previous data demonstrating that the E5 transmembrane domain is sufficient for PDGF-R binding, these results suggest that E5 and PDGF-R transmembrane alpha helices are alone responsible for governing their specific interaction, and for inducing the subsequent activation of this signal transduction pathway.

A1-133 A GENERAL STRATEGY FOR CONTROLLING THE ACTIVATION OF SRC-LIKE TYROSINE KINASES USING THE CELL PERMEABLE SYNTHETIC LIGAND FK1012.

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One of the earliest events following T cell receptor (TCR) stimulation is the tyrosine phosphorylation of multiple cellular substrates. Biochemical and genetic evidence has implicated two src-family protein tyrosine kinases (PTK), p59fyn and p56lck, in the induction of the TCR signaling cascade. Elucidation of the distinct roles of these PTKs, however, is complicated by the plethora of signaling events initiated upon TCR crosslinking. We therefore devised a novel strategy allowing the conditional activation of individual src-kinases. This model was based upon the observation that N-terminal myristilation and membrane localization of src-kinases is essential for their signaling potential. Thus, two chimeric proteins were designed: i.) fusion proteins formed between the FK506 binding molecule FKBP and constitutively active versions of lck and fyn lacking their N-terminal myristilation domain , ii.) a docking protein consisting of the c-src myristilation site joined to three FKBP dimerization domains. Consequently, addition of the synthetic dimerizing ligand FK1012 should tether the constitutively active kinases to the plasma membrane, thus allowing their interaction with downstream substrates. Transient expression analysis in Tag Jurkat cells revealed that membrane targeting of a catalytically active version of lck and/or fyn upon addition of FK1012 is sufficient to induce activation of NF-AT, Oct/OAP, and AP-1 responsive reporter gene constructs independent of TCR crosslinking. The functional roles of the SH3, SH2 and kinase domains were explored by expression of a series of lck and fyn mutants. Neither the SH3 domain nor the SH2 domain contributed to the activation of the reporter gene constructs, but a functional tyrosine kinase domain was essential for reporter gene expression. Thus drug induced recruitment of only the kinase domain of lck and/or fyn to the plasma membrane is required and sufficient to invoke downstream signaling events and activation of NF-AT, Oct/OAP or AP-1 transcription factor complexes.

A1-134 Role of Ras-mediated signal transduction in cell differentiation and migration in *C. elegans*

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We use *C. elegans* vulval induction as a sensitive and specific assay to identify components in a Ras-mediated signal transduction pathway and to analyze the role of this pathway in controlling cell differentiation and other developmental processes. *let-23 RTK*, *let-60 ras*, *lin-45 Raf* and many other genes plays key roles in transducing a signal.that induces three of the six equipotent precursor cells to differentiate into vulval cells.

To identify new genes that act downstream of Ras in the signalling pathway, we have isolated more than 75 mutations (in >10 genes) that suppress either the Multivulva phenotype of an activated *let-60 ras* mutation or the Vulvaless phenotype of a dominant negative *let-60 ras* mutation. We have cloned three of these genes (*sur-1, 2, 3*) that appear to act downstream of Ras. The *sur-1* gene encodes a MAP kinase that can be functionally replaced by a mammalian MAP kinase. The *sur-2* gene encodes a novel protein that is essential for vulval cell fate specification and appears to act downstream of *sur-1* mpk. Genetic and molecular analyses of other genes including several negative factors are in progress.

The Ras-mediated signal transduction pathway plays roles in many other aspects in *C. elegans* development. We have discovered that *let-60 ras*, *lin-45 raf* and several other genes are required for proper migration of sex-myoblasts (SMs). During L2 larval stage, two SMs migrate anteriorly from the tail region to the middle of the gonad which is thought to provide an attractive and a repulsive signal for positioning the cells during the migration. Mutations in several genes of the Ras pathway cause either more posteriorly located SMs or more random distribution of SMs. An activated *let-60 ras* mutation does not disrupt SM migration but synergistically causes a rather specific SM migration defect when it is in cis to a loss-of-function mutation of *let-60 ras*. The activated *let-60 ras* mutation also enhances SM migration defects of mutations in several other genes in the pathway. A mutation in the *sur-3* gene appears to mimic the effect of gonad elimination (random SM location). Our results suggest that Ras-mediated pathway plays key roles in transducing both the attractive and the repulsive migration signals from the gonad. We are also carrying out mosaic analysis of *let-60ras* to study its roles in various developmental processes. Our studies in *C. elegans* should contribute significantly to the understanding of animal development and the causes of cancer.

A1-135 INCREASED CELL-SUBSTRATUM ADHESION AND ACTIVATION OF SRC FAMILY KINASES BY PROTEIN TYROSINE PHOSPHATASE α

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The protein tyrosine phosphatase family of enzymes include a structurally diverse collection of molecules dedicated to the regulation of tyrosine phosphorylation within cells.

Protein tyrosine phosphatase α (PTP-α) is a widely expressed receptor-like PTP that is constitutively tyrosine phosphorylated and alternatively spliced so as to produce two isoforms with different extracellular domains. The alternatively spliced isoform contains a 9 amino acid insert just N-terminal to the transmembrane domain. This sequence contains a furin-like protease recognition sequence, suggesting that proteolytic cleavage of the extracellular domain of PTP-α may constitute a method of regulation of this enzyme.

To explore the physiological function of this PTPase we overexpressed PTP-α in the epidermoid cell line A431. Overexpression leads to the physical association with, and activation of, members of the src family of kinases.

Activation of src family kinases within this cell type results in increased tyrosine phosphorylation of the src kinase substrates paxillin and focal adhesion kinase. Interestingly, paxillin from cells overexpressing PTP-α, co-immunoprecipitates elevated levels of the protein kinase csk. Increased association between the src kinase substrate paxillin and csk may reflect a method of feedback control of src kinase activity within these cells.

Furthermore, we show that overexpression of PTP-α results in increased cell-substratum adhesion. These results suggest that PTP-α can regulate cell adhesion and support a role for src kinases in integrin-ECM mediated adhesion.

A1-136 IDENTIFICATION AND CHARACTERIZATION OF FOCAL ADHESION KINASE-ASSOCIATED PROTEINS.

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Focal Adhesion Kinase, or pp125^{FAK}, has been implicated in multiple signal transduction processes, including cellular transformation by pp60^{src}, integrin-induced cytoplasmic signalling, stimulation of cells with neuropeptides, lysophosphatidic acid, and growth factors, and cross-linking of basophil Fc receptors. The role for pp125^{FAK} in these processes is undetermined. In order to elucidate the function of pp125^{FAK}, we have attempted to identify cellular proteins which interact with pp125^{FAK} and may serve as regulators or effectors of pp125^{FAK} function. Using gel overlay/farwestern blot analysis we have identified a direct association between pp125^{FAK} and the tyrosine phosphorylated focal adhesion protein paxillin. This association is direct and occurs both *in vivo* and *in vitro*. The paxillin binding site resides in the carboxy-terminus of pp125^{FAK} and appears to extensively overlap the previously defined focal adhesion targeting sequence of pp125^{FAK}. By expression cloning we have isolated a cDNA encoding a second potential pp125^{FAK} binding protein. Homology search using the putative amino acid sequence indicates this is a novel cDNA. We predict that the protein encoded by this gene will play a key role in the transmission of signals from focal adhesions to the cytoplasm following activation of pp125^{FAK} in response to various cellular stimuli.

A1-137 **MEMBRANE LOCALIZATION REGULATES THE GUANINE NUCLEOTIDE EXCHANGE ACTIVITY OF SOS.**

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The guanine nucleotide exchange factor Sos activates the GTP-binding protein Ras by catalyzing the exchange of GDP for GTP. Sos is thought to be brought to the membrane in the vicinity of Ras by binding to Grb-2, whose SH2 domains bind to phosphotyrosine motifs on receptors or receptor-associated molecules. Sos was constitutively localized to the plasma membrane by the addition of a src myristylation peptide and its effect on Ras-dependent signalling was analyzed. Addition of phorbol ester or constitutively active Ras activates AP-1-dependent transcription. Membrane-bound Sos activated AP-1-dependent transcription and also synergized with either constitutively active calcineurin or ionomycin to activate NF-AT-dependent transcription in T cells. This effect of Sos was blocked by expression of dominant negative N17 Ras. Mutation of Sos C-terminal proline-rich sequences, blocking the interaction with Grb-2 SH3 domains, had no effect on Sos activity. This indicates that Sos can function independent of Grb-2 interaction when constitutively localized to the plasma membrane. A cell permeable, dimeric, synthetic ligand of FK506 (FK1012A) and its intracellular binding domain, the immunophilin FKBP, were used to inducibly control the association of soluble Sos molecules with the plasma membrane. Soluble Sos was fused to FKBP and coexpressed in cells with membrane-bound myristylated FKBPs. Addition of dimeric ligand resulted in the localization of soluble Sos with intracellular membranes and the activation of guanine nucleotide exchange activity. These data demonstrate that targeting Sos to the plasma membrane *per se*, facilitates its activation of Ras. In addition, this method of ligand-induced relocalization and activation of a nucleotide exchange factor illustrates a method that may be applied to the specific regulation of other Ras-like GTP binding proteins.

A1-138 CELL-CECLE-DEPENDENT REGULATION OF p185^{neu}: A RELATIONSHIP BETWEEN DISRUPTION OF THIS REGULATION AND TRANSFORMATION.

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Structure and function of p185^{neu} receptor tyrosine kinase was found to be regulated in a cell-cycle-dependent manner. In M phase, p185^{neu} is hyperphosphorylated at serine and/or threonine residues. The phosphotyrosine (PY) content of p185^{neu} is at its highest level in G0/G1 phase, decreases through S and G2 phases, and reaches its lowest level in M phase. Phospholipase C-gamma (PLC-γ) and GTPase-activating protein (GAP), substrates of p185^{neu}, also have a similar profile of PY content during the cell cycle. These results, along with *in vitro* immune complex kinase assays, suggest that the tyrosine kinase activity of p185^{neu} is least active in M phase. Interestingly, the mutation-activated neu oncogene (neu*) -encoded protein product, p185^{neu*}, escaped from the cell-cycle regulation. Taken together, we demonstrate in this report that the structure and function of p185^{neu} are regulated in a cell-cycle-dependent manner, yet p185^{neu*} escapes from this regulation and remains active through the cell cycle. Disruption of this cell-cycle regulation may define a novel mechanism for the p185^{neu*}-mediated cellular transformation.

A1-139 ASSOCIATION OF THE P85 REGULATORY SUBUNIT OF PI 3-KINASE WITH TUBULIN,

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The protein-tyrosine kinase regulated PI 3-kinase, is a heterodimer composed of a regulatory subunit, p85 and a catalytic subunit, p110. p85 contains several domains that have been shown to mediate protein-protein interactions. PI 3-kinase has been implicated in mitogenesis and transformation. Recent studies have shown that PI 3-kinase may be important for internalization of receptor tyrosine kinases and/or targeting of receptor to a specific subcellular compartment. In addition PI 3-kinase has been shown to be homologous to VPS34p, a protein implicated in vesicular sorting in yeast. We had previously shown that PI 3-kinase co-localizes with microtubules by cytoimmunofluorescence using an antibody raised against p85. All the above findings indicate that PI 3-kinase may interact directly or indirectly with the microtubule network.

Here we show that PI 3-kinase associates with tubulin, the major component of microtubules. GST fusion protein containing full length p85 (GST-85) precipitated tubulin from cell lysates. This association was not prevented by the removal of microtubule-associated proteins indicating that tubulin binds directly to the p85 subunit of PI 3-kinase. GST fusion proteins containing either the N-terminal SH2 domain (GST-NSH2) or the C-terminal SH2 domain (GST-CSH2) failed to precipitate tubulin form cell lysates. In addition, binding of tubulin to full length p85 was not blocked by pre-incubating GST-85 with phosphorylated peptides that block phosphotyrosine-mediated interactions with the SH2 domains of p85. These results argued against an SH2-mediated interaction. Tubulin, however, was precipitated by a GST-fusion protein containing the NSH2, CSH2 and the inter-SH2 region of p85, suggesting that the interaction between p85 and tubulin probably occurs via the inter-SH2 region. It is of interest that this same region has been shown to mediate the interaction between p85 and p110. Furthermore, we were able to coimmunoprecipitate p85 and tubulin from cell lysates which indicates that this interaction exists in intact cells. Further work is underway to understand how this interaction may affect PI 3-kinase activity or alternatively, how PI 3-kinase may affect microtubule dynamics.

A1-140 THE PH DOMAIN OF BRUTON TYROSINE KINASE INTERACTS WITH PROTEIN KINASE C. Toshiaki Kawakami, Libo Yao and Yuko Kawakami, Division of Immunobiology, La Jolla Institute for Allergy and Immunology, La Jolla, CA 92037

Bruton tyrosine kinase (Btk), which is variously mutated in X-linked agammaglobulinemia patients and X-linked immunodeficient (*xid*) mice, has the pleckstrin homology (PH) domain at its amino terminus. Btk is phosphorylated on serine, threonine and tyrosine residues, and enzymatically activated by cross-linking of the high-affinity IgE receptor (FcεRI). PH domains are found in many signaling proteins, including serine/threonine kinases, GTPases, GTPase activating proteins, guanine nucleotide exchange factors, and phospholipase C. The PH domain of Btk expressed as a bacterial fusion protein directly interacts with protein kinase C (PKC) in mast cell lysates. Evidence was obtained that Btk is physically associated with PKC in intact murine mast cells as well. Both Ca^{2+}-dependent (α, βI, and βII) and Ca^{2+}-independent PKC isoforms (ε and ζ) in mast cells interact with the PH domain of Btk in vitro, and PKC βI is associated with Btk in vivo. Btk served as a substrate of PKC and its enzymatic activity was down-regulated by PKC-mediated phosphorylation. Furthermore, depletion or inhibition of PKC with pharmacological agents resulted in an enhancement of the tyrosine phosphorylation of Btk induced by FcεRI cross-linking, suggesting important roles played by PH domain-mediated protein-protein interactions among signaling molecules.

A1-141 REGULATION OF HUMAN c-*fgr* PROTO-ONCOGENE EXPRESSION IN MYELOID CELLS, Panagiotis Kefalas, Timothy R.P. Brown, David R. Katz and Paul M. Brickell, Departments of Molecular Pathology and Immunology, University College London Medical School, Windeyer Building, Cleveland Street, London, W1P 6DB, UK

Studies of normal and leukaemic myeloid cells show that the expression of the c-*fgr* proto-oncogene, which encodes a member of the *src* family of protein tyrosine kinases, is activated during the terminal differentiation of monocytes and granulocytes. In accordance with this, the differentiation of the human U937 promonocyte cell line in respone to phorbol myristate acetate (PMA) is accompanied by c-*fgr* mRNA accumulation. In our studies we have investigated the regulation of c-*fgr* transcription during U937 differentiation. Various fragments of the upstream region of the major myeloid-specific transcriptional start site of the human c-*fgr* gene were cloned into a luciferase reporter plasmid. These constructs were transfected into undifferentiated U937 cells and luciferase activity was assayed in undifferentiated cells and in cells induced to differentiate with PMA. We have shown that a DNA fragment extending from -1688 to -752 (with respect to the major myeloid transcription initiation site) is responsive to PMA, which implies that activation of the c-*fgr* promoter upon myeloid cell differentiation via PMA requires sequences within this region.

A1-142 Specific Recruitment of the Hematopoietic Phosphotyrosyl Phosphatase SH-PTP1 to the EPO-R Causes Dephosphorylation of JAK2, Ursula Klingmüller[1,2], Ulrike Lorenz[3], Lewis C. Cantley[2], Benjamin G. Neel[3], and Harvey F. Lodish[1]

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Protein-tyrosine phosphorylation and dephosphorylation are antagonizing forces in the response to binding of growth factors to cell surface receptors. The erythropoietin receptor (EPO-R) lacks intrinsic catalytic activity, but upon ligand binding triggers the activation of the associated cytosolic protein-tyrosine kinase JAK2. Tyrosine phosphorylation of cellular proteins, including the EPO-R, is transient and returns to basal levels approximately 30 min. after ligand addition, indicating a role for a phosphotyrosine phosphatase in termination of signaling.

One likely candidate is the hematopoietic phosphotyrosyl phosphatase SH-PTP1. To address this idea five out of eight tyrosine (Y) residues in the cytoplasmic domain of the EPO-R were mutated to phenylalanine (F). Expression of the recombinant EPO-R cDNAs in the pro-B cell line Ba/F3 showed that after EPO addition each of the mutant EPO-Rs became tyrosine phosphorylated and was able to transduce a proliferative signal. Co-immunoprecipitation and binding experiments showed that SH-PTP1 binds, via its SH2 domains, specifically to one tyrosine residue in the EPO-R cytoplasmic domain. In cell lines expressing the respective Y to F mutant EPO-R autophosphorylation of JAK2 was prolonged. Co-expression in insect Sf9 cells, mediated by baculovirus infection, revealed that SH-PTP1 specifically dephosphorylates JAK2 and that the extent of JAK2 autophosphorylation reflects the ability of JAK2 to phosphorylate an exogenous substrate such as the EPO-R. Taken together, these findings indicate that the recruitment of SH-PTP1 to the EPO-R leads to dephosphorylation and therefore inactivation of JAK2. These results imply that activation of SH-PTP1 by binding to the EPO-R is crucial for termination of the proliferative signal generated by activation of JAK2.

A1-143 ISOLATION OF THE DROSOPHILA HOMOLOG OF HUMAN SHC. Ka-Man Venus Lai[1,2], J.P. Olivier[1,2], M. Henkemeyer[1], J. O'Bryan[1,] P.G. Pelicci[3] and T. Pawson[1,2]. [1]Mt. Sinai Hospital, 600 University Avenue, Toronto, Ont., Canada M5G 1X5. [2]Department of Molecular and Medical Genetics, University of Toronto. [3]Istituto Clinica Medica I, Policlinico Monteluce, University of Perugia, 06100 Perugia, Italy.

The human *shc* gene encodes a protein product with a well conserved SH2 domain, several Pro/Gly rich motifs and no identified catalytic activity. Recent studies have demonstrated that overexpression of Shc in tissue culture cell lines induce cell transformation and neurite outgrowth. When these cells are activated, Shc becomes phosphorylated on tyrosine by receptor tyrosine kinases (eg.EGFR) or by cytoplasmic tyrosine kinases(eg.v-Src and v-Fps). Upon phosphorylation, Shc rapidly forms complexes with other signal transduction molecules, such as the SH2 domain of Grb2/sem-5/drk. Although EGFR has been directly linked to the Ras pathway in a Grb2/sem-5/drk-dependent manner, the role of Shc in this pathway or alternative ones has yet to be determined.

In order to investigate the role of Shc in signal transduction, we have isolated both the murine *shc* (m*shc*) and the Drosophila *shc* (d*shc*) homologs. As expected, both proteins are Pro/Gly rich and contain the SH2 domain. The Drosophila homolog of Shc was isolated from an expression library using the anti-human Shc antibody. Analysis of the predicted amino acid sequences revealed 62% similarity (46% identity) with the human Shc product. We have mapped the chromosome location of d*shc* by *in situ* hybridization and are looking for mutations and P-element insertions that have been mapped to the same region.

The conservation of the *shc* gene through evolution implies it has an important role in signal transduction. We plan to use these two clones to pursue parallel genetic analysis of Shc function both in *Drosophila* and in mouse. Whole mount RNA *in situ* hybridization suggests that the distribution of d*shc* is ubiquitous. We have raised antibody to study the expression of d*shc* gene product. Finally, we are introducing a targeted null mutation into the mouse *shc* gene by homologous recombination.

A1-144 TUMOUR INDUCTION BY TYROSINE PHOSPHORYLATION OF THE PRODUCT OF THE CBL ONCOGENE. Wallace Y. Langdon, Christopher E. Andoniou and Christine B.F. Thien, Department of Biochemistry, The University of Western Australia, Nedlands, Western Australia 6009, Australia

v-*cbl* is the transforming gene of a murine retrovirus which induces pre-B cell lymphomas and myelogenous leukemias. It encodes 40 kd of a gag fusion protein which is localized in the cytoplasm and nucleus of infected cells. The c-*cbl* oncogene encodes a 120 kd cytoplasmic protein and its overexpression is not associated with tumourigenesis. The c-*cbl* sequence has shown that v-*cbl* was generated by a truncation that removed 60% of the C-terminus. The function of cbl is not known and its sequence has not revealed definitive clues.

In this study we carried out experiments to identify the position within cbl where the transition occurs between non-tumourigenic and tumourigenic forms by expressing a range of C-terminally truncated proteins. These experiments focussed attention on a region of 17 amino acids which is deleted from cbl in the 70Z/3 pre-B lymphoma due to a splice acceptor site mutation. This mutation activates cbl's tumourigenic potential and induces its tyrosine phosphorylation. Furthermore the deletion of either of two tyrosine residues within this region (Y368 and Y371) is sufficient to induce tyrosine phosphorylation of cbl and fibroblast transformation. We also show that the expression of the v-*abl* and *bcr-abl* oncogenes results in the induction of cbl tyrosine phosphorylation and that abl and cbl associate *in vivo*. These findings demonstrate that tyrosine phosphorylated cbl promotes tumourigenesis and that cbl is a downstream target of the bcr-abl and v-abl kinases.

A1-145 **pp60^{src} PHOSPHORYLATES CONNEXIN43, A GAP JUNCTION PROTEIN**, Alan F. Lau[1], Lenora W.M. Loo[1], John Berestecky[2] and Martha Y. Kanemitsu[1], [1]Molecular Carcinogenesis, Cancer Research Center, [2]Mathematics-Science Department, Kapiolani Community College, University of Hawaii, 96813.

The *v-src* oncogene of the Rous sarcoma virus produces a rapid, marked decrease in gap junctional communication in fibroblasts, which correlates with the tyrosine phosphorylation of the plasma membrane gap junction protein, connexin43 (Cx43). To determine if Cx43 is a substrate of pp60^{src}, we examined pp60^{src}'s ability to phosphorylate Cx43 in *in vitro* kinase reactions. Because low levels of Cx43 are present in fibroblast cells, a Cx43 recombinant baculovirus was prepared and expressed in Sf9 insect cells to high levels. Cx43 and kinase-active pp60$^{c\text{-}src}$ were immunoaffinity purified from recombinant baculovirus-infected Sf9 cells using monoclonal antibodies. Activated pp60$^{c\text{-}src}$ not only phosphorylated Cx43 on tyrosine *in vitro*, but the resulting *in vitro* phosphotryptic peptides appeared to represent a subset of peptides observed in the 2D peptide maps of Cx43 isolated from cells metabolically-labeled with $^{32}P_i$. Similar results were also obtained using a cytoplasmic, C-terminal portion of Cx43 fused to GST and expressed in bacteria. Immunodepletion of pp60^{src} from *in vitro* kinase reactions demonstrated that phosphorylation of Cx43 was strictly dependent upon pp60^{src}'s kinase activity. Co-infection of Sf9 insect cells with Cx43 and pp60$^{v\text{-}src}$ recombinant baculoviruses also resulted in the phosphorylation of Cx43 on tyrosine. Taken together, these results strongly suggests that pp60^{src} phosphorylates Cx43 directly. Because the presence of phosphotyrosine on Cx43 correlates with the disruption of gap junctional communication, these results support the concept that pp60^{src} regulates Cx43 function by tyrosine phosphorylation.

A1-146 Participation of Igα/Igβ, Lyn, Syk, and a 120 kDa phosphoprotein in the signal transduction pathways mediated by the B cell antigen receptor.
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A major signaling receptor in B lymphocytes, the B cell antigen receptor complex (BCR), consists of antigen-specific surface immunoglobulin and an associated heterodimer, Igα/Igβ, essential for downstream signaling. Protein tyrosine kinases (PTK) including members of the Src-family kinases (Lyn and Blk) and the spleen tyrosine kinase (Syk) interact with the BCR, are activated after BCR-crosslinking, and are required for or contribute to signaling pathways such as activation of PLCγ1 and release of intracellular free Ca2+ ([Ca2+]i). However, it has not been clear just how these PTK interact with the BCR, each other, or other signaling components. We have detected a direct physical association with Lyn and Syk (Sidorenko et al., submitted, 1994). This interaction depends on the maturational stage of the B cells in that Lyn-Syk interaction is only detectable in mature B cells but not in early B cell precursors. Syk from early B cells cannot interact with Lyn from early or mature B cells suggesting Syk may have to be modified before it can associate with Lyn. Within 20 sec after sIg crosslinking a tyrosine-phosphorylated protein the size of Syk (72 kDa) could be found in Lyn immunoprecipitates and tyrosine phosphorylated proteins the sizes of Lyn (53 and 55 kDa) were present in Syk immunoprecipitates. A GST-fusion protein with the SH2 domain of Lyn (GST-LynSH2) specifically precipitated Syk from lysates of B cells stimulated via the BCR. This interaction was dependent on tyrosine phosphorylation of Syk, since GST-LynSH2 protein did not precipitate non-tyrosine-phosphorylated Syk from resting B cells. These results suggest the in vivo association of Lyn and Syk could be mediated in part by the SH2 domain of Lyn. Syk interacts with other phosphoproteins including Igα/Igβ heterodimers and a 120 kDa molecule, pp120. Igα/Igβ heterodimers and Syk can be found associated even in unstimulated B cells, but after BCR stimulation the amount of Syk associating with the Igα/Igβ heterodimers increases dramatically, apparently due to recruitment of Syk from the cytosol. This recruitment is accompanied by a concomitant augmentation in tyrosine phosphorylation of Syk molecules associated with Igα/Igβ. The 120 kDa protein associated with Syk also associates with PLCγ1, and immunoprecipitates of PLCγ1 contain both pp120 and Syk (Sidorenko et al., submitted, 1994), suggesting pp120 may serve as a bridge between Syk and Syk-dependent activation of [Ca2+]i release in B lymphocytes. (Supported by NIH grants GM42508 and GM37905. C.L.L. is a Leukemia Society of America Special Fellow).

A1-147 DIMERIZATION OF THE EGF RECEPTOR BY A SINGLE MOLECULE OF EGF. Mark A. Lemmon, John E. Ladbury, Min Zhou, Dalia Pinchasi, Axel Ullrich+, Irit Lax, and Joseph Schlessinger, Department of Pharmacology, New York University Medical Center, 550 First Avenue, New York, NY 10016, and +Max-Planck Institut für Biochemie, Am Klopferspitz 18A, 82152 Martinsried, Germany.

Growth factors of the EGF family are monomeric, and activate their cognate receptors by inducing dimerization. The mechanism of this ligand-induced receptor dimerization is not clear. In the case of human growth hormone (hGH), the monomeric ligand is actually bivalent, and binds simultaneously to two hGH-receptor molecules. Studies of hGH highlight the importance of a detailed knowledge of ligand:receptor stoichiometry for the understanding of ligand-induced receptor activation. We have studied the stoichiometry and thermodynamics of EGF binding to the soluble extracellular domain of its receptor (sEGFR), using isothermal titration calorimetry and other techniques. EGF shows two modes of binding to its receptor, one with high affinity ($K_D \approx 10$ nM) and one with lower affinity ($K_D \approx 200$ nM). Surprisingly, each of these binding modes occurs with a stoichiometry that corresponds to 1:2 (EGF:receptor). This finding indicates that the high-affinity complex contains two receptor molecules and a single ligand, as was seen in the case of hGH bound to its receptor. Binding of EGF to sEGFR also caused receptor dimerization as revealed by gel filtration analyses and covalent cross-linking studies.

A 200-residue subdomain (domain 3) of the receptor, previously shown to be important in ligand recognition, was isolated by limited proteolysis, and was found to bind EGF with a simple 1:1 stoichiometry. The affinity of this binding was similar to that observed for the low affinity interaction of EGF with sEGFR, and domain 3 did not dimerize upon binding of EGF. We suggest that this subdomain represents one site for EGF binding in the EGFR extracellular domain. Another subdomain (domain 1), related by internal sequence homology, may correspond to a second EGF binding site. Indeed our calorimetric studies have also detected a state in which 2 molecules of EGF are bound simultaneously to 1 molecule of sEGFR. We propose from these studies that the high-affinity complex between EGF and its receptor involves simultaneous binding of a single EGF molecule to two receptor molecules. We suggest that the interaction with EGF occurs via domain 3 of one receptor molecule, and domain 1 of the other. Binding of EGF to both receptors would result in the ligand-induced stabilization of the active dimeric form of EGFR.

A1-148 MULTIPLE CYTOKINES STIMULATE THE TYROSINE PHOSPHORYLATION OF A 52 kDa Shc ASSOCIATED PROTEIN. Ling Liu, Jacqueline E. Damen, and Gerald Krystal, Terry Fox Laboratory, BC Cancer Research Centre, Vancouver Canada.
Shc is a ubiquitously expressed adaptor protein that contains, in addition to a C-terminal SH2 domain, a region homologous to collagen $\alpha 1$. Following growth factor stimulation it becomes tyrosine phosphorylated and activates Ras. This activation is thought to be mediated, at least in part, by the association of the tyrosine phosphorylated form of Shc with Grb-2-Sos1 complexes. We recently reported that interleukin-3, steel factor and erythropoietin all induce the binding of a common 145 kDa protein to the tyrosine phosphorylated form of Shc and this association appears hemopoietic cell specific and inhibitable with a YXN containing phosphopeptide. We have now further characterized the proteins that become tyrosine phosphorylated and associated with Shc following stimulation with these cytokines and found that, in addition, to p145, a 52 kDa tyrosine phosphorylated protein also associates with Shc in response to all three cytokines. This protein was not detected in one-dimensional Laemmli gels of anti-Shc immunoprecipitates because of its co-migration with the p52 and p46 forms of Shc. Anti-phosphotyrosine blots of two-dimensional O'Farrell gels demonstrated that this 52 kDa protein +possessed an isoelectric point of 6.2 and silver stained gels of anti-Shc immunoprecipitates revealed that it was constitutively associated with Shc. Although it is possible that the 52 kDa protein is a member of the Src family, the level of ^{32}P incorporated into this protein was extremely low in immune complex assays suggesting that it is most likely not a kinase. The fact that Shc appears to associate with 145- and 52-kDa tyrosine phosphorylated proteins following cytokine stimulation suggests a more complex role for Shc than has been previously thought in regulating Ras activity and perhaps other as yet unidentified activities as well.

A1-149 MULTIPLE HEMATOPOEITIC SIGNAL TRANSDUCTION PATHWAYS ARE NEGATIVELY REGULATED BY THE SH2-CONTAINING TYROSINE PHOSPHATASE SH-PTP1

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The phosphotyrosine phosphatase SH-PTP1 (also known as PTP1C, SHP, HCP) is expressed primarily in hematopoeitic cells of all lineages. Mutations in SH-PTP1 cause the motheaten (*me*) mouse, which shows a panoply of hematopoeitic abnormalities, including increased sensitivity of erythroid precursor cells to erythropoeitin (Epo), B and T cell abnormalities, and myeloid hyperproliferation.

We have found that SH-PTP1 associates via its N-terminal SH2 domain with the ligand-activated EPO receptor (EpoR). Using a panel of EpoR mutants, we have mapped the site of interaction to pY429 of the EpoR. Expression of a pY429 -> F EpoR mutant results in specific differences in the kinetics of Epo-dependent tyrosyl phosphorylation.

Previously, we have reported that SH-PTP1 becomes tyrosyl phosphorylated in response to T cell receptor (TCR) and/or CD4/CD8 crosslinking. We have found that activation of motheaten thymocytes leads to altered kinetics of stimulated kinase activities and tyrosyl phosphorylation compared to normal littermate controls. Moreover, we have observed that although thymocyte development, as assessed by flow cytometry using multiple cell surface markers, is normal in *me/me* mice, motheaten thymocytes hyperproliferate in response to TCR stimulation. A post-proliferation flow cytometry analysis reveals expansion of different populations in normal and motheaten mice.

Others have reported that SH-PTP1 becomes tyrosyl phosphorylated in the BAC.125 macrophage cell line. We have confirmed this result in primary macrophages and found that, in addition, SH-PTP1 associates with a 130 kD tyrosyl phosphoprotein in these cells. A protein of this MW is constitutively hyperphosphorylated in macrophages of *me/me* mice, as are several other phosphotyrosyl proteins. Motheaten macrophages hyperproliferate in response to CSF-1 as well as to GM-CSF.

Taken together, our data suggest a negative regulatory role for SH-PTP1 in several hematopoeitic signaling pathways.

A1-150 SYNERGISTIC INTERACTIONS BETWEEN HTLV-1 TAX AND ONCOGENIC RAS ON PROENKEPHALIN GENE TRANSCRIPTION, Kenneth G. Low and Michael J. Comb, Laboratory of Molecular Neurobiology, Massachusetts General Hospital, Charlestown, MA 02129.

The transactivation of viral and cellular genes by the HTLV-1 Tax protein is greatly enhanced by activators of second messenger signaling pathways which suggests interactions between Tax and components of these pathways. Previous studies indicate that oncogenic ras and Tax cooperate to induce neoplastic transformation of cells. In order to examine the effects of oncogenic ras and Tax on proenkephalin gene transcription, human Jurkat T-lymphocytes were co-transfected with a CAT reporter construct containing the human proenkephalin gene promoter with oncogenic ras and/or a CMV expression construct for Tax. The co-transfection of oncogenic ras alone did not significantly alter the level of proenkephalin gene transcription over basal levels. Expression of Tax alone increased level of proenkephalin gene transcription 2-fold over basal levels. When oncogenic ras was co-expressed with Tax, proenkephalin gene transcription was increased 37-fold over basal levels. This synergistic activation of proenkephalin gene transcription by oncogenic ras and Tax suggest interactions between Tax and components of the ras-dependent signaling pathway. On-going studies are focused on identifying these components of the ras-dependent signaling pathway which activate or are activated by Tax.

A1-151 EFFECT OF CELLULAR EXPRESSION OF PLECKSTRIN HOMOLOGY DOMAINS ON Gi-COUPLED RECEPTOR SIGNALING. Louis M. Luttrell, Kazushige Touhara, Brian E. Hawes, Tim van Biesen, Sabrina T. Exum and Robert J. Lefkowitz. Departments of Medicine and Biochemistry and the Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC 27710.
Pleckstrin homology (PH) domains are 90 to 110 amino acid regions of protein sequence homology that are found in a variety of proteins involved in signal transduction and growth control. We have previously reported [Touhara, K., *et al.* (1994) *J. Biol Chem* 269: 10217-10220] that the PH domains of several proteins, including βARK1, PL-Cγ, IRS-1, Ras-GRF and Ras-GAP, expressed as GST-fusion proteins, can reversibly bind purified bovine brain Gβγ subunits *in vitro* with varying affinity. To determine whether PH domain peptides would behave as antagonists of Gβγ subunit-mediated signal transduction in intact cells, plasmid minigene constructs encoding these PH domains were prepared which permit transient cellular expression of the peptides. Pertussis toxin-sensitive, Gβγ subunit-mediated phosphatidylinositol (PI) hydrolysis was significantly inhibited in COS-7 cells transiently coexpressing the α2-C10 adrenergic receptor (AR) and each of the PH domain peptides, with the βARK1, PL-Cγ and IRS-1 PH domains exhibiting the greatest activity. Pertussis toxin-insensitive, Gqα subunit-mediated PI hydrolysis via coexpressed α1B AR was unaffected, suggesting that the inhibitory effect of the PH domains was Gβγ subunit-specific. Stimulation of the MAP kinase pathway by Gi-coupled receptors in COS-7 cells has been reported to involve activation of *Ras* and to be independent of protein kinase C. Since several proteins involved in Ras activation contain PH domains, the effect of PH domain expression on α2-C10 AR-mediated phosphorylation of MAP kinase was determined. Coexpression of the βARK1 and Ras-GRF PH domains resulted in marked inhibition of MAP kinase phosphorylation. In contrast, the PL-Cγ, IRS-1, and Ras-GAP PH domains had no significant effect. MAP kinase phosphorylation mediated by the Gqα subunit-coupled α1B AR, which is PK-C dependent, was unaffected by coexpression of any of the PH domains. These data suggest that PH domains behave as specific antagonists of Gβγ-mediated signaling in intact cells, and that interactions between PH domains and Gβγ subunits or structurally related proteins, may play a role in the activation of mitogenic signaling pathways by G protein-coupled receptors.

A1-152 FGF-8 PROTEIN ISOFORMS DIFFER IN ONCOGENIC POTENTIAL, Craig A. MacArthur[1], Deepa B. Shankar[2], Avril Lawshé[1] and Gregory M. Shackleford[2,3], Department of Pediatrics[1], Washington University School of Medicine, St. Louis, MO 63110, and Departments of Microbiology[2] and Pediatrics[3], Childrens Hospital Los Angeles and University of Southern California School of Medicine, Los Angeles, CA 90027.

We have recently identified a member of the fibroblast growth factor (FGF) gene family, *Fgf-8*, as a gene that is activated by mouse mammary tumor virus (MMTV) insertion in approximately 10% of tumors from MMTV-infected *Wnt-1* transgenic mice. When the gene structure was examined, *Fgf-8* was found to consist of five coding exons that can be alternatively spliced to generate four potential mRNAs. These mRNAs are predicted to code for secreted growth factors that differ in their mature amino termini, but are identical in the carboxyl 2/3 of the proteins. We have evidence that mRNA for three of the four potential isoforms exist in normal mouse testes, during mouse development, and in mammary tumors from MMTV-infected *Wnt-1* transgenic mice that express *Fgf-8*, using reverse transcription-polymerase chain reaction (RT-PCR) and ribonuclease protection assays. The three cDNAs were obtained by "exon-trapping" and RT-PCR and have been cloned into a plasmid vector that results in high frequency of selected G418-resistant ($G418^r$) cells expressing the transfected *Fgf-8* cDNA. We have examined the oncogenic potencies of the encoded FGF-8 protein isoforms by stable transfection of NIH-3T3 and C57MG cells with the *Fgf-8* cDNA plasmids. NIH-3T3 cells transfected with the control plasmid are morphologically flat and are not tumorigenic in nude mice. In contrast, NIH-3T3 cells transfected with plasmid encoding FGF-8b are transformed morphologically and form tumors rapidly (7 days) in all injected nude mice. NIH-3T3 cells transfected with plasmids encoding FGF-8a or FGF-8c are weakly tumorigenic (50-75% of animals injected with latency of 4-6 weeks) in nude mice. NIH-3T3 cells with FGF-8a are not transformed morphologically, while those with FGF-8c are mildly transformed. Tumors have been isolated from the nude mice, and the $G418^r$ cells from the tumors found to express the transfected *Fgf-8* cDNA, even after several weeks of tumorigenesis in the cases of FGF-8a and FGF-8c. These results suggest that the FGF-8 protein isoforms possess different oncogenic potencies, likely because of different affinities of the FGF-8 isoforms for the various FGF receptors.

A1-153 EFFECT OF βGBP ON GROWTH FACTOR SIGNALLING. Livio Mallucci and Valerie Wells, Department of Microbiology (Laboratory of Cellular and Molecular Biology), Guy's Medical School, London Bridge, London SE1 9RT.

The β-galactoside binding protein (βGBP) is a negative cell growth regulatory molecule and a cytostatic factor which controls exit from Go and traverse through G2 with mechanisms involving ligand-receptor interaction (1,2). The structural gene for βGBP has been cloned (3) and mapped (4) to the E region of mouse chromosome 15 and to the q12 - q13 region of human chromosome 22, a syntenic group which contains the sis/PDGFB homologues and which is known to undergo deletions and translocations in a number of oncogenic conditions. The signalling pathway initiating from the βGBP receptor has not yet been elucidated but tyrosine kinase receptor pathways are interfered with by β GBP. The effect of βGBP on Go cells is characterised by lack of c-fos and c-jun expression after growth factor stimulation, also free E2F remains at basal levels and there is no c-myc induction; MAP kinase is not activated but EGF receptor phosphorylation does occur. This indicates that β GBP interferes with key transducing element(s) (ras?) which connects with different signalling events.

References, 1) Cell (1991) 64 91-97;2) BBA (1992) 1221 239-244; 3) BBA (1991) 1089 54-60; 4) Genomics (1993) 15 216-218.

A1-154 IDENTIFICATION OF THE MAJOR TYROSINE PHOSPHORYLATED SUBSTRATE IN SIGNALLING COMPLEXES FORMED AFTER ENGAGEMENT OF Fcγ RECEPTORS, Antonio Marcilla, Alka Agarwal, Octavio M. Rivero-Lezcano and Keith C. Robbins, Laboratory of Cellular Development and Oncology, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20852-4330.

We have recently identified the protein product of the c-cbl protooncogene as an SH3 binding protein in a cDNA expression library prepared from macrophages. To investigate the possibility that $p120^{cbl}$ might be involved in signalling pathways of myelomonocytic cells, HL60 cells were treated with a variety of ligands known to induce tyrosine phosphorylation. Lysates of such cells were examined for the presence of tyrosine phosphorylated cbl. Our findings demonstrate that cbl is tyrosine phosphorylated upon Fcγ receptor engagement and represents the major tyrosine phosphorylated protein observed in this signalling pathway. $p120^{cbl}$ was also found complexed with a number of previously identified signalling molecules. The nature of these complexes will be discussed.

A1-155 BIOLOGICAL FUNCTION OF THE HUMAN CHOLECYSTOKININ-B/GASTRIN RECEPTOR AND ITS SIGNALING PATHWAY INVOLVING TYROSINE KINASES, Toshimitsu Matsui, Taizo Taniguchi, Tohru Murayama, Nobuko Iwata, Mitsuhiro Ito, Kazuo Chihara, Third Division, Department of Medicine, Kobe University School of medicine, Kobe 650, Japan

The receptors for brain-gut peptide hormones, cholecystokinin (CCK) and gastrin are expressed in a variety of human tumor cells. Recently, we have cloned a human CCK-B/gastrin receptor cDNA and demonstrated the trophic effect of CCK-8 and gastrin I. In the present study, we investigated the molecular basis of signal transduction pathway of the guanine nucleotide regulatory protein (G protein)-coupled receptor. Human CCK-B/gastrin receptor expressed in NIH3T3 cells coupled efficiently to phosphoinositide hydrolysis and mobilization of intracellular Ca^{2+}, and transduced mitogenic signals assessed by [^{3}H]thymidine incorporation in a ligand-dependent manner. CCK-8 induced tyrosine phosphorylation of several protein species. Among them, $p60^{c\text{-}src}$ and MAP kinase were tyrosine phosphorylated and activated in response to CCK-8, as was induced by platelet derived growth factor (PDGF). In contrast, tyrosine phosphorylation of p125 FAK was remarkably induced by CCK-8 but not by PDGF. CCK-8 rapidly reorganized actin stress fibers, while PDGF initially induced membrane ruffling followed by less stress fiber formation. The micro-injection of either *rho* GDP dissociation inhibitor or *botulinum* C3 which impairs the function of *rho* p21 inhibited the stress fiber formations by CCK-8, but not the membrane ruffling. Moreover, CCK-8 as well as PDGF has been demonstrated to activate *ras* p21. These results suggest that the CCK-B/gastrin receptors expressed on human tumor cells might regulate not only cell growth but also cell motility or adhesion by cross-talking with the tyrosine kinase cascades and reorganizing actin cytoskeletons.

A1-156 REGULATION OF MEMBRANE LOCALIZATION OF CDC42Hs BY rhoGDI, Sandra J. McCallum, Jon W. Erickson and Richard A. Cerione, Department of Pharmacology, Cornell University, Ithaca NY, 14850.

CDC42Hs is a 22kd GTP binding protein which belongs to the ras superfamily. In yeast, CDC42Sc has been genetically linked to the assembly of the bud site during cell division, but in mammalian systems, the function of CDC42Hs is unknown. CDC42 was purified initially from plasma membranes and has also been shown to exist in a cytosolic complex with the Guanine nucleotide Dissociation Inhibitor, rhoGDI (Regazzi et al, JBC 267(25),17512-19,1992). Recently, we have shown CDC42 to be primarily localized to the Golgi apparatus based on immunofluorescence in normal rat kidney (NRK) cells and cell fractionation studies. Thus, an important question concerns whether CDC42 binds to specific target proteins in plasma membranes and Golgi and, if so, how are these binding events regulated?

One potential regulator of the membrane binding activity of CDC42 is the rhoGDI which has been shown to remove CDC42 from plasma membranes (Leonard et al, JBC 267(32), 22860-68,1992) and to inhibit its insertion into plasma membranes for subsequent carboxymethylation (Backlund, BBRC 196(2) 53442,1993). Here, we show that rhoGDI can also remove CDC42 from purified Golgi membranes in a nucleotide independent manner. However, it appears that the percentage of CDC42 pulled out by rhoGDI in Golgi is lower than that for CDC42 overexpressed in SF9 insect cell membranes. This difference may indicate the presence of a specific CDC42 associated protein in the Golgi apparatus which inhibits its removal from membranes to some extent. Currently, we are attempting to further characterize this putative CDC42 associated Golgi protein.

A1-157 INHIBITION OF V-SRC KINASE ACTIVITY IN RAT-1 CELLS BY AN INHIBITOR OF NON-VOLTAGE GATED CALCIUM INFLUX, Scott E. McNeil and Karin D. Rodland, Dept. of Cell Biology and Anatomy, Oregon Health Sciences University, Portland, OR 97201.

The transforming oncogene v-src is a constitutively active tyrosine kinase which causes dramatic intracellular and extracellular changes when expressed in cells. Among these changes are increased activity of the MAP kinases, loss of mitotic regulation, increased amounts of tyrosine phosphorylated proteins, and disruption of normal signalling pathways. More recent reports also suggest a correlation between concentration of the intracellular messenger calcium and activity of the src kinase family. Calcium is well documented in its role in activation of various serine/threonine kinases and is required for normal cell cycle progression. In this study we demonstrate that an inhibitor of non-voltage gated calcium influx, SK&F 96365, a known anti-proliferative compound, is an effective inhibitor of v-src kinase activity. Rat1 fibroblasts which have been transformed with v-src exhibit a pronounced decrease in global tyrosine phosphorylation when treated with SK&F 96365. More specifically, v-src isolated from cells treated with SK&F 96365 had decreased activity in an *in vitro* kinase assay. The *in vivo* decrease could not be duplicated by growing cells in the presence of the calcium ATPase inhibitor thapsigargin or with the calcium chelator BAPTA. Our preliminary data suggest that the decrease in v-src kinase activity cannot be attributed solely to decreased intracellular calcium concentrations.

A1-158 A MAJOR ROLE FOR PKCη IN MEDIATING RESPONSES TO TUMOR PROMOTERS IN EL4 THYMOMA CELLS, Kathryn E. Meier, Sandra A. Fulwood, and Heather M. Turnquist, Department of Pharmacology, Medical University of South Carolina, Charleston, SC 29425-2251.

The EL4 thymoma cell line exists in forms that are either sensitive ("wild-type") or resistant ("variant") to phorbol ester (PMA). In wild-type (WT) cells, PMA induces activation of the MAP kinase pathway, transcription of IL-2, and cell death. In variant cells, PMA does not induce any of these responses even though these cells express activatable PKC, MEK, and MAP kinase. We developed a "new variant" (NV) cell line by incubating WT cells with PMA. NV cells exhibited the same phenotype, with regard to kinase activation, IL-2 synthesis, and toxicity, as the variant cell line previously developed in other laboratories. Toxicity to PMA in WT cells resulted from non-programmed cell death that was preceded by morphological changes. NV cells did not show morphological responses to PMA. MAP kinase activation, morphologic changes, and toxicity were seen in WT cells treated with a variety of PKC activators, including thymeleatoxin, bryostatin 5, indolactam V, and prostatin. Some of these compounds are reported to be selective for cPKCs, and most are weak tumor promoters. Relative to WT cells, NV cells underexpress PKCη, an nPKC isoform. Translocation of PKCη to cell membranes, followed by its down-regulation, was seen in WT cells in response to all of the PKC activators. The small amount of PKCη expressed in NV cells was also translocated and down-regulated in response to these agents. Taken together, the results suggest that activation of PKCη is critical for responsiveness and toxicity to phorbol esters in EL4 cells. Under-expression of this isoform appears to be one of the factors responsible for conferring resistance to phorbol esters in variant cells. (Supported by NIH CA58640)

A1-159 PROTEIN TYROSINE PHOSPHATASE EPSILON (PTPε) IS REGULATED IN CELLS OF THE MONOCYTE/MACROPHAGE LINEAGE AND ASSOCIATES WITH p53/56^{lyn}

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Cytokines play an important role in the generation of immune reponses. IFNγ, for example, has been shown to activate macrophages, increasing MHC class II expression, augmenting the production of chemotactic factors and other pro-inflammatory cytokines, etc. To study regulation of PTPase expression in monocytic cells, murine RAW264.7 cells were used in a PCR-based screening system to identify PTPases upregulated in response to IFNγ stimulation. This screen revealed that PTPε mRNA expression was upregulated in both the RAW264.7, and human HL-60 cell lines. Hybridizing transcripts of ~6.5 kb, as well as 1.8 and 2.1 kb (possibly due to alternate splicing) were seen. Upregulation of PTPε mRNA was first seen at 3 hrs, and was maximal after 20 hrs (10-fold increase) of exposure to IFNγ. Immunoblotting revealed a 97 kDa protein in cell lysates. As the predicted size from the translated cDNA sequence was 86 kDa, this suggested that PTPε was glycosylated, likely at the two potential N-linked glycosylation sites within the extracellular domain. Using co-immunoprecipitation and GST-PTPε fusion protein affinity chromatography techniques we found evidence that PTPε associates with p53/56^{lyn} in these monocytic cell lines. These findings suggest that PTPε has a role within signal transduction pathways involving this *src*-family kinase.

A1-160 INTERACTION BETWEEN MEMBERS OF THE cAMP SIGNALING PATHWAY IN *Saccharomyces cerevisiae*,

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In the yeast *Saccharomyces cerevisiae*, two genes, *RAS*1 and *RAS*2, code for the RAS protein. The yeast *RAS* protein has been shown to be involved in the cAMP signal transduction pathway. In this pathway the RAS protein acts primarily on adenylyl cyclase. Adenylyl cyclase is a protein of approximate Mr 200,000 encoded by the *CYR*1 locus. The cellular role of adenylyl cyclase in yeast is to convert ATP into cAMP in response to nutritional and environmental stimuli. Adenylyl cyclase has been found to be associated with a protein of Mr 70,000 referred to as cyclase associated protein or CAP.

The experiments presented address questions concerning the nature of the interaction among members of the cAMP signaling pathway in yeast. The site on adenylyl cyclase that binds to CAP is mapped to the C-terminal 259 amino acids of adenylyl cyclase. This region of adenylyl cyclase is also shown to bind a protein of Mr 40,000. It is demonstrated that the C-terminal 67 amino acids of adenylyl cyclase are required for RAS stimulation of cAMP production. Experiments are presented describing the interaction between CAP and the src homology 3 (SH3) domains of two yeast proteins CDC25 and actin binding protein 1 (ABP1). In one set of experiments. an interaction between CAP and the SH3 domains of ABP1 and CDC25 is demonstrated using recombinant yeast CAP and bacterially expressed GST-SH3 domains. In a second set of experiments, an interaction between native yeast CAP and SH3 domains is characterized through the demonstration that recombinant GST-SH3 fusion proteins immobilized on glutathione agarose beads can specifically precipitate CAP from a solubilized yeast extract.

A1-161 CLONING AND CHARACTERISATION OF cDNAs ENCODING A NOVEL NON RECEPTOR TYROSINE KINASE, brk, EXPRESSED IN HUMAN BREAST TUMOURS.

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It is now well established that protein tyrosine kinases play an important role in regulating proliferation and differentiation. Using a polymerase chain reaction based differential screening approach, we have isolated and characterised a cDNA from a human metastatic breast tumour representing a novel protein tyrosine kinase (brk). Sequencing of brk cDNAs isolated from T-47D and MCF-7 human breast tumour cell lines indicate that they encode a protein with a predicted molecular weight of 52kDa that has the features of a novel non-receptor tyrosine kinase, including amino terminal SH3 and SH2 domains. When synthesised in recombinant baculovirus and bacterial expression systems, brk protein products are capable of autophosphorylation on tyrosine residues. Initial expression studies have detected low levels of brk transcripts in some human breast tumours and breast tumour cell lines, but not in normal breast tissue. In addition introduction of a eukaryotic expression vector containing the brk cDNA into NIH3T3 cells results in efficient focus formation in soft agar.

A1-162 Differential induction of early genes in A431 cells by PDGF receptor mutants.

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In an effort to clarify the signaling pathways used by the platelet-derived growth factor (PDGF) β receptor (βPDGFR), we have constructed a series of phosphorylation site receptor mutants interacting with a limited number of effectors. The mutants included an F5 receptor that did not associate with PLCγ1, GAP, PI3K or the phosphotyrosine phosphatase Syp/SH-PTP2; as well as four additional mutants that could stably associate with only one of these proteins. When expressed in HepG2 cells (a human hepatocarcinoma cell line), we found that the F5 receptor was nearly unable to initiate DNA synthesis, and that repairing the binding site for either PLCγ or PI3K, but not the other two proteins, restored the majority of the DNA synthesis response. These observations indicated that the proteins that associate with the βPDGFR via the PI3K and PLCγ sites direct the receptor's mitogenic response, and we wanted to know if these effectors signaled through common or independent pathways.

To investigate these possibilities we examined the ability of the above described series of receptor mutants to stimulate the expression of a panel of immediate early genes. The βPDGFR mutants were expressed in A-431 cells (a human epidermoid carcinoma cell line), and the PDGF-dependent induction of c-*fos*, c-*jun*, *KC*, and *rhoB* was investigated by Northern Blot analysis. The F5, Y771 (binds GAP) and Y1009 (binds Syp) receptors were largely unable to activate any of these genes, while the Y40/51 (binds PI3K) and Y1021 (binds PLCγ) showed markedly distinct responses. The Y1021 receptor was able to fully activate c-*fos*, c-*jun*, and *KC*, but not the TPA-insensitive *rhoB* ; whereas the Y40/51 receptor induced *rhoB* maximally, c-*fos* partially, but was unable to stimulate the expression of either *KC* or c-*jun*. An in-depth comparison of which responsive elements in the c-*fos* promoter are induced by the Y1021 and Y40/51 receptors will be presented. These studies suggest that the proteins that associate with the activated βPDGFR are responsible for the induction of immediate early genes using independent signaling pathways.

A1-163 CSK ASSOCIATES WITH A TYROSINE-PHOSPHORYLATED PROTEIN THAT IS HIGHLY RELATED TO GAP-ASSOCIATED P62,

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CSK suppresses the kinase activities of Src family members by phosphorylation of a conserved tyrosine residue in the C-terminus. In resting fibroblasts, Src is heavily phosphorylated at this site. It is well established that wild type (wt) Src is localized in perinuclear regions and is found associated with membranes, while activated Src is found in adhesion structures termed podosomes. CSK itself lacks the myristylation signal that the Src family members have and appears to be cytosolic. We believe that CSK is recruited to Src complexes following activation of Src. This hypothesis is supported by recent immunofluorescence localization studies demonstrating that CSK is cytosolic when co-expressed with wt Src, yet is localized in podosomes when expressed with activated Src (Howell and Cooper, MCB 14:5402).

To identify proteins mediating this translocation we have analyzed proteins that are associated with CSK in a NIH3T3 cell line stably expressing v-Src as a "model" of activated Src. At least 8 proteins co-immunoprecipitate with CSK as detected by anti-P.Tyr blotting. Two of the most prominent bands are an unknown diffusely migrating protein of ~97 kDa and a doublet at ~63-65 kDa. The 63-65 kDa doublet clearly comigrates with the GTPase-activating protein (GAP)-associated p62 doublet (GAP-A.p62). Although good antibodies to GAP-A.p62 are unavailable, evidence that the CSK-associated protein and GAP-A.p62 are closely related if not identical was obtained by phosphotryptic peptide mapping comparisons. GAP-A.p62 is co-immunoprecipitated by 2 different affinity-purified anti-peptide CSK antibodies, indicating that its precipitation is not due to cross-reactivity of the antibodies. GAP-A.p62 is one of the most prominent phosphotyrosine-containing proteins that binds to GST-CSK SH2 fusion protein in solution, and mutations in the CSK SH2 domain that have been reported to abolish SH2 binding decrease binding to GAP-A.p62. This indicates that the association is mediated at least in part by the SH2 domain. We believe that CSK binds to GAP-A.p62 directly because (i) GST-CSK SH2 binds strongly to GAP-A.p62 in SDS-denatured cell lysates, and (ii) biotinylated GST-CSK SH2 used as a probe recognizes GAP-A.p62 on a blot. We are currently analyzing other conditions under which GAP-A.p62 associates with CSK.

A1-164 CLONING AND SEQUENCING OF *cph* cDNA REVEAL HOMOLOGY WITH GDP-EXCHANGE FACTORS,

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Treatment of Syrian hamster embryo fibroblasts with a single dose of 3-methylcholanthrene resulted in the activation of cellular sequences with transforming potential. Serial transfection into NIH/3T3 cells of DNA from chemically initiated hamster cells allowed the isolation by cosmid rescue of a genomic clone encompassing these sequences, which were further identified as a novel oncogene, termed *cph* (Velasco et al., Oncogene 9: 2065-2069, 1994). The cloned *cph* oncogene was able to transform NIH/3T3 cells but also showed a synergistic action with H-*ras* in the transformation of murine fibroblasts. Gene expression analyses performed with various *cph* genomic fragments identified in normal and neoplastic hamster embryo cells, as well as in transformed NIH/3T3 cells, the presence of various *cph*-related transcripts: a major mRNA of about 2.5 kb, and several other larger species. Screening with *cph* genomic probes of a λ-Zap-II cDNA library prepared from mRNA of neoplastic Syrian hamster embryo fibroblasts allowed the isolation of various cDNA clones with inserts long enough to represent the most abundant *cph* (2.5 kb) transcript. Nucleotide sequence determination of the entire insert of one of these clones (pcDNA-19) demonstrated that: (a) the *cph* cDNA does not show any significant global homology to sequences deposited in established databanks, confirming that *cph* is indeed a novel gene, (b) it contains an open reading frame with coding capacity for a protein of about 26 kDa, which was indeed in vitro translated from pcDNA-19, and (c) the cph protein deduced from the nucleotide sequence contains a dbl-homologous (DH) domain with about 22.7% identity (49.2% similarity when conservative substitutions are considered) with the DH domain of the human dbl protein, and also similar to the DH domains of other proteins of yeast or human origin. Because DH domains have been identified primarily among GDP-exhange factors, it is possible that the cph protein may be a new member of that class of proteins.

A1-165 SYNTHETIC HEPARIN SACCHARIDES BIND FIBROBLAST GROWTH FACTORS AND ACTIVATE FGF RECEPTORS: BIOLOGIC ACTIVITY AND CRYSTAL STRUCTURE,

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Fibroblast growth factors (FGFs) are unique among polypeptide growth factors in that they require a polysaccharide cofactor, heparin, for high affinity receptor binding and subsequent activation. The mechanism by which heparin activates FGF is not known. However, it has been proposed that heparin may be involved in the dimerization of two FGF molecules or of one FGF molecule and an FGF receptor (FGFR). To test these models and to define the molecular mechanism by which heparin activates FGF we have synthesized and assayed small synthetic heparin molecules corresponding to structures found in native heparin. We have discovered that non-sulfated heparin di- and trisaccharides can bind to and activate FGF, and induce FGF to bind to and activate the FGF receptor. The small size of these oligosaccharides and the crystal structure of a complex between oligosaccharide and basic FGF (bFGF) place constraints on possible mechanisms by which heparin can activate FGF-dependent pathways. Furthermore, the structural data and biologic activity suggests that other small molecules that can be more readily synthesized could be used to modulate FGF activity.

A1-166 REDUCTION OF TGF-ß SENSITIVITY FOLLOWING EXPRESSION OF A TGF-ß RECEPTOR TYPE II ANTISENSE,

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The transforming growth factors beta (TGF-ß) are a family of peptides which are involved in the regulation of cell growth and differentiation. The membrane anchored receptors which mediate TGF-ß action can be divided into signaling (type I and type II) and non-signaling (type III and endoglin) groups. It has been suggested that the loss of sensitivity to growth inhibition to endogenous TGF-ß may contribute to the process of carcinogenesis in epithelial systems. Since TGF-ß receptor type II (TGF-ßRII) had been shown to mediate TGF-ß-induced growth inhibition, it is possible that a reduced TGF-ßRII content could promote cell growth and cancer progression. To explore this possibility, we have tested the ability to block TGF-ßRII expression by expressing a complementary TGF-ßRII antisense strand.

The growth of mink lung Mv1Lu cells is potently inhibited by TGF-ß1. Stable transfectants of Mv1Lu cells bearing the TGF-ßRII antisense gene were 3 to 4 times less sensitive to TGF-ß1-induced growth inhibition as compared to mock transfected cells or cells transfected with the sense construction. The stable transfectants were transfected with a reporter plasmid carrying the luciferase gene under control of a TGF-ß inducible promoter. This reporter gene provided an alternate assay of the sensitivity of the cells to TGF-ß. Measurements of the effect of exogenous TGF-ß on luciferase activity showed that expression of TGF-ßRII antisense abolishes the TGF-ß-induced increased in luciferase activity. Moreover, Cos-1 cells transfected with an expression vector coding for the TGF-ßRII demonstrated a decrease of up to 70% of functional receptor when co-transfected with an TGF-ßRII antisense construction, as evidenced by a ligand-binding assay with iodinated TGF-ß1. These results show that an antisense RNA complementary to the TGF-ßRII can decrease the response of the cells to TGF-ß. Similar results were obtained when a truncated version of the TGF-ßRII lacking the intracellular signaling domain was expressed in stably transfected cells. This indicate that this truncated receptor acts as a dominant negative mutant.

A1-167 **PHOSPHORYLATION AND ACTIVATION OF THE IGF-I RECEPTOR IN *SRC* TRANSFORMED CELLS,**

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Our laboratory has previously identified the Insulin-Like Growth Factor receptor (IGF-I receptor) as a protein whose tyrosine phosphorylation correlates with transformation by *src*. The IGF-I receptor exhibits an elevated level of tyrosine phosphorylation in *src* transformed cells even in the absence of IGF-I treatment. The *src*-induced phosphorylation of the receptor correlates with an increase in the *in vitro* tyrosine kinase activity of the receptor, which is dependent on tyrosine phosphorylation, as treatment with a tyrosine-specific phosphatase lowers receptor activity. In addition, an IGF-I receptor mutant defective for kinase activity exhibits constitutive tyrosine phosphorylation in src-transformed cells indicating that the src-induced tyrosine phosphorylation of the receptor is not dependent on receptor autophosphorylation. The src-induced sites of IGF-I receptor tyrosine phosphorylation were identified.

Since $pp60^{v\text{-}src}$ can directly phosphorylate insulin-family receptors and since phosphorylation of the IGF-I receptor correlates with phenotypic transformation in cells transformed by various *src* mutants, we believe our findings suggest a novel pathway by which $pp60^{v\text{-}src}$ (and presumably other *src*-family members) can alter cellular regulation, namely by intracellular, ligand-independent activation of growth-factor receptors.

A1-168 ACTIVATING MUTATED KIT RECEPTOR MEDIATES MAST CELL PROLIFERATION, DIFFERENTIATION AND TRANSFORMATIOM, Xiānhua Piao and Alan Bernstein, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada M5G 1X5

The Kit receptor tyrosine kinase plays a crucial role in hematopoiesis, especially in mast cell (MC) growth and differentiation. We have previously shown that the Kit receptor is constitutively activated in the mouse mastcytoma cell line P815. Recently, Kitamura's group has found this activation is due to a point mutation of Asp814 to Tyr in the kinase domain. In order to determine the transforming potential and biological effects of this mutantion on mast cells, the D814Y substitution was introduced into both Kit and KitA isoforms (Kit-DY and KitA-DY). The KitA isoform contains a 4 amino acid insert upstream of the transmembrane domain. The two mutants were then introduced into the IL-3-dependent immature mast cell line IC2. Both mutants promoted IC2 cell growth and differention in a ligand-independent manner, as measured by ^{3}H-TdR incoporation, cell size, granularity and MC protease-4, 5, and 6 expression; however KitA-DY did so to a lesser degree. Moreover, IC2/Kit-DY cells formed colonies in methylcellulose in the absence of growth factor. In contrast, the colony-forming effeciency of IC2/KitA-DY cells was 5-fold less. Interestingly, when IC2/Kit-DY cells were introduced intravenously into syngeneic DBA/2 mice, 100% of the mice developed mastocytomas in the liver within 2 months, whereas only 20% of the IC2/KitA-DY mice did, with much fewer tumor nodules. These data argue that the single point mutation in the Kit kinase domain is responsible for the oncogenic activation of the Kit receptor. They also demonstrate functional differences between the highly similar isoforms of Kit and KitA.

A1-169 A SINGLE AMINO ACID EXCHANGE IS RESPONSIBLE FOR CONSTITUTIVE ACTIVATION OF THE 124 v-mos KINASE.

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The viral forms of the c-Mos protein kinase (HT1 v-Mos and 124 v-Mos) are distinguished from the proto-oncogene product c-Mos by an N-terminal extension of 31 amino acids derived from the c-mos gene and retroviral *env* sequences. In addition, 124 v-Mos but not HT1 v-Mos carries 12 amino acid substitutions.

We have compared the kinase activities of the wild-type c-Mos protein and of two v-Mos proteins (strain HT1 and MSV124) after expression in insect cells. Only the 124 v-Mos protein showed kinase activity *in vitro* as measured by autophosphorylation, vimentin phosphorylation or by phosphorylation and activation of MAP kinase kinase. By domain swapping and site-directed mutagenesis we identified a single point mutation in the 124 v-Mos protein ($Arg^{145}\rightarrow Gly$) which is responsible for its constitutive activity. This residue is located in the α-helix C of the kinase domain close to the ATP binding fold and is conserved in all known c-Mos proteins. Introduction of the corresponding mutation into HT1 v-Mos and into murine c-Mos activated both proteins for autophosphorylation, vimentin phosphorylation and for signalling via MAP kinase kinase *in vitro*. We hypothesize that the $Arg^{145}\rightarrow Gly$ mutation found in 124 v-Mos mimicks a conformational change which might be an obligatory step in the activation of c-Mos *in vivo*.

A1-170 ACTIVATED RAC1 IS ONCOGENIC IN FIBROBLASTS, R.-G. Qiu, F. McCormick and M. Symons, Onyx Pharmaceuticals, Richmond, CA 94806

We have investigated the role of rac1, a small GTP-binding protein, in the control of cell proliferation in stably transfected Rat1 fibroblasts. Cell lines overexpressing rac1V12, a constitutively activated mutant, grew to higher saturation density, showed enhanced growth in low serum, proliferated in soft agar and induced tumors in nude mice. In contrast, cells overexpressing rac1N17, a dominant negative mutant, grew to lower saturation density, displayed a slower growth rate and did not grow in soft agar. Thus, rac1 controls cell proliferation and overexpression of rac1V12 induces loss of contact inhibition, anchorage independence and tumorigenicity. The rac1V12 lines showed constitutive ruffling activity, even in the absence of serum or upon reaching confluence. Induction of ruffling by growth factors was inhibited in rac1N17 lines, conforming previous studies (Ridley et al, Cell 70, 401). Interestingly, rac1V12 lines had a high percentage of multinucleate cells, reminiscent of fibroblasts transformed by the dbl oncogene. In summary, our results indicate that overexpressed rac1V12 is transforming and that this oncogenic property may result from a deregulation of actin dynamics.

A1-171 INTERACTION OF Shc WITH Grb2 REGULATES THE Grb2 ASSOCIATION WITH mSOS. K.S.Ravichandran, U.Lorenz, S.E.Shoelson, and S.J.Burakoff. Division of Pediatric Oncology, Dana-Farber Cancer Institute and the Department of Pediatrics, Harvard Medical School, Boston, MA.

The adapter protein Shc has been implicated in Ras signaling via many receptors including the antigen receptors on T and B cells as well as the receptors for interleukin-2, interleukin-3, erythropoietin and insulin. One of the mechanisms of T cell receptor (TCR)-mediated, tyrosine-kinase dependent Ras activation, involves the simultaneous interaction of phosphorylated Shc with the TCR-ζ chain and with a second adapter protein Grb2. Grb2, in turn, interacts with the Ras guanine nucleotide exchange factor, mSOS, thereby leading to Ras activation. Although it has been reported that in fibroblasts growth factor stimulation does not alter the levels of the Grb2:mSOS complex, we observe that TCR stimulation leads to a significant increase in the level of Grb2 associated with mSOS. This enhanced Grb2:mSOS association, is regulated through the SH2 domain of Grb2 and occurs via the previously characterized SH3:proline-rich sequence interaction. The following observations support a role for Shc in regulating the Grb2:mSOS association: (1) a phosphopeptide corresponding to the sequence surrounding Tyr^{317} of Shc, which displaces Shc from Grb2, abolished the enhanced association between Grb2 and mSOS; (2) addition of phosphorylated Shc to unactivated T cell lysates was sufficient to enhance the interaction of Grb2 with mSOS. Furthermore, fusion proteins encoding different domains of Shc indicated that the collagen-homology (CH) domain of Shc alone (which includes the Tyr^{317} site), can mediate this effect. Thus, the Shc-mediated regulation of Grb2:mSOS association may provide a means for controlling the extent of Ras activation following receptor stimulation.

A1-172 ALTERED REGULATION OF CELL CYCLE CONTROL PROTEINS IN MOS TRANSFORMED CELLS,

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The mos proto-oncogene encodes a protein kinase that is normally only expressed at significant levels in germ cells and is known to function during oocyte maturation. Inappropriate expression of mos in somatic cells can result in neoplastic transformation. We have investigated the effects of serum withdrawal on the growth characteristics of v-*mos*-transformed NIH3T3 cells and their ability to down-regulate cell cycle control proteins under these conditions. We found that v-*mos*-transformed NIH3T3 cells were able to undergo cell cycle arrest with a G0/G1 DNA content in serum-free medium. However, they entered the cell cycle 4-6 hours earlier than their nontransformed counterparts in response to the readdition of serum and were not arrested in a true quiescent state. These serum starved v-*mos*-transformed cells were unable to down-regulate various cell cycle control proteins, such as $p34^{cdc2}$, cyclin A, cyclin D1, and cyclin E. The elevated cyclins and cdks resulted in active kinase complexes even in an arrested state. We found that the inability to down-regulate $p34^{cdc2}$ was at the transcriptional level and that a transcription factor responsible for controlling its expression, E2F, was not in complexes normally present in G0/G1 arrested cells. In fact, serum starved v-*mos*-transformed cells contained E2F complexes that resemble S phase complexes in nontransformed cells. This defective regulation of cell cycle control proteins following the withdrawal of serum represents a mechanism for the uncontrolled growth of transformed cells that may be shared with other transforming oncogenes.

A1-173 DIFFERENTIAL PALMITOYLATION OF THE TWO ISOFORMS OF THE SRC FAMILY KINASE, HCK AFFECTS THEIR LOCALIZATION TO CAVEOLAE.

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Hematopoietic cell kinase (HCK) is one of nine members of the SRC family of protein tyrosine kinases that are believed to play critical roles in cellular signalling. Expression of HCK is especially prominent in the myelomonocytic lineage of hematopoietic cells, rising sharply as the cells differentiate to granulocytes and macrophages. There are two HCK isoforms, p59HCK and p61HCK, that are generated by selective utilization of two inframe initiation codons from a single mRNA species. Subcellular fractionation studies revealed that p59HCK localized exclusively to the membrane fraction, whereas, p61HCK was localized largely in the cytoplasm. We sought to examine properties that could explain this difference in localization and observed that p59HCK, in addition to being myristoylated, was also palmitoylated. Palmitoylation occurred at $cysteine^3$, a residue that is not conserved in p61HCK. Using site directed mutagenesis to modify p61HCK to contain a cysteine residue at position 3, the encoded product was now palmitoylated and exhibited a higher affinity for the membrane fraction. Myristoylation of gly^2 was a prerequisite for the palmitoylation of cys^3. These results suggest that the dual acylation of HCK contributes to its localization to membranes.

The surfaces of most cells are covered by small flask-shaped membrane invaginations called caveolae. Recently, a growing body of biochemical and morphological evidence indicates that a variety of molecules known to function directly or indirectly in signal transduction are enriched in caveolae. Using biochemical fractionations to isolate caveolae from a variety of cell lines we determined that a significant portion of p59HCK co-localized with the caveolae fraction. Palmitoylation of p59HCK was required for the inclusion in caveolae. When p61HCK was modified to contain a cys^3, it also was enriched in caveolae. We conclude that dual acylation of the N-terminal met-gly-cys motif of HCK and other SRC family protein tyrosine kinases is required for their localization to caveolae.

A1-174 DIFFERENTIAL EXPRESSION OF PROTO-TRKA AND p75LNGFR ON PC12 CELLS

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Different antibodies to the extracellular and intracellular domains of TrkA have been used to study the expression of TrkA in PC12 cells and its fate subsequent to NGF addition. In exponentially-growing cultures, all cells express TrkA intracellularly. This labelling is perinuclear, characteristic of membrane-targetted proteins. When paraformaldehyde-fixed cells are used, approximately 10 - 30 % of the cells are labelled at the cell surface. Using intact cells, a similar fraction of the population is labelled. This label is located at the membrane, but can also be observed in the cytoplasm and nucleus. Expression of p75 at the cell surface appears to be detectable on 70 - 90% of the cell population. When double labelling is performed, several patterns of labelling are observed including: cells with TrkA alone, p75 alone and both p75 and TrkA. These results suggest that there is, in the very least, a differential accessibility of the TrkA molecules at the surface of cells in an exponentially-growing population. These observations open the way for further investigations of the mechanism of action of NGF, in particular the signal transduction pathway via TrkA or p75 homodimers or heterodimers of TrkA with p75.

Cytoplasm: The Connectors II; Nucleus: The Responder

A1-200 RESCUE OF BCR-ABL POINT MUTANTS WITH DOWNSTREAM ACTING GENES SUPPORTS A MULTI-SIGNAL MODEL FOR TRANSFORMATION

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A complementation strategy was developed to define the signaling pathways activated by the BCR-ABL tyrosine kinase. Transformation inactive point mutants of BCR-ABL were tested for complementation with a panel of downstream acting genes. Single point mutations in the src-homology 2 (SH2) domain, the major tyrosine autophosphorylation site of the kinase domain and the Grb-2 binding site in the BCR region impaired transformation of fibroblasts by BCR-ABL. Hyperexpression of c-MYC, c-H-ras and cyclin D1 efficiently restored transformation activity to selected BCR-ABL mutants. Other genes, such as c-fos and c-jun, were incapable of rescuing transformation activity of BCR-ABL mutants. This data supports a model where BCR-ABL activates multiple independent pathways for transformation. This complementation strategy should be useful for discerning signaling pathways activated by other oncogenes.

A1-201 LATE-G1 EFFECTS OF C-MYC ON TGFß1-INDUCED INHIBITION OF MOUSE KERATINOCYTES, Mark G. Alexandrow and Harold L. Moses, Department of Cell Biology and The Vanderbilt Cancer Center, Vanderbilt University School of Medicine, Nashville, TN 37232-2175

The mechanisms by which TGFß1 arrests cell growth are still poorly understood. Evidence has suggested that targets of TGFß1-induced inhibitory signals may include certain G1 cyclins, cyclin-dependent kinases, and the product of the proto-oncogene c-*myc*. We have found that overexpression of c-Myc using the estrogen-inducible mycER protein blocks TGFß1-induced growth arrest of mouse keratinocytes (BALB/MK). Unexpectedly, c-Myc is able to block TGFß1 only when the mycER protein is induced in the early part of G1; late-G1 induction of mycER does not affect the ability of MK cells to be inhibited by TGFß1. Additional data show that MK cells are sensitive to growth inhibition by TGFß1 throughout the entire pre-replicative G1 period and that the MK cells lose sensitivity to TGFß1 as they enter S-phase. Interestingly, TGFß1 is able to inhibit MK cells even when it is added in late G1, a period of time when MK cells no longer require serum or *de novo* type II RNA synthesis. In addition, TGFß1 can inhibit entry into S-phase when it is added to cells in late G1 in the presence of inhibitors of type II RNA polymerase. These data imply that, at least in late G1, TGFß1 is not inhibiting cell cycle progression by downregulating or stimulating expression of genes at the transcriptional level, but is more likely inhibiting entry into S-phase through post-translational effects on target proteins. The ability of c-Myc to block TGFß1-induced growth arrest in early, but not late, G1 suggests that c-Myc may function, in part, in the normal regulation of these TGFß1-sensitive factors. Overexpression of c-Myc at the appropriate time in G1 may affect the function and/or levels of these factors in late-G1 such that TGFß1 is unable to completely abrogate cell cycle progression.

A1-202 STUDIES ON THE INTERACTIONS BETWEEN THE HEPATITIS B VIRUS X PROTEIN AND LEUCINE ZIPPER PROTEINS. O. M. Andrisani, J.S. Williams and N. L. Dodge, Dept. of Physiology and Pharmacology, School of Vet. Medicine, Purdue University, W. Lafayette, IN 47907

The Hepatitis B virus genome encodes a promiscuous transactivator protein called pX which transactivates a variety of viral and cellular genes under control by diverse cis-acting elements. pX does not directly bind DNA, thus direct protein-protein interactions with cellular transcription factors is a mode of pX action.

Direct protein-protein interactions have been demonstrated to exist between the cellular protein CREB and the HBV X protein. pX interacts via the bZip domain of the CREB protein; the interactions between CREB and pX increases the affinity of CREB for the CRE site by one order of magnitude, although pX does not alter the dimerization rate of CREB, suggesting that pX targets the basic, DNA binding region of CREB.

In this study we are examining the interaction of pX with another leucine zipper protein, NF-Il6. NF-Il6 is involved in the expression of the acute phase response genes in the liver and also plays a role in the gene expression of HIV. We have observed that the X protein enhances the binding of NF-Il6 to NF-Il6 binding sites present within the LTR of HIV. The mechanism of this enhancement is presented. Similarly, the functional significance of the observed *in vitro* enhancement has been assessed by *in vivo* functional assays.

A1-203 ISOLATION AND CHARACTERIZATION OF PROTEINS THAT INTERACT WITH THE HUMAN N-MYC PROTOONCOGENE, Jianxin Bao and Antonis S. Zervos, CBRC, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA 02129.

The yeast two hybrid system has been very useful in isolating proteins that interact with Max-the heteromeric partner of the Myc protein. We have used a similar approach to isolate genes whose protein products interact specifically with the human N-myc. Since expression of the N-myc is restricted to the nervous system, we screened a human fetal brain cDNA library. We isolated a number of N-myc interactor proteins, and grouped them together based on their specificity and strength of interaction.

We focused our studies on one of the groups represented by a helix-loop-helix protein. This protein, in yeast, interacts specifically with the c-terminus of N-myc but not with C-myc or other helix-loop-helix or leucine zipper proteins. The expression of this gene is restricted to the brain and some parts of spinal cord. It is also expressed in cell lines that have amplified N-myc. This protein may represent a new and highly specific partner for N-myc, the function of which we are currently studying. It is interested to note that this gene is also amplified in at least one human neuroblastoma cell line.

Other families of N-myc interactors consist of novel proteins but unlike the gene described above their expression is ubiquitous and they are able to interact with Myc as well as Max proteins.

Our results imply N-myc may have a distinct function different from that of C-myc due to specific association with protein(s) that are present exclusively in the nervous system.

A1-204 GATA-1 AND C-MYB CROSSTALK DURING RED BLOOD CELL DIFFERENTIATION

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Several lines of evidence demonstrated the impact of GATA-1 transcription factor and of the c-myb proto-oncogene product for erythroid cell development and differentiation. During normal red blood cell maturation c-myb transcription gets downregulated, while GATA-1 expression increases. Accordingly, high and constitutive c-myb expression efficiently blocks terminal differentiation, while GATA-1 promotes this process.

To understand molecular mechanisms underlying erythroid cell differentiation, chicken erythroid progenitors were used. Cells were grown in tissue culture and following induction of differentiation analysed for several morphological parameters, specific changes in gene expression and the activity of red cell-specific transcription factors.

We found that by the time when c-myb mRNA is efficiently downregulated, GATA-1 DNA-binding activity is strongly increased. Furthermore, we demonstrate that GATA-1 protein directly interacts with regulatory sequences of the c-myb gene. By employing various strategies 3 major GATA-1 binding sites were identified within the CpG island of c-myb promoter. GATA-1 protein effectively binds to these sites *in vitro* as demonstrated by band shift assay. One of the GATA-1 binding sites resides at position -30 with respect to the major mRNA initiation sites, while two binding sites are located more distal. Our experiments therefore suggest that in differentiating red blood cells c-myb proto-oncogene expression is negatively regulated by GATA-1 protein.

A1-205 THE ASSOCIATION OF Raf-1 KINASE WITH p21Ras AND PHOSPHOLIPIDS : MULTIPLE DOMAINS OF INTERACTION.

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The physical interaction between p21Ras and Raf-1 kinase is a critical step in the signal transduction cascade. The association of Raf-1 with Ras-GTP results in translocation of Raf-1 from the cytosol to the plasma membrane. Subsequent interactions with membrane lipids and/or proteins lead to activation of Raf-1 by a mechanism that is not fully understood. The present study focused on identifying regions of Raf-1 that were important for binding to Ras-GTP and to phospholipids. Using deletion mutants of human Raf-1 kinase, expressed as GST-fusion proteins, at least two domains in the N-terminal region of Raf-1 were found to contribute significantly towards its binding to Ras. Removal of amino acid residues 52-64 of Raf-1 completely abolished Raf:Ras interaction, independent of the COOH-terminal end of the mutant proteins; residues 2-64 alone were not sufficient for binding Ras. The second domain encompassed residues 126-147 of Raf-1. The binding interaction was significantly enhanced from Raf 2-126 to Raf 2-130, and again between Raf 2-130 and Raf 2-131. Maximal interaction was obtained with a further extension of only 3 amino acids (Raf 2-134) and was maintained in Raf 2-147. A GST-fusion protein coding for only the cysteine-rich domain of Raf-1 (residues 128-196) bound to Ras with about 4-fold lower affinity compared to Raf 2-147. On an ELISA format assay, full-length Raf-1 bound specifically to PS and PA. The cysteine-rich domain of Raf-1 bound preferentially to PS over PA ; conversely, a carboxyl-terminal fragment of Raf-1 (amino acid residues 273-648) bound preferentially to PA over PS. Microinjection of both the cysteine-rich domain and the COOH-terminal domain fragment of Raf-1 into stage VI *Xenopus laevis* oocytes delayed the insulin-induced, Ras mediated, germinal vesicle breakdown (GVBD) of the oocytes; Raf2-64 was ineffective. The inhibition of GVBD caused by the cysteine-rich domain of Raf-1 was overcome by co-injection with oncogenic (^{61}Leu)Ras. The cysteine-rich domain of Raf-1 did not affect progesterone induced GVBD in the oocytes. Our data suggest that the binding of Raf-1 to Ras is regulated by at least two distinct domains on Raf-1. The cysteine-rich region of Raf-1 is adjacent to one of the two domains and is capable of specific interaction with phosphatidylserine, whereas a phosphatidic acid binding site appears to exist in the catalytic domain. This binding of Raf-1 to PA may be a signal-regulated event caused by the transient activation of phospholipase D by the receptor tyrosine kinase signaling pathway. Such protein-lipid interactions may lead to conformational switches in Raf-1, ultimately leading to its activation at the plasma membrane.

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A1-206 HORMONAL REGULATION OF TRANSCRIPTION FACTOR GENES (C-FOS, C-JUN, JUN-B, AND EGR-1) IN THE ADULT RAT UTERUS IS TISSUE SPECIFIC.

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The proto-oncogenes *c-fos*, *c-jun*, and *jun*-B are members of a family of genes that encode proteins which dimerize and act as the transcription factor, AP-1. *Egr*-1 is a member of another family of genes that encode proteins with DNA-binding zinc finger motifs, these are also believed to act as transcriptional factors. Expression of each of these four genes is rapidly induced by mitogenic stimuli, suggesting an association with cell proliferation. Estrogen and progesterone control cellular proliferation and differentiation in the uterus. It is known that estradiol induces transcription of a number of immediate early genes, including *c-fos*, *c-jun*, *jun*-B and *egr*-1, in the uterus of immature rats. We have examined the expression of these genes in the adult ovariectomized rat uterus by Northern analysis of RNA isolated from the luminal epithelium or from the uterus as a whole. In these animals, only the luminal epithelium responds to the mitogenic stimulus provided by a single injection of estradiol. We found that estradiol induced dramatic, rapid expression of all four genes in the uterus as a whole; concurrent progesterone treatment did not modify these estrogen responses. However, the epithelium responded quite differently. Steady state levels of *c-jun* mRNA were reduced in the epithelium of estradiol-treated animals; this effect was overcome by concomitant progesterone treatment. Our previous work suggests that estradiol directly represses transcription of *c-jun* in the epithelium (Endocrinology 134:1820, 1994). Estradiol treatment yielded increased levels of mRNAs for *c-fos* and *jun*-B in the epithelium and this was unaffected by progesterone. Levels of *egr*-1 mRNA were undetectable in the epithelial extract and they were unchanged by hormone. Thus, although these four genes are coordinately regulated by mitogenic stimuli in a number of cell types, within the uterine epithelium they show quite diverse regulation, dissociated from the mitogenic response. (supported in part by a grant from NIH, HD23244)

A1-207 SPARC AND THROMBOSPONDIN GENES ARE REPRESSED BY C-*JUN* AND *RAS* ONCOGENES IN RAT EMBRYO FIBROBLASTS.

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Activated Ha-Ras and cJun cooperate to transform primary Rat Embryo Fibroblasts (REF) and, in the same cells, Ras causes a marked increase in the ability of cJun to activate transcription from AP1-dependent promotors (Binétruy et al., 1991, Nature,*351*,122). Ras leads to a considerable and specific increase in the level of phosphorylation of serines 63 and 73 localised within the cJun activation domain. Furthermore, site-directed mutagenesis indicates that phosphorylation of these serines is essential for stimulation of cJun activity and for cooperation with Ha-ras in oncogenic transformation (Smeal et al., 1991, Nature, *354*, 494). These experiments demonstrate that cJun is a downstream target for a phosphorylation cascade involved in cell proliferation and transformation.

In an attempt to identify specific c-Jun targets we constructed a subtracted cDNA library from mRNAs purified from REF cells and performed differential screening experiments with subtracted probes. After transitory overexpression of c-*jun*, we identified several differentially repressed clones corresponding to SPARC and thrombospondin genes. mRNAs and proteins of these genes were also found repressed in c-Jun and Ras stably-transformed cell lines by comparison to normal fibroblasts. These results identify new c-Jun targets and suggest new regulatory roles for these extracellular matrix proteins in the control of cell growth.

A1-208 STRUCTURE/FUNCTION ANALYSIS OF ISGF3 COMPLEX FORMATION, Hans A.R. Bluyssen, Regina Raz and David E. Levy, Department of Pathology, New York University Medical Center, New York, NY 10016.

The members of the STAT family undergo combinatorial interactions, mediated through SH2 and phosphotyrosyl peptide interactions. They also show the ability to interact with transcription factors from other families, as evidenced in ISGF3 where STAT113 and STAT91 interact with the DNA-binding protein p48. These multiple interactions suggest a potential for forming and delivering transcription complexes to the nucleus specific to a wide array of different ligands and different DNA target sites.

We studied in more detail the ability of STAT proteins to interact both within the family and outside the family. Homo- and hetero-dimerization of different STAT proteins (STAT113, STAT91/STAT84, APRF) were tested in correlation with the ability to interact with p48. In this respect we found that upon IFN-γ treatment, a STAT91 homodimer joins with p48 to make a complex, that is able to bind the ISRE.This indicates that, in addition to STAT91 being important in the IFN-γ pathway via binding to the GAS DNA sequence, there is a second IFN-γ response pathway, which includes p48 in addition to STAT91, that is directed to ISRE-containing genes. STAT113 homodimers as well as STAT113-STAT91 heterodimers are also capable of interacting with p48 and bind the ISRE. Interestingly, the stability of these complexes appears much greater as compared to those containing STAT91 homodimers and p48, indicating that STAT113 interactions are favored above STAT91 interactions. In these STAT-p48 type of complexes, APRF-STAT113 heterodimers are not formed. Similarly, homodimers of APRF do not interact with p48.

This indicates that the highly homologous proteins of the STAT family show differences in their ability to interact in multimeric complexes. These differences could determine DNA-binding specificity and control sensitivity to distinct signal transduction pathways.The protein domains that specify the interactions among STAT family members and STAT -p48 interactions, will be discussed.

A1-209 MODULATION OF HTLV-I LTR PROMOTER EXPRESSION BY TAX AND PKA VIA cAMP RESPONSE ELEMENT BINDING PROTEIN CREB AND CRE MODULATOR PROTEIN CREM, J. Bodor and J. F. Habener, Laboratory of Molecular Endocrinology, Massachusetts General Hospital, Howard Hughes Medical Institute, Harvard Medical School, Boston, MA 02114

The human T-cell leukemia virus causes neoplasia by immortalization of T-cells resulting in marked lymphocytosis. The HTLV-I genome consists of 5' and 3' long terminal repeated sequences (LTRs) that contain three imperfect 21 bp repeats located within 200 bps of the promoter. Each of the 21 bp repeats contains a cAMP response element motif, TGACGT/AC/G/A/T (CRE) that governs the response of the LTR to the viral-encoded transactivator protein Tax and to cAMP-activated protein kinase-A (PKA). We find that CRE-binding protein CREB and CRE-modulator protein CREMτα bind to the CREs in the 21 bp repeats of the HTLV-I LTR. Analyses by UV-crosslinking and PAGE of proteins in nuclear extracts of human peripheral T-cells bound to the CREs show proteins similar and/or identical to CREB activator and CREM repressor isoforms. Transfection of a CREM transrepressor to NIH 3T3 cells with a HTLV-I LTR promoter-CAT reporter repressed both Tax and PKA stimulation of transcription. Moreover, immunoblots of nuclear proteins in peripheral T-cells detect both CREB and CREM isoforms. A potential role of CREB in activation of the HTLV-I LTR was examined further in transfection-expression assays by using GAL-CREB fusion proteins in which the bZIP DNA-binding domain of CREB was replaced by the GAL4 binding domain and cis reporter plasmids in which the CREs of the HTLV-I LTR were replaced by binding sites for GAL4. In these experiments, co-expression of both Tax and PKA stimulated transcription via GAL CREB. Notably, mutation of the serine-119 in CREB, phosphorylated by PKA and essential for the generation of the transactivation functions, retained transcriptional responsivity to Tax but not to PKA, suggesting that in human peripheral blood T-cells both CREB and CREM interact to modulate expression of transcription of the HTLV-I gene in response to Tax and cAMP activation of PKA. These findings suggest that CREB and CREM may modulate initiation and maintenance of HTLV-I latency in adult T-cell leukemia.

A1-210 INVOLVEMENT OF THE CYTOSKELETON IN EGF-INDUCED SIGNAL TRANSDUCTION, J. Boonstra, M.A.G. van der Heijden, P.J. Rijken, M. Nievers, S. van Delft, P.M.P. van Bergen en Henegouwen, Department of Molecular Cell Biology, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands.

The high affinity class of EGF receptor is associated to the cytoskeleton, and in addition this class has been demonstrated to be responsible for signal transduction. These observation suggest that the association of the EGF-receptor to the cytoskeleton plays a role in EGF induced signal transduction.

Using purified EGF-receptors and purified actin we have demonstrated that the EGF receptor is an actin binding protein. The actin binding domain was subsequently identified by competition studies with specific antibodies and synthetic peptides and by homology to the actin binding domain of profilin, a well known actin binding protein. Subsequently, EGF receptor mutants were constructed that lack the actin binding domain, and these mutants were stably transfected in mouse fibroblasts. Analysis of the signal transduction cascade as induced by EGF in these mutants, revealed that the actin binding of the EGF receptor is not involved in the induction of the early responses such as phosphorylation and activation of phospholipase C, activation of PI kinases, MAP kinase and phospholipase A2, and the expression of fos and jun oncogenes. But the binding of the receptor to actin appears to be of importance for the feed-back regulation of receptor kinase activity.

Activation of the EGF receptor by binding of EGF causes also a drastic reorganization of the actin microfilament system, including depolimerization of stress fibers and polimerization of cortical actin. Most prominent is the formation of membrane ruffles immediately after receptor activation. The membrane ruffle formation is dependent on actin polymerization. Furthermore, using confocal laser microscopy-evidence is obtained that the membrane ruffles constitute the site of EGF-induced signal transduction.

A1-211 THE p100/p52/NF-kB SUBUNIT IS SPECIFICALLY EXPRESSED AT HIGH LEVELS IN BREAST CANCER CELL LINES AND TUMORS. Vincent Bours, Emmanuel Dejardin, Giuseppina Bonizzi, Marie-Paule Merville and Vincent Castronovo. Metastasis Research Laboratory, University of Liège, BELGIUM.

NF-kB is a pleiotropic transcription factor which controls the expression of many genes and viruses. Several observations have suggested that it could be involved in carcinogenesis and in the development of metastasis in humans. To investigate the possibility that members of the NF-kB family could participate in the molecular control of the transformed phenotype, we examined the expression of these proteins in human breast cancer cell lines and tumors. Western Immunoblots demonstrated high expression of p52 and its precursor p100 in several breast cancer cell lines while cultured primary breast epithelial cells do not express these proteins. We screened 18 tumors and we observed significant expression of the p100/p52 protein in 11 of them (61%). In two patients we were able to compare the expression of p52 and its precursor in the tumor and in the adjacent non cancerous tissue; in both cases we demonstrated specific expression of p100/p52 in the tumor. In many of these cases, we saw high expression of the p100 precursor compared to the p52 truncated protein. We confirmed our data by immunostaining histologic slides of breast cancers with a monoclonal anti-p52 antibody revealed by a secondary antibody complexed to peroxydase. We indeed showed high expressed of p100/p52 in the cytoplasm of cancer cells. Our data strengthen the hypothesis that NF-kB could be involved in carcinogenesis and suggest that the p100/p52 NF-kB subunit could play a role in the development of human breast cancers.

A1-212 INACTIVATION OF *FOSB* BY TARGETED MUTATION IN MICE, Jennifer R. Brown and Michael E. Greenberg, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115

The c-*fos* family of transcription factors, which includes *fosB*, is induced rapidly and transiently at the transcriptional level by many different extracellular stimuli. This induction is thought to mediate proliferation and differentiation triggered by these stimuli. The *fosB* gene encodes both full-length and truncated forms of a transcription factor which dimerizes with Jun family proteins to affect gene expression. Embryonic stem cell lines carrying one mutated copy of *fosB* at the normal chromosomal locus have been generated by positive-negative selection and identified at a frequency of 1 in 35 $G418^R$, $FIAU^R$ clones. Chimeric animals derived from two different cell lines have transmitted the embryonic stem cell DNA to their offspring, resulting in mice heterozygous for *fosB* mutation. These heterozygous mice appear to be normal, viable and fertile. Interbreeding of heterozygous mice should give rise to mice homozygous for *fosB* mutation. The phenotype of these mice, which is now being characterized, will reflect the unique functions of *fosB* in mouse development. Animals carrying the *fosB* mutation are also being mated to mice which lack c-*fos* in order to elucidate functions of the *fos* family which are shared by c-*fos* and *fosB*. The results of these matings will be presented at the meeting.

A1-213 DIFFERENTIATION OF PC12 CELLS EXPRESSING A DOWN REGULATION-DEFECTIVE MUTANT OF THE EPIDERMAL GROWTH FACTOR RECEPTOR BY EGF: CONVERSION OF A PROLIFERATIVE SIGNAL TO A DIFFERENTIATION SIGNAL.
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The rat pheochromocytoma cell line, PC12, is used as a model system for studying neuronal differentiation. When PC12 cells are stimulated with nerve growth factor (NGF), the cells undergo a differentiation response which leads to the cells adopting a "sympathetic"-like neuronal phenotype. In contrast, stimulation of PC12 cells with epidermal growth factor (EGF) leads to a mitogenic response. Both the NGF receptor and the EGF receptor are receptor protein-tyrosine kinases. When stimulated with their respective ligands, the tyrosine kinase activity of these receptors is activated leading to the activation of the cascade of intracellular signalling proteins which in turn direct the changes in gene expression, cellular metabolism and cellular morphology. What determines the cellular response to a specific growth factor and where the specificity in signalling lies between specific growth factors is a fundamental question. We hypothesised that one difference in the signalling mechanism between NGF and EGF is the duration of receptor activation. That is, upon ligand binding, the EGFR is rapidly down-regulated whereas the NGFR is not. We have established PC12 cell lines expressing a C-terminal truncated version of the human EGF receptor. This receptor is truncated at amino acid 973, which results in the removal of the C-terminal regulatory loop of the receptor. We have also established PC12 lines expressing the wild-type human EGF receptor. When PC12 cells or PC12 cells expressing the wild-type human EGF receptor are stimulated with EGF the cells are not induced to differentiate. In contrast, when PC12 cells expressing the truncated EGF receptor are stimulated with EGF, the cells are induced to differentiate. Currently, we are attempting to identify the signalling pathways which mediate this conversion from a mitogenic response to a differentiation response.

A1-214 C-JUN EXPRESSION IN OVARIAN CANCER CELLS
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In order to study the expression of c-jun in ovarian cancer, RNA was purified from the ovarian teratocarcinoma cell line PA-1 and the adenocarcinoma cell lines NIH-OVCAR 3, SKOV3, UCLA 222, A2780, UCI-101 and UCI-107. The mRNA was immobilized on microtiter plates with oligodeoxythymidine covalently bound to the plastic. Reverse transcriptase PCR was performed using the primers CCGCTGTCCCCCATCGACATGG and CTGTTTAAGCTGCGCCACCTG, which are sequences common to both c-jun and jun B. Southern blots of the PCR products were probed with oligonucleotides specific for either c-jun or jun B. The percentage of c-jun was determined by comparing the intensities of the bands on the autoradiograms with the intensities of simultaneously amplified mixtures of c-jun and jun B in known ratios. These experiments showed that PA-1, SKOV3, UCLA-222, and NIHOVCAR 3 all had c-jun mRNA representing from 55% to over 95% of the total c-jun plus jun B mRNA. Immunoprecipitation with two different c-Jun specific antibodies revealed c-Jun expression in all of these lines. In A2780, UCI-101 and UCI-107, the percentage of c-jun mRNA was 40% or less and c-Jun protein was not measurable in these lines. Thus, c-Jun expression correlates with a higher percentage of c-jun mRNA. Jun B protein was not detected in any of these lines. The inability to detect c-Jun or Jun B most likely reflected insufficient sensitivity of the immunoprecipitation procedure. Three normal mesothelial cell lines all expressed c-Jun protein. c-Jun expressing ovarian carcinoma lines and mesothelial cells grow more slowly than c-Jun negative lines. Furthermore, two c-Jun negative lines over express p53 protein and one, UCI-101, was more resistant to cis-platinum. The possible role of c-Jun as a negative regulator of ovarian cancer cell growth will be explored further by transfecting these cells with c-jun cDNA.

A1-215 **PURIFICATION AND CHARACTERIZATION OF THE 70 kDa 40S RIBOSOMAL PROTEIN S6 KINASE FROM MATURE SEASTAR OOCYTES, Lorin A. Charlton and Steven L. Pelech, Kinetek site, Biomedical Research Centre, University of British Columbia, Suite 500-520 West 6 Avenue, Vancouver, B.C., V5Z 1A1, Canada.**

Serine hyperphosphorylation of the 40S ribosomal protein S6 has been shown to be a common phenomenon in a variety of cell types upon exposure to an array of mitogenic stimuli. We have detected an S6 kinase activity that becomes elevated ~10-fold during sea star oocyte maturation. We have purified this S6 kinase over 18,000-fold with a recovery of ~4% using sequential column chromatography steps. The kinase migrates as an ~67 kDa silver-stained band on SDS-PAGE gels, and immunoblots with a variety of p70 S6 kinase antibodies. The purified enzyme was characterized with respect to its substrate consensus sequence for phosphorylation. Basic residues (arginines) at the -2 and +2 positions from the phosphorylated serine were determined to be critical for recognition by the kinase (RXSXR). Additional amino terminal arginine residues were shown to have an inhibitory effect on kinase activity and substitution of the phosphorylatable serine to threonine reduced the affinity of the kinase for the substrate. The best peptide substrates had k_m values in the range of 0.20-0.30 μM, and V_{max} values in the range of 2,000-6,000 pmol per min per ml. The kinase was shown to be susceptible to inhibition by Ca^{2+}, Zn^{2+}, Mn^{2+}, and NaF, while heparin, spermine and β-glycerophosphate had no effect on enzyme activity. Further characterization of the physiological mechanism of action of the kinase has been undertaken via biological experiments. Rapamycin, an immunosuppressant drug has been shown to block p70 S6 kinase activity in other model systems. Oocyte maturation and S6 kinase activity in seastar are also inhibited in response to rapamycin treatment. **(Supported by M.R.C. of Canada)**

A1-216 FUNCTIONAL CHARACTERIZATION OF THE BRAIN-SPECIFIC FGF-1 PROMOTER, FGF-1.B, Ing-Ming Chiu and René L. Myers, Department of Internal Medicine and Comprehensive Cancer Center, The Ohio State University, Columbus, OH 43210

We have previously shown that the expression of alternatively spliced human acidic fibroblast growth factor (aFGF/FGF-1) transcripts is regulated in a tissue/cell line specific manner via multiple promoters. The brain specific promoter, FGF-1.B, is a non-TATA promoter and contains multiple transcription start sites. To identify the cis-regulatory elements in the FGF-1.B promoter, we constructed a series of promoter deletions fused to the luciferase reporter gene. The transcriptional activity of these recombinants was determined following transfection into an FGF-1.B positive glioblastoma cell line, U1240MG and a 1.B negative cell line, U1242MG. Results of transient transfections indicate several elements which are involved in the positive and negative regulation of FGF-1.B expression. The core promoter is located in a 40 bp region, showing activity in U1240MG but not in U1242MG cells. Two positive and one potential negative regulatory elements are located within the 540 bp region 5' to the major transcription start site and are designated regulatory region-1 (RR-1), RR-2 and RR-3. Electrophoretic mobility shift assays (EMSA) using radiolabeled probes for the 1.B promoter region have identified sequence specific binding sites in RR-1, RR-2 and RR-3. Footprinting analysis by methylation interference of the positive regulatory element RR-3 identified three guanine residues which are important for protein binding. Mutants of RR-3 show abolished binding to nuclear proteins and showed diminished luciferase reporter activity. These results confirmed RR-3 as a transcriptional enhancer element. The effects seen are specific for the U1240MG cell line supporting a role for RR-3 in the tissue specific regulation of FGF-1.B. Studies are ongoing to further identify these and other interactions, to characterize the protein(s) and to ascertain the mechanism of transcriptional activation of the brain specific FGF-1.B transcript.

A1-217 ROLE OF KIR, A NOVEL RAS-LIKE PROTEIN, IN BCR/ABL-LEUKEMOGENESIS. Lucie Cohen, Randolph Mohr, Douglas Saffran and Owen N. Witte. Department of Microbiology and Human Genetics, University of California, Los Angeles, CA 90024

The t(9:22) chromosomal translocation associated with chronic myelogenous leukemia (CML) encodes a chimaeric protein which possesses constitutive tyrosine kinase activity and oncogenic potential. At least two genes are known to be transcriptionally induced by BCR/ABL signaling pathways, the c-myc proto-oncogene which functions downstream of BCR/ABL, and kir, a novel ras-like gene located at the vicinity of c-myc on chromosome 8q. Kir was cloned by differential screening of genes present in fully malignant versus growth factor independent cell lines expressing wild type or mutant forms of BCR/ABL respectively. Kir on its own is not oncogenic, but can be converted to an oncogene. Random mutagenesis has been used to isolate oncogenic mutants of kir. One of these mutants carries a stop codon mutation and encodes a protein with a truncated carboxy terminal domain which may result in relocation of the protein. The characterization of these mutants will be presented. The correlation between kir overexpression and the highly tumorogenic and metastatic phenotype of BCR/ABL-transformed cells suggests that kir may be involved in processes of invasion or metastasis. To assess the role of kir *in vivo*, freshly isolated bone marrow cells from male Balb/C mice were infected with retroviruses co-expressing Kir and wild-type or mutant BCR/ABL proteins, and injected into Balb/c syngenic female mice. The effects of kir on BCR/ABL-tumorogenicity *in vivo* will be described.

A1-218
High Level Expression of Rsu-1 Inhibits the GAP Activity of Neurofibromin.
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Rsu-1/rsp-1 cDNA, when expressed under the control of a heterologous promoter, suppresses transformation of NIH3T3 cells by Ki-ras and Ha-ras but not v-src, v-mos or v-raf. Expression of Rsu-1 under the control of an inducible promoter was used to examine the effect of p33 Rsu-1 on Ras dependent pathways in several cell backgrounds. Rsu-1 was introduced into NIH3T3 fibroblasts and the pheochromocytoma cell line PC12 under the control of a dexamethasone inducible MMTV promoter, and Rsu-1 was expressed under the control of a metallothionein promoter in a human glioblastoma cell line,U251. Analysis of the level of GTP versus GDP-bound Ras in these cell lines indicated that the level of GTP-bound Ras increased upon induction of Rsu-1 expression in all three cell backgrounds. An assay to detect Ras GTPase activating activity in the cell lysates indicated that the level of GAP-like activity declined in the Rsu-1-U251 transfectants following induction of Rsu-1 expression. While a decrease in GAP-like activity was not evident directly in lysates of Rsu-1-NIH3T3 cells, depletion of GAP from the lysates with anti GAP antibody revealed that lysates from Rsu-1 infectant cells contained 80% less GTPase activating activity than the uninduced or control cell lines depleted of GAP. These results suggested that the inhibition of Ras GAP-like activity resulted from loss of neurofibromin function. Analysis of the level of neurofibromin in the Rsu-1-U251 transfectant by both Western blotting and immunoprecipitation indicated that the level of this protein was greatly reduced following 48 hours of Rsu-1 induction. The effect of Rsu-1 expression and decreased Ras GAP-like activity on Ras dependent signal transduction pathways was examined using Raf-1 and Erk-2 hyperphosphorylation as an assay. NIH3T3- and PC12- Rsu-1 transfectants, expressing high levels of Rsu-1, showed an increase in Raf-1 and Erk-2 phosphorylation upon growth factor (EGF,NGF) or TPA treatment when compared to uninduced or control cell lines. These results suggest that Rsu-1 acts downstream of Ras and is involved in the regulation of neurofibromin. Because the amino terminus of p33 Rsu-1 exhibits structural homology to a series of leucine based repeats found in yeast adenylyl cyclase, located in the region through which Ras activates adenylyl cyclase in Sa.cerevisiae, it is likely that high level expression of p33 Rsu-1 prevents interaction between Ras and "downstream" targets like neurofibromin. Because high level expression of Rsu-1 inhibits transformation of fibroblasts by Ras, it suggests that neurofibromin is an important Ras effector in this process.

A1-219 GROWTH INHIBITION AND TRANSCRIPTIONAL ACTIVATION BY GAL4-IκB IN SACCHARO-MYCES CEREVISIAE, Jessica Downs, Patrice Morin & Thomas Gilmore, Biology Department, Boston University, Boston, MA 02215

The IκB family of proteins regulates the activity of the Rel/NF-κB family of eukaryotic transcription factors by a direct protein-protein interaction. Expression of a fusion protein (GAL4-p40) containing the DNA-binding domain of GAL4 (aa 1-147) fused to chicken IκB-α (p40) can inhibit the growth of S. cerevisiae and can activate transcription from GAL4 site-containing promoters. We have isolated several temperature-sensitive (ts) mutants in p40 that are ts for growth inhibition and transcriptional activation in S. cerevisiae, suggesting that GAL4-p40 activates transcription and inhibits growth through the same sequences. GAL4-p40 must be bound to DNA to inhibit the growth of yeast; forms of p40 that do not bind DNA do not inhibit growth. Using high copy suppression, we have isolated three S. cerevisiae genes that can suppress growth inhibition by GAL4-p40, but that do not suppress growth inhibition by GAL4-VP16. Transcriptional activation by GAL4-p40 appears to occur by recruitment of a transcriptional activator to the promoter, and we have reconstituted an activating GAL4-p40/Rel complex in vivo in Drosophila Schneider cells. We are currently attempting to identify a yeast transcriptional activating protein that interacts with p40 and that is likely to be important for yeast cell growth.

A1-220 MOLECULAR CHARACTERIZATION OF THE GM-CSF RECEPTOR ALPHA SUBUNIT, Sean E. Doyle, Lucienne V. Ronco, and Judith C. Gasson, Department of Biological Chemistry, Molecular Biology Institute, and Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, California 90024
Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a glycoprotein cytokine that induces the proliferation and maturation of immature hematopoietic cells and the functional activity of mature granulocytes and monocytes. GM-CSF elicits these responses through the heterodimeric GM-CSF receptor (GM-CSFR). Both the alpha (84 kDa) and beta (120 kDa) subunits of the GM-CSF receptor are members of the cytokine receptor superfamily. The alpha subunit binds GM-CSF both at a low affinity as a monomer and at a high affinity when heterodimerized with the beta subunit. The beta subunit is incapable of binding ligand, but is absolutely required for GM-CSF-induced signal transduction. We have performed a mutational analysis of the structurally conserved motifs present in the alpha subunit to identify the regions required for GM-CSF signal transduction and found that cysteines #2 and #3 of the four conserved extracellular cysteine residues are required for low-affinity binding of GM-CSF. However, when the cysteine #2 mutant was co-expressed with the beta subunit, GM-CSF bound with wild-type affinity, indicating that the GM-CSFR exists as a preformed binary complex on the cell surface (Ronco et. al., 1994). Interestingly, factor-dependent cell lines stably transfected with either of these mutant alpha subunits and the wild-type beta subunit displayed a proliferative response identical to cell lines expressing wild-type alpha and beta subunits. The 54-amino acid intracellular domain of the GM-CSFR alpha subunit is essential for GM-CSF-stimulated signal transduction. Mutant GM-CSF alpha subunits with 18- and 25-amino acid intracellular domains have been constructed and stably transfected into the 32Dc13cell line, along with the wild-type beta subunit. Preliminary results indicate that neither of these mutants is capable of mediating a proliferative response. Further experiments to elucidate the precise residues present in the GM-CSFR alpha subunit required for signal transduction are in progress.

A1-221 THE ROLE OF THE CACCC AND THE DRE MOTIFS IN *DROSOPHILA RAS2/ROP* ONCOGENE REGULATION, Raquel A.B. Duarte, Kurt Lightfoot and Orit Segev, Department of Zoology, University of the Witwatersrand, Private Bag 3, JHB, RSA
The *Drosophila ras2* promoter is an authentic bidirectional promoter governing the expression of both the *Dras2* and *rop* (ras opposite) genes by a single mechanism. The *rop* gene shares sequence homology with the *S. cerevisiae* genes SLY1, SEC1 and SLP1 which are involved in yeast vesicle trafficking. SLY1 is a downstream effector of the YPT1 gene (a *ras*-like gene) hinting that *rop* may be a downstream effector of *Dras2*. A remarkably simple mechanism of dual gene expression by a bidirectional promoter may therefore coordinate the need for both gene products in a particular tissue. Characterisation of the *Dras2/rop* promoter has revealed that a unitary complex interacts with two promoter sub-domains encompassing a CACCC and DRE (*Drosophila* DNA replication related element of the PCNA and DNA polymerase α genes) motif. This unitary complex is composed of two transcription factors designated Factors A and B. Detailed mutational analysis has revealed that the CACCC core sequence is vital for *Dras2/rop* promoter recognition by Factor B. The cytosine residues at positions -27,-25,-24 and -23 were observed to play a critical role in Factor B recognition. Factor B, which is distinct from Sp1 has been purified as a 43kD polypeptide. The 20kD Factor A interacts with the DRE with a GC pair (-53 and -52) being pinpointed as cardinal for binding. We are currently in the process of cloning these two genes. The very presence of a functional DRE in the *ras2/rop* promoter underlines the involvement of *ras* in cell proliferation. It is conceivable that the final targets of a *ras* signal transduction pathway (eg. PCNA and DNA polymerase α genes) are regulated by a similar mechanism to *ras* itself.

A1-222 **A STUDY OF IL-2-DEPENDENT SIGNAL TRANSDUCTION THROUGH THE ADAPTER PROTEIN SHC: ANALYSIS OF TYROSINE PHOSPHORYLATION AND RECEPTOR ASSOCIATION**, Gerald A. Evans, James A. Johnston, John J. O'Shea, Rebecca Erwin, O. M. Zack Howard, and William L. Farrar, Biological Carcinogenesis and Development Program, Program Resources Inc./DynCorp; Laboratory of Experimental Immunology; Laboratory of Molecular Immunoregulation, National Cancer Institute, Frederick Cancer Research and Development Center, Frederick, MD 21702
Among the dominant tyrosine kinases activated by IL-2 in T cells are the src-family members lck and fyn and the JAK family kinase JAK3. Because IL-2 signal couples to the SHC/GRB2/SOS pathway via tyrosine phosphorylation of SHC we determined the dominant tyrosine kinase activity responsible for SHC phosphorylation and the mechanism of SHC association with the IL-2 receptor complex. A comparison of the ability of lck, fyn or JAK3 to phosphorylate SHC in vitro revealed that the dominant activity responsible for this phosphorylation in response to IL-2 is JAK3. Fyn was shown to enhance SHC phosphorylation only slightly while the phosphorylation of SHC by lck was undetectable. Further, phosphorylation of SHC by JAK3 in vitro was reduced when tyrosine phosphorylated SHC from IL-2 stimulated cells was used as a substrate and JAK3 phosphorylation of SHC induced the association of SHC and GRB2. Anti-phosphotyrosine immunoblotting of SHC after in vitro assay, verified that JAK3 phosphorylation of SHC is indeed on tyrosine and further that IL-2 activation of JAK3 greatly enhances its ability to phosphorylate SHC in vitro. These studies show that the activation of JAK3 is responsible for the tyrosine phosphorylation of SHC in T cells by IL-2. In several other systems, it has been shown that SHC associates with a tyrosine phosphorylated receptor or receptor associated protein bringing it into proximity for phosphorylation by the receptor tyrosine kinase. Because JAK3 associates with the IL-2 receptor complex, and is the most heavily tyrosine phosphorylated protein in the complex after IL-2 treatment, we asked whether SHC association with the IL-2 receptor was via binding to the IL-2 receptor beta chain or JAK3. Using various IL-2 receptor beta chain truncations transfected into Baf/3 cells we show that the acid rich domain of the IL-2 receptor beta chain is essential for mediating SHC phosphorylation and SHC association with the IL-2 receptor in response to IL-2. However this region is not essential for activation of JAK3 suggesting that this is not a prerequisite for SHC binding. We are also unable to detect significant amounts of SHC associated with JAK3. Additional evidence for a lack of involvement of JAK3 in SHC receptor association is found when T cells are treated with IL-4. This results in rapid activation and tyrosine phosphorylation of JAK3 without a corresponding phosphoryolation of SHC. Together this data suggests that SHC specifically associates with one or more phosphorylated tyrosine residues in the acid rich region of the IL-2 receptor beta chain and is then phosphorylated by the tyrosine kinase JAK3.

A1-223 ACCUMULATION AND RELEASE OF BIOACTIVE PHOSPHOINOSITIDE METABOLITES IN RAS-TRANSFORMED FIBROBLASTS, Falasca M., Carvelli A., Iurisci C. and Corda D. Istituto di Ricerche Farmacologiche "Mario Negri", Consorzio Mario Negri Sud, Laboratory of Cellular and Molecular Endocrinology, 66030 Santa Maria Imbaro, Chieti, Italy.

Increased levels of phospholipid metabolites, produced by different phospholipases, have been related to the presence of oncogenic ras-p21. We have recently reported that ras-transformed thyroid cells have a sustained activity of phospholipase A2 (PLA2) (1,2) specific for the membrane phosphoinositides (3). As a consequence, in ras-transformed thyroid cells, an accumulation of phosphoinositide metabolites such as lysophosphatidylinositol (LysoPtdIns) and glycerophosphoinositol-4-phosphate (GroPIns-4-P), both possessing biological activities, could be demonstrated. In particular, GroPIns-4-P inhibited the stimulation of adenylyl cyclase in different cell lines, probably interacting with Gs-protein (2).
We have now analyzed the levels of phosphoinositide metabolites in normal (FRT-fibro) and in ras-transformed fibrobalsts (FRT-fibro Ha-ras). In the latter higher levels of LysoPtdIns, GroPIns-4-P and of inositol 1:2-cyclic phosphate (Ins-1:2-cycP) could be resolved. No evidence for the accumulation of other inositol polyphosphates was found. Both LysoPtdIns and GroPIns-4-P were mitogenic in FRT-fibro Ha-ras. Interestingly, in ras-transformed but not in normal fibroblasts, GroPIns-4-P and Ins-1:2-cycP were secreted into the extracellular medium. Similarly, Ins-1:2-cycP was present in the extracellular medium of ras-trasformed thyroid cell lines, but not in their normal counterparts. These data suggest that GroPIns-4-P and Ins-1:2-cycP migth also function as paracrine mitogenic factors, thus inducing hormone-independent cell proliferation upon ras-induced cell transformation.
1) Valitutti, S., et al.(1991) Cell. Signal. 3: 321-332.
2) Iacovelli, L., et al. (1993) J. Biol. Chem. 268, 20402-20407.
3) Falasca, M. and Corda, D. (1994) Eur. J. Biochem. 221, 383-389.
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A1-224 HEMATOPOETIC TRANSFORMATION BY THE BCR/ABL KINASE IS INDEPENDENT OF ITS DIRECT INTERACTION WITH THE GRB2 ADAPTOR MOLECULE.

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The Chronic Myelogenous Leukemia-associated tyrosine kinase BCR/ABL is capable of transforming both lymphoid and fibroblast cells. Disruption of either c-Myc or c-Ras signaling pathways block BCR/ABL mediated transformation. To determine the minimal number of signals which are required for transformation, we have constructed a panel of point mutations within different functional domains of the BCR/ABL kinase. All of these mutants are defective in fibroblast transformation. One of these point mutations (Y177F) has previously been shown to block the direct binding of BCR/ABL to the Grb2 adaptor molecule. It has been proposed that the Grb2 molecule directly links BCR/ABL signaling and the ras pathway. In contrast to the fibroblast model, each of the single BCR/ABL point mutants, including Y177F, can effectively transform bone marrow in culture and cause factor independent growth of both myeloid and lymphoid cell lines. BCR/ABL activation of ras in hematopoetic cells is independent of the Y177F Grb2 binding site. In addition, the SH3 and SH2 containing adaptor molecules SHC and CRK-L are activated in all lymphoid cell lines transformed by BCR/ABL point mutants, and may provide alternate pathways for ras activation. These results demonstrate the diversity of signals provided by BCR/ABL, and that the requirements for BCR/ABL transformation of hematopoetic cells are distinct from fibroblast cells.

A1-225 Dissecting Molecular Carcinogenesis by Epidermal Gene Targeting: Cooperativity Between ras^{Ha}, fos and TGFα

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By employing an epidermal-specific targeting vector, based on the human keratin K1 gene (HK1), transgenic mice have been developed that express ras^{Ha}, fos and TGFα exclusively in the epidermis, to assess their roles and synergism in multistage skin carcinogenesis. Singly these transgenes induced epidermal hyperplasia and later the onset of benign, regression-prone papillomas. Thus, a requirement existed for other events to achieve tumor autonomy and malignant conversion. To explore oncogene synergism, mating experiments were performed and in HK1.ras/fos or HK1.fos/α genotypes papilloma autonomy was achieved. Furthermore, in TGFα/fos cooperation, TGFα was observed to have both an initiation-like role, replacing ras^{Ha} activation in cooperation with fos, and also a promotion role, accelerating the wound-dependent preneoplastic fos phenotypes. However, in TGFα/ras^{Ha} cooperation an apparent redundancy exists, since to date HK1.ras/α tumors were not only benign, but also remained prone to regression. In addition, the low frequency of spontaneous carcinomas observed to date suggests a genetic stability that will allow the creation of the triple genotype to assess the requirements for malignant conversion. Thus, these transgene experiments highlight the necessity for events in the early stages of multistage carcinogenesis in mouse skin, and provide evidence for the existence of alternative pathways of carcinogenesis that are available to a specific oncoprotein.

A1-226 ATF3: A TRANSCRIPTION FACTOR INDUCED BY PHYSIOLOGICAL STRESSES, Tsonwin Hai[1,2,3], Benjamin P.C. Chen[1] and Curt D. Wolfgang[1], [1]Ohio State Biochemistry Program, [2]Department of Medical Biochemistry and [3]Neurobiotechnology Center, Ohio State University, Columbus, OH 43210

ATF3 is a member of the ATF/CREB family of transcription factors. Members of this family share a similar DNA binding domain, the basic region/leucine zipper (bZip) domain, and bind to the same consensus site, GTACGTCA. In this report, we describe that ATF3 is induced by a variety of extra-cellular stimuli. Using *in situ* hybridization, we demonstrate that ATF3 mRNA level increases two hours after seizure induction, wounding, liver regeneration, and carbon tetrachloride treatment. We observed a substantial increase of ATF3 mRNA level in the following areas after specific treatments: (1) dentate gyrus, after treating rats with pentylenetetrazole (PTZ) to induce seizure, (2) healing skin, after acute incision of the rat skin, and (3) liver, after partial hepatectomy to induce liver regeneration or after intragastrical injection of carbon tetrachloride to induce acute liver damage. These observations indicate that ATF3 is induced by many stimuli and we propose that one of its functions is to react to certain physiological stresses and mediate specific responses. Since ATF3 is induced in a variety of tissues, we propose that the specificity of the response is determined by the cellular context and the interacting proteins in a given tissue. As a first step toward testing this hypothesis, we isolated several ATF3 interacting proteins. One of them is the bZip protein, Chop10/gadd153, which forms heterodimer with ATF3 through the leucine zipper region. As demonstrated previously by other investigators, Chop10/gadd153 is induced by serum starvation and DNA damage. In this report, we demonstrate that the DNA damaging agent, methyl methanesulfonate (MMS), induces both ATF3 and Chop10/gadd153 in a cascade manner: It induces ATF3 first, then Chop10/gadd153. We also demonstrate that dimerization of Chop10/gadd153 with ATF3 results in a heterodimer that, unlike the ATF3 homodimer, does not bind to the ATF consensus site, suggesting a functional significance of this heterodimerization.

A1-227 A HUMAN PROTEIN, SELECTED FOR ITS ABILITY TO INTERFERE WITH Ras FUNCTION, INTERACTS DIRECTLY WITH Ras AND COMPETES WITH Raf1, Limin Han and John Colicelli, Department of Biological Chemistry and Laboratory of Structural Biology and Molecular Medicine, UCLA School of Medicine, Los Angeles, CA 90024

A human gene *rin1* (Ras interaction) was identified by virtue of its ability to interfere with the function of *RAS2*, when expressed in yeast. The functional block is located at the level of the effector itself, since it does not block activation mutations further downstream in the same pathway. We demonstrate, with *in vivo* and *in vitro* experiments, that the protein encoded by *rin1* can interact directly with yeast Ras2p. Rin1 also interacts strongly with human H-Ras protein. The interaction between Ras and Rin1 is enhanced when Ras is bound to GTP rather than GDP. In addition, Rin1 is not able to interact with either an effector mutant or a dominant negative mutant of H-Ras. Thus, Rin1 displays a human H-Ras interaction profile that is the same as that seen for Raf1 and yeast adenylyl cyclase, two known effectors of Ras. It is also demonstrated that Raf1 directly competes with Rin1 for binding to H-Ras *in vitro*. Unlike Raf1, however, Rin1 fails to interact with Rap1A, a Ras-like protein. Rin1 resides primarily at the plasma membrane, where H-Ras is localized. These data are consistent with Rin1 functioning as a distinct mammalian effector or regulator of H-Ras.

A1-228 THE 5' FLANKING REGION OF CYCLIN D2 CONTAINS FOUR E-BOX ELEMENTS AND PROMOTES TRANSCRIPTION, J. Hanley-Hyde and Steven I. Reed, The Scripps Research Institute, La Jolla, CA 92037, and Sabine Mai, Basel Institute for Immunology, CH 4005, Basel, Switzerland

A 4.4 kbp genomic fragment containing the 5' flanking sequences of murine cyclin D2 has four E-box elements. Each of these sites is specifically bound by both c-*Myc* and *Myn* (the murine MAX homologue) as demonstrated by electrophoretic mobility shift assays and supershifts using antibodies against either of these proteins. Specificity was shown by competition for binding with double stranded oligonucleotides that included the E-box motif CACGTG, but not by those that lacked this motif. Fragments of this region promote transcription as determined by using a luciferase reporter gene construct in transient transfection assays of cells that express high levels of c-*Myc*. The relative contribution of each E-box element to the activity of this promoter is currently being addressed using site-directed mutagenesis of these sites, individually and in combination.

A1-229 A QUAIL LONG-TERM CELL CULTURE TRANSFORMED BY *JUN*, Markus Hartl, Peter K. Vogt* and Klaus Bister, Institute of Biochemistry, University of Innsbruck, A-6020 Innsbruck, Austria, *The Scripps Research Institute, La Jolla, CA 92037.

A chimeric construct (VCD) consisting of parts from viral *jun*, chicken c-*jun* and chicken *jun*D, was cloned into the replication-competent retroviral RCAS vector. This construct, RCAS-VCD, was found to have a higher focus forming potential in avian fibroblasts than the equivalent construct RCAS-VJ-1, expressing viral *jun*. DNAs from RCAS-VCD and RCAS-VJ-1 were transfected into primary quail embryo fibroblasts. Cells derived from one RCAS-VCD induced focus survived cell crisis, which became manifest after 15 passages, and could be expanded into a long term culture. This cell line, termed VCD, has been passaged for over 100 times so far. The cells grow to very high densities and then pile up into lumps of rounded cells. Southern blot analysis of genomic DNA from VCD cells showed proviral integration of the RCAS construct without detectable rearrangements. Northern and Western blot analyses confirmed correct expression of expected RNAs and of Jun protein from integrated RCAS-VCD. The long term culture releases a transforming virus into the culture medium with a titer of 10^5 focus forming units per ml, as tested on primary quail embryo fibroblasts. The transformed phenotype of VCD cells was verified by agar colony formation. VCD cells are capable of anchorage-independent growth with a cloning efficiency of 10 %. *jun*-transformed VCD cells provide a constant source of homogeneous cellular material for the investigation of the molecular mechanisms of *jun*-induced cell transformation and for the identification of direct and indirect targets of Jun protein function.

A1-230 INTERFERON-ALPHA 2B DOWNREGULATION OF ONCOGENES H-RAS, C-RAF-2, C-KIT, C-MYC, C-MYB, AND C-FOS IN ESKOL, A HAIRY CELL LEUKEMIC LINE RESULTS IN TEMPORAL PERTURBATION OF SIGNAL TRANSDUCTION CASCADE, William H. Harvey, Omar S. Harb, Steven T. Kosak, J. Christian Sheaffer, Lisa R. Lowe and Nyla A. Heerema, Department of Biology, Earlham College, Richmond, IN 47374 and Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN 46202-5121

ESKOL, a B-lymphoblastoid cell line consisting of late differentiated cells, resembles hairy cell leukemia (HCL). It is pseudodiploid with a deleted 7q and an unbalanced translocation between chromosomes 4 and 6. It was screened by Northern hybridization for oncogenes, including H-ras, c-raf-2 (c-raf1p1), c-kit, c-myc, c-myb, c-fos, Fim-1, c-jun, ski, and c-mos, that are believed to contribute to B-cell differentiation and maturation. Interferon-alpha-2b (IFN) downregulates the expression of H-ras, c-raf-2, c-kit, c-myc, c-myb, c-fos, as determined by Northern hybridization of RNA isolated from cells harvested at time points during a 30 h time course. Downregulation of oncogenes H-ras, c-raf 2, c-kit, whose proteins are associated with cell surfaces or are cytosolar transducers, occurs before those oncogenes c-myc, c-myb, and c-fos,whose products are DNA binding proteins. This suggests a temporal perturbation of signal transduction by IFN. No change in oncogene expression occurs in non-treated cells nor were these oncogenes expressed in the non-transformed B-lymphoblast cell line, Wil-2, under the same treatment regimen. The basis for the IFN perturbation is not understood; yet the role of these oncogenes as signal transducers in differentiation and proliferation of human hematopoietic progenitors is unfolding, and ESKOL is an excellent system in which to study this phenomenon.

A1-231 DIFFERENT MODE OF AP1/JUN ACTIVATION BY RAS AND PKCδ, Syu-ichi Hirai, Yasushi Izumi, Yoko Tamai, Shin-ichi Osada, Keiko Mizuno, Atsushi Suzuki and Shigeo Ohno, Departmenet of Molecular Biology, Yokohama City University School of Medicine, Yokohama, 236 Japan.

Modulation of gene expression by 12-O-tetradecanoylphorbol-13-acetate (TPA) is thought to be mediated by protein kinase C (PKC), a major cellular receptor for TPA. We found that PKCδ is the most effective signal transducer from TPA to TRE among three PKC isozymes (PKCα,δ,ε) detected in NIH3T3 cells. To investigate the mutual relationship between PKCδ- and Ras-dependent signal transduction pathways to a TRE-binding transcription factor, AP1/Jun, we constructed constitutively active and dominant negative mutants of PKCδ. Activated Ras induced reporter-gene expression in collaboration with over-expressed c-Jun or JunD, and this induction was insensitive to the dominant negative PKCδ. On the other hand, reporter gene expression induced by the constitutively active PKCδ was severely inhibited by dominant negative Ras as well as by the dominant negative PKCδ. Thus, Ras activation must be indispensable for PKCδ to activate AP1/Jun. In fact, constitutively active PKCδ activates ERK and *c-fos* promoter that are known to be activated by Ras. However, activated Ras enhances the expression of TRE-tk-CAT gene in NIH3T3 cells only moderately, and the over-expression of c-Jun or JunD is required for the activation of this reporter gene to the level attained by the over-expression of constitutively active PKCδ. On the other had, constitutively active PKCδ showed full activation of reporter-gene expression by itself and over-expression of c-Jun or JunD showed just a moderate effect on the activation of reporter-gene expression. These suggest the presence of an additional, Ras-independent, signaling pathway downstream of PKCδ to activate endogenous AP1/Jun. Interestingly, over-expression of JunB abolishes the effect of constitutively active PKCδ, indicating that JunB cannot be activated by signaling system driven by PKCδ and acts as a negative regulator of TRE. In spite of its remarkable ability of constitutively active PKCδ to activate TRE-tk-CAT expression, this mutant suppressed cell growth.

A1-232 TRANSLATIONAL REPRESSION: A STRATEGY FOR CELLULAR CONTROL OF PIM-1 PROTEIN LEVELS, Debra S. Hoover, Denise G.Wingett, Raymond Reeves, Nancy S. Magnuson, Department of Genetics and Cell Biology, Department of Microbiology, Washington State University, Pullman, WA 99164

Pim-1 is an oncogenic serine-threonine kinase expressed predominantly in hematopoietic tissue. Data suggests a role for Pim-1 involvement in signal transduction pathways directing both cell growth/differentiation and apoptosis. *pim-1* mRNA is inducible in response to various growth factors and mitogens. Pim-1 protein, however, is not induced under the same conditions. This suggests translational regulation may be occurring. Consistent with this hypothesis, the 5' untranslated region (UTR) of *pim-1* mRNA is unusually long (400bp.) and approximately 76 % GC rich. GC richness, which creates extensive secondary structure in mRNA, has been shown to repress translation. Here we show that Pim-1 translation is repressed both *in vitro* and *in vivo* by elements within the 5'UTR. Furthermore, we demonstrate a factor dependent mechanism for relief of translational repression *in vivo*. Overcoming this translational repression of Pim-1 protein may be involved in the malignant transformation of hematopoietic tissue.

A1-233 ACTIVATION OF REL/NFκB TRANSCRIPTION FACTORS BY CD40-MEDIATED SIGNALING AND ITS IMPLICATION IN BLOCKING B CELL APOPTOSIS INDUCED BY ANTIGEN RECEPTOR CROSSLINKING, Jun-ichiro Inoue, Takaomi Ishida, Norihiko Kobayashi and Tadashi Yamamoto, Department of Oncology, The Institute of Medical Science, The University of Tokyo, Tokyo 108, Japan

CD40 is expressed on both immature and mature B cell and its association with CD40 ligand (CD40L) on T cell is required for B cell activation, immunoglobulin class switching and rescue of B cell from apoptosis induced by surface immunoglobulin crosslinking. CD40 shows structural homology with a family of cell-surface receptors, including tumor necrosis factor (TNF) receptor, FAS antigen and the low-affinity nerve growth factor receptor. Especially, 45 amino acids stretch within cytoplasmic domain is significantly conserved among these receptors. Since TNF receptor-mediated signal has been demonstrated to activate Rel/NFkB transcription factors, we addressed the question of whether CD40-mediated signal could lead to the activation of these transcription factors. Cotransfection of CD40 and CD40L expression vectors or combination of CD40 expression plasmid transfection and stimulation by anti-CD40 antibody results in the activation of Rel/NFkB transcription factors in B and T cell lines. Using series of deletion mutants of CD40, we have mapped the cytoplasmic domain sufficient for the Rel/NFkB activation from amino acid no.216 to 245 including half of the 45 amino acids conserved box described above. To demonstrate the domain sufficient for the blocking apoptosis induced by crosslinking of antigen receptor, we have established WEHI 231 cell clones which express mutants of CD40. Although wild type CD40 rescues cells from apoptosis, a mutant with a portion of cytoplasmic domain (216 to 245) which can activate Rel/NFkB does not rescue cells. This study indicates that at least two kinds of signal including the activation of Rel/NFkB are transferred from CD40 and these signals could be required for the biological function of CD40 and the communication between B and T cell.

A1-234 A NOVEL cDNA ENCODING A PROTEIN THAT BINDS THE CYTOPLASMIC DOMAIN OF CD40, Shinji Irie, Takaaki Sato and John C. Reed, La Jolla Cancer Research Foundation, La Jolla, CA 92037

CD40 is a 50 kDa transmembrane glycoprotein expressed on B lymphocytes, epithelial cells and some carcinoma cell lines. It is a member of the tumor necrosis factor receptor superfamily. Cross-linking of CD40 with immobilized anti-CD40 antibody or via interaction with cells expressing a ligand for CD40 results in B cell proliferation, protection from apoptosis, secretion of IgE following isotype switching and enhanced tyrosine phosphorylation of proteins. Activation of a protein tyrosine kinase appears to be important for the transduction of CD40 signals. As the CD40 cytoplasmic domain does not contain any obvious enzymatic activity, it is likely that the CD40 cytroplasmic domain interacts with proteins required for its signal transduction function.

Here we report the cloning of cDNAs encoding a protein that binds to the CD40 cytoplasmic domain, based on use of a yeast two-hybrid approach. 31 million cDNA clones of human B cell library were screened and one clone that specifically bound to CD40 cytoplasmic domain was obtained. The interaction of CD40 and the protein encoded by the novel cDNA was biochemically confirmed, using in vitro translated protein and recombinant GST-CD40. Studies are underway to determine whether the protein encoded by the cDNA participates directly or indirectly in mechanisms utilized by CD40 to affect its biological responses.

A1-235 DIFFERENTIATION ASSOCIATED CHANGES IN C-MYC/MAX PROTEIN COMPLEXES AT THE E-BOX SEQUENCES OF TWO MYC TARGET GENES: DHFR AND NPY, **Annika Jalava[1] and Sabine Mai[2], [1]Turku Center for Biotechnology Box 123, FIN-20521 Turku, Finland, [2]Basel Institute for Immunology, Grenzacherstr. 487, CH-4005 Basel, Switzerland.**

We have previously described that the two E-boxes at the dihydrofolate reductase (DHFR) promoter bind c-Myc/Max heterodimers (Mai and Jalava, 1994, NAR 22: 2264-2273) in extracts of SH-SY5Y human neuroblastoma cells. During the chemically-induced neuronal differentiation of these cells, the binding of the c-Myc/Max protein complexes decreases concomitant with the reduced rate of proliferation and DNA synthesis. In the same time the differentiation associated gene, neuropeptide tyrosine (NPY), is rapidly induced. We have recently shown that the induction of NPY mRNA is associated with the modulation of Jun and Fos protein complexes at the AP-1 like motif at the NPY promoter (Jalava and Mai, 1994, Oncogene 9, 2369-2375). In addition, the 5′ flanking region of the NPY gene reveals an E-box sequence, which is shown in this study to bind both c-Myc and Max proteins. The differentiation-dependent modulation of the Myc/Max protein complexes at the E-box sequences of both DHFR and NPY is compared in two distinct differentiation model systems, in SH-SY5Y human neuroblastoma and in mouse F9 teratocarcinoma cells.

A1-236 REGULATION OF CYCLIN A TRANSCRIPTION BY CYCLIN D1 AND BY VIRAL ONCOGENES,Pidder Jansen-Dürr, W. Zwerschke, A. Schulze, K. Zerfass, D. Spitkovsky, B. Henglein[1]; Deutsches Krebsforschungszentrum, D-69120 Heidelberg/GERMANY and [1]CMG/CNRS, Gif-Sur-Yvette/FRANCE
In mammalian cells, progression through the G1 phase of the cell cycle is characterized by the subsequent activation of a set of regulatory genes encoding G1 cyclins. At the G1/S boundary, further cell cycle progression requires the transcriptional activation of the cyclin A gene, the product of which may be directly involved in DNA replication. We undertook a molecular dissection of the human cyclin A promoter. We identified a novel E2F binding site in the cyclin A promoter, which mediates induction of cyclin A gene transcription by cyclin D1, a G1 cyclin. The same promoter element mediates regulation of cyclin A transcription during the cell cycle in NIH3T3 cells. Cyclin D1-dependent promoter activation is blocked by the kinase inhibitor p16 and can be restored by cdk4, indicating that it involves a phosphorylation event. As the cyclin D1 gene is activated by serum growth factors, these findings establish a regulatory cascade linking mitogenic stimuli to the onset of S phase.
Expression of the human papillomavirus 16 E7 oncogene in NIH3T3 cells leads to constitutive expression of the cyclin A and cyclin E genes in the absence of external growth factors. The ability of E7 to promote high level expression of the cyclin A and cyclin E genes in NIH3T3 cells cosegregates with its ability to fully transform these cells. These findings define deregulated expression of particular G1 cyclins as a possible mechanism for cell transformation by HPV 16. The results of additional experiments, addressing mechanistic aspects of the observed modulation of cyclin gene expression by E7, will be discussed.

A1-237 INDUCTION OF c-*fos* AND c-*jun* PROTOONCOGENE EXPRESSION IN PULMONARY TARGET CELLS BY ASBESTOS FIBERS. Yvonne M.W. Janssen, Nicholas H. Heintz and Brooke T. Mossman, Department of Pathology, University of Vermont College of Medicine, Burlington VT 05403
Asbestos fibers cause persistent dose-dependent increases in mRNA levels of c-*fos* and c-*jun* protooncogenes in rat pleural mesothelial cells (RPM) and hamster tracheal epithelial cells (HTE) the progenitor cells of asbestos-induced mesothelioma and bronchogenic carcinoma respectively (Heintz et al, PNAS, 90, 1993). Increases in c-*fos* and c-*jun* mRNA levels are accompanied by increases in AP-1 DNA binding activity and are not observed after exposure of non-carcinogenic particulates. Exposure to asbestos depletes cellular glutathione (GSH) pools, an effect abolished by preexposure to the GSH precursor, N-acetyl-L-cysteine (NAC). In addition, NAC decreases asbestos-mediated induction of c-*fos* and c-*jun* mRNA levels in a dose-dependent fashion. Since active oxygen species (AOS) appear to be involved in asbestos-induced disease, H_2O_2, and xanthine plus xanthine oxidase, a generating system of AOS, were examined for their ability to induce c-*fos* and c-*jun*. Alterations in protooncogene mRNA levels varied with cell type, and patterns were different from those induced by asbestos. Increases of NF-KB DNA binding activity that are inhibitable by NAC are also observed in both cell types after exposure to asbestos. These data suggest that asbestos fibers trigger multiple signalling pathways in target cells of disease which may be controlled by the cellular redox status. Persistent activation of the transcription factors, AP-1, and NF-KB, may contribute to chronic inflammation and proliferation, features of asbestos-induced pulmonary diseases.

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A1-238 **HTLV-I TAX-MEDIATED ACTIVATION OF NF-κB FROM NOVEL p100 (NF-κB2)-INHIBITED CYTOPLASMIC RESERVOIRS**, Tomohiko Kanno, Guido Franzoso and Ulrich Siebenlist, Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892

The human T-cell leukemia virus type I (HTLV-I) Tax protein transforms T cells through induced expression of many cellular genes, including those encoding the growth-related proteins interleukin 2 and the α-chain of its receptor. Induction of these genes is mediated, at least in part, through Tax-dependent posttranslational activation of NF-κB, typically heterodimers of p50 (NF-κB1) and p65 (RelA). The preexisting NF-κB proteins are retained in the cytoplasm of cells by association with inhibitory ankyrin-motif containing IκB proteins, primarily IκB-α, but including also the precursor proteins p105 (NF-κB1) and p100 (NF-κB2). We demonstrate the existence of a previously undescribed multimeric cytoplasmic complex in which NF-κB dimers are associated with the p100 inhibitor in a manner dependent on the precursor protein's ankyrin domain. We also demonstrate an antagonistic effect of Tax on the cytoplasmic sequestration function of p100; this in turn leads to nuclear translocation of the liberated NF-κB dimers. Tax may exert these effects through the physical association with p100. Tax also relieves the p100-mediated inhibition of DNA binding by p50/p65 heterodimers *in vitro*. The results demonstrate a novel mechanism by which Tax may activate NF-κB in T cells.

A1-239 **REPRESSION OF HOST CELL TRANSCRIPTION BY THE VSV-MATRIX PROTEIN** Yuka Kanno, Manfred Schubert*, and Keiko Ozato, Laboratory of Molecular Growth Regulation, Laboratory of Viral and Molecular Pathogenesis*, National Institutes of Health, Bethesda, MD 20892
The matrix (M) protein of the vesicular stomatitis virus (VSV) has a regulatory role both in viral transcription and host cell transcription. Rapid and global inhibition of host cell transcription occurs following VSV infection, and M protein alone without any other viral component is capable of inhibiting host transcription to some degree. To investigate the nature of M protein-mediated transcriptional repression further, we performed reporter assays in L929 cells using M protein expression vectors and luciferase (Luc) reporter plasmids. Expression of the wild type M protein led to 10-fold inhibition in tk-Luc reporter activity. The M protein devoid of the N-terminal 32 amino acids elicited similar inhibition, however the M protein without the C-terminal 56 amino acids did not elicit inhibition. Similar results were obtained with reporters having interferon stimulated response elements fused to the tk promoter. RNA protection analysis with transfected cells confirmed that the M protein and the N-terminally truncated counterpart inhibit Luc mRNA synthesis. Inability of the C-terminal truncated M protein to inhibit reporter activity was not due to the low expression, nor to the altered intracellular localization, because both the wild type M protein and C-terminal truncated M protein were found in the nuclear fraction of transfected cells to a comparable amount by Western blot analysis. We are currently investigating a potential interaction of M protein with cellular transcription factors, in particular the importance of the C-terminal domain of the M protein in transcriptional repression.

A1-240 *INTERACTION OF JAK1 AND JAK3 TYROSINE KINASES WITH THE IL2 RECEPTOR COMPLEX.*

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The tyrosine kinases JAK1 and JAK3 have been shown to be involved in interleukin-2 (IL2) receptor signaling. Here, we show for the first time that IL2 stimulated the catalytic activity of JAK1, in addition to the established activation of JAK3. IL2 induced tyrosine phosphorylation of JAK1 and JAK3 with similar rapid and transient kinetics, suggesting coactivation rather than sequential induction of the two enzymes. However, the quantitative recruitment of JAK3 by IL2 was distinctly higher than that of JAK1, implying that JAK1 and JAK3 interact with IL2 receptors in a nonequimolar stoichiometry. This conclusion was based upon a 15-fold or higher degree of IL2-induced tyrosine phosphorylation of JAK3, more readily detectable levels of immunoreactive JAK3 and JAK3 autokinase in activated receptor complexes, as well as more readily detectable levels of IL2 receptor components in JAK3 immunoprecipitates than in JAK1 immunoprecipitates from stimulated cells. No effect of IL2 on JAK2 or TYK2 was observed. Analysis using antibodies to either IL2 receptor β- or γ-chains (IL2Rβ and IL2Rγ) revealed that the extent of coimmunoprecipitation of JAK1 and JAK3 with IL2 receptors increased significantly at 20 min of IL2 exposure, well after initial formation of heterodimeric receptor complexes and peak tyrosine phosphorylation levels of JAKs had been reached. This second *phase of stabilization between JAK enzymes and the aggregated* receptor components persisted for several hours and may be the result of intermolecular crosslinking by phosphotyrosyl-binding SH2-domain proteins. Furthermore, the correlation between the use of IL2Rγ and JAK3 by cytokines was also extended to include IL9, in addition to IL2, IL4 and IL7, as previously reported.

A1-241 ANALYSIS OF THE INT-3 ONCOGENE, A TRUNCATED FORM OF A NOTCH-LIKE TRANSMEMBRANE RECEPTOR,

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The *int-3* gene was originally defined as a target for insertional activation in MMTV-induced mouse mammary tumors. The gene encodes the intracellular portion of a putative transmembrane protein belonging to the Notch/Lin-12 protein family. By analogy to studies of Notch proteins, the intracellular part of the int-3 protein is thought to act as a gain of function mutation. Expression of the intracellular portion leads to transformation of mouse mammary epithelial cells, and is associated with high incidence of mammary adenocarcinomas when expressed as a transgene in a transgenic mouse strain. We have been assessing the biochemical and biological properties of the int-3 protein in mammary epithelial cells. Towards this goal, we have cloned a cDNA encoding for the intracellular part of the int-3 protein. Our clone contains a membrane spanning region and the intracellular portion containing a series of ankyrin-repeats. This cDNA was fused to an influenza hemagluttinin (HA) epitope to allow detection of the gene product with an anti-HA monoclonal antibody. Immunofluorescence analysis indicates that the truncated *int-3* oncoprotein resides in the nucleus suggesting that, as proposed for other Notch proteins, the intracellular portion may function in the nucleus. Mutational analysis has narrowed down a domain harboring a putative nuclear localization signal. In addition, we have cloned cDNAs encoding part of the extracellular domain. The primary structure of the extracellular domain contains a series of EGF-like repeats and Lin-12 repeats characteristic of other Notch protein family members.

A1-242 **DEREGULATION OF PAX-5 GENE EXPRESSION IN MEDULLOBLASTOMA**

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The developmental control genes of the PAX family are expressed in specific regions of the brain and contribute to the patterning of particular brain structures. A growing body of evidence indicates that one of the mechanisms by which these genes act is controlling the proliferation of neuroectodermal precursor cells during development. Pax genes seem to act as true oncogenes *in vitro* and in naturally occurring tumors: (i) ectopic overexpression of PAX genes elicits transformation of rat fibroblasts, and (ii) PAX-3 and PAX-7 genes are consistently activated in alveolar *rhabdomyosarcoma, a malignant tumor of childhood characterized* by specific chromosomal translocations involving either of the two PAX genes. For these reasons, we set out to analyze the expression of PAX family members in a comprehensive collection of surgically removed medulloblastomas. In addition, we have investigated expression of the homeobox containing *Engrailed-1* and *-2* genes, which are expressed in the embryonic mid-hindbrain region giving rise to the cerebellum. One or more members of the PAX gene family and one or both Engrailed genes were found to be expressed in all medulloblastomas analyzed. The most consistently expressed members of the family were PAX-5 and PAX-6. Furthermore, our data indicate that (i) PAX-5 provides a useful diagnostic marker for the identification of medulloblastoma cells, and (ii) that deregulation of PAX-5 expression may be causally involved in the genesis of this tumor. The latter hypothesis is amenable to experimental verification by targeting expression of PAX-5 gene to the appropriate cellular compartments in transgenic mice.

A1-243 REGULATION OF ANCHORAGE-INDEPENDENT GROWTH IN ONCOGENE-RESISTANT MUTANT CELL LINES,

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We have previously reported the isolation of mutant rat fibroblast cell lines that fail to form colonies in soft agar or methylcellulose when infected with a v-H-*ras* - expressing retrovirus, yet still undergo transformation-related morphological alterations in response to this oncogene (Krauss *et al*, MCB 12:3117, 1992). Similar to data reported by others (Guadagno *et al*, J. Cell. Biol. 115:1419, 1991), we find that control and mutant cell lines that have not been infected with the *ras* virus and that cannot proliferate in semi-solid medium, arrest in the G1 phase of the cell cycle when seeded into methylcellulose. *ras* virus-infected control cells proliferate in an anchorage-independent fashion and their cell cycle distribution in methylcellulose reflects active growth, with cells in all phases of the cell cycle. To our surprise, when the *ras*-infected mutant cells are seeded into methylcellulose, they retain a cell cycle distribution that resembles a growing population, despite their failure to proliferate. Thus, the mutation in these cells that prevents them from acquiring the anchorage-independent growth phenotype in response to *ras* does not simply restore adhesion-mediated regulation of exit from G1. Furthermore, conditioned medium (CM) from control cells contains an activity that specifically corrects the defect in the mutant lines, rendering them capable of growth in soft agar in response to *ras*. The major activity in CM, designated transformation-restoring factor (TRF), is ~1300 MW, lipid insoluble, and heat, protease, acid and base stable. Additionally, TRF production is itself regulated by transformation. We predict that these mutants are deficient in a pathway controlled by *ras* and TRF, and that this pathway plays a role in progression through multiple phases of the cell cycle.

A1-244 ROLE OF RAF IN DIFFERENTIATION OF NEURONAL CELLS.

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In order to study signal transduction pathways leading to cellular differentiation, we have generated conditionally-immortalized neuronal cell lines from rat hippocampal neurons by transduction of cells with a retrovirus expressing a temperature-sensitive SV40 large T antigen. Cell lines derived by these procedures proliferate in culture in response to EGF at the permissive temperature, and differentiate in response to bFGF or PDBu at the nonpermissive temperature. Upon differentiation, the hippocampal cells expressed neuron-specific proteins, were capable of generating action potentials, and formed synapses with primary rat neurons in co-culture. Treatment of one cell line, H19-7, with the differentiating agent bFGF resulted in the activation of c-Ras, c-Raf-1 and MAP kinase. To evaluate the role of Raf-1 in neuronal cell differentiation, we stably transfected H19-7 with v-raf and an oncogenic human raf-1/estrogen receptor fusion gene (Δraf-1:ER). Activation of Raf kinase in the transfectants was confirmed by both the band shift of Raf-1 protein in gels and *in vitro* phosphorylation of MEK, a RAF-1 substrate. Several of the v-raf transfectants became differentiated at both permissive and nonpermissive temperatures, although at the nonpermissive temperature the cells differentiated more rapidly and completely. In the presence of estrodial, Δraf:hER transfectants expressed a differentiation phenotype similar to that of the v-raf transfectants. However, no activation of MAP kinase (erks 1 and 2) was detected following activation of the Raf-ER fusion protein by estradiol. These results suggest that Raf is capable of inducing differentiation of a neuronal cell line through a MAP kinase-independent pathway.

A1-245 DNA BINDING BY NF-κB/Rel PROTEINS IS DIFFERENTIALLY MODULATED BY CONFORMATIONAL CONTEXT OF THE BINDING SITE,

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NF-κB/Rel is a family of pleiotropic transcriptional regulators involved in the control of constitutive and inducible expression of numerous eukaryotic genes. Although members of NF-κB family share the same Rel homology region which is responsible for dimerization, cytoplasmic retention and DNA binding, distinct NF-κB complexes bind various κB sites with different affinity, suggesting the importance of nucleotide context. Since in the living cell DNA is packed into chromatin, and the typical promoter and enhancer contain multiple protein-binding sites, we chose to investigate how 3-dimensional DNA configuration may contribute to the specificity of NF-κB binding. To simulate 3-dimensional DNA configuration in the cell, we used A-tract-containing minicircles of nucleosomal size with variously oriented κB sites. In binding assays performed with extracts from transfected 293 cells, homodimers of both p52 and p65 demonstrated significant variation in binding affinity towards differently prebent target sites, while p50 showed no effect. In general, bending orientations preferred by p52 were not those preferred by p65 and vice versa, and the ratio of binding to the sites bent in preferred versus non preferred orientation was about a factor of ten. The effects observed may be a combined contribution of prebending of the kB site in the minicircle, and of steric restrictions for the binding of a large complex to a minicircle with internal radius of about 6 nm. We propose that both types of effects may be relevant to the process of binding the transcription factors to the target sites located in the crowded eukaryotic promoter regions occupied by other bound factors and nucleosomes.

A1-246 KINETICS OF MYC, MAX AND MAD INTERACTIONS

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An important function of the c-myc protein is to transcriptionally activate a set of genes required for cellular proliferation. This activity is mediated primarily by a heterodimeric complex composed of the c-myc protein and its binding partner max. In addition to its participation in the myc:max complex, the max protein can form a max:max homodimer as well as a heterodimer with another protein called mad. The max:max and mad:max complexes may function as neutral or negative regulators of transcription. As part of a program to identify an inhibitor of the interaction between myc and max, we have initiated a project to characterize the kinetic parameters of the formation of myc:max, max:max and mad:max dimers. Human myc, max and mad cDNAs were isolated and cloned into bacterial and mammalian expression vectors. Recombinant peptides representing either full length proteins, or the dimerization domains alone have been prepared. Using these recombinant proteins we have analyzed myc, max and mad interactions using three independent methods: dimerization in solution, DNA binding (gel shift assay), and real-time biospecific interaction analysis using the Pharmacia Biacore system. The solution dimerization assay and the gel shift assay allow equilibrium measurements of dimerization reactions to be made, while the Biacore system directly measures the kinetics of complex formation. Using these techniques, we have begun to analyze each of the significant protein-protein interactions as well as the interaction of dimers with oligonucleotides representing the myc DNA binding sequence. Our initial results indicate that the Kd of the myc-max interaction is about 1 X 10-7 M, while the Kd of the interaction between the max homodimer and the DNA binding site is about 2 X 10-8 M. A comparative analysis of the kinetic parameters of each of the relevant interactions in the myc regulatory system will be presented.

A1-247 PHOSPHORYLATION OF NFKB1-p50 IS INVOLVED IN NF-κB ACTIVATION AND STABLE DNA BINDING.

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We have previously shown that NF-κB/Rel family members are physically associated phosphoproteins, and p105 and p50 are hyperphosphorylated after NF-κB activation. In this report, we further studied the phosphorylation involved in NF-κB activation in Jurkat T cells responding to phorbol myristate acetate (PMA) and phytohaemagglutinin (PHA). Immediately following stimulation, p50 is hyperphosphorylated, and a phosphorylated form of p50 (pp50) is translocated from the cytoplasm to the nucleus. The kinetics of this nuclear translocation paralleled that of the appearance of an active κB DNA-binding complex. An at least 30-fold higher level of κB DNA-binding was detected in pp50 than p50. The enhanced binding could be attributed to a much greater stability detected in the complex consisting of κB DNA and pp50, but not p50. These results suggest that phosphorylation of p50, and perhaps other family members as well, may be involved in the activation of NF-κB/Rel family transcription factors.

A1-248 **Blockage of Ras signaling pathway by interferon gamma in macrophage in response to colony stimulating factor-1**

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Interferons (IFN) exert anti-proliferative effects on many cell types. The underlying molecular mechanism, however, is unclear. The studies here show that the growth-arrest effects of IFN-γ overrides the mitogenic function of colony-stimulating factor-1 (CSF-1) and phorbol ester in macrophages, as measured by early gene expression, [^{3}H]-thymidine incorporation and cell proliferation. While the CSF-1 receptor phosphorylation, turnover and CSF-1-stimulated increase in diacylglycerol (DAG) production were not affected, IFN-γ blocks CSF-1- and phorbol ester-stimulated activation of Ras, Raf-1 and MAP kinases. This inhibition could be detected as early as 1 minute after IFN-γ treatment and was insensitive to cycloheximide. The IFN-γ-mediated inhibition acts upstream of Ras and downstream of DAG, and PKC inhibitors H7 and staurosporine block the signaling by CSF-1 and PMA. Results of experiments using anti-sense oligo technique indicated that PKC-δ is required for CSF-1-stimulated mitogenesis. These data suggest that either PKC-δ or some yet unidentified mediator(s) between Ras and PKC is the target for the inhibition by IFN-γ. Thus, these studies have revealed a novel mechanism by which IFN-γ may execute its anti-proliferative function.

A1-249 **THE ROLE OF NERF, A NEW MEMBER OF THE ETS FAMILY, AND OTHER ETS RELATED FACTORS IN B AND T CELLS.** Towia A. Libermann[1], Peter Oettgen[1], Yasmin Akbarali[1], Jay Boltax[1], and Chuck Kunsch[2], [1]Department of Medicine, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215 and [2]Human Genome Sciences, Inc., Rockville, MD 20850.
An intriguing question regarding immune system development is how embryonic pluripotent cells differentiate into specific cell types at programmed time points and which developmental defects of the immune system lead to leukemia and lymphoma formation. One approach is to analyze the transcriptional control mechanisms of developmentally regulated genes. To achieve this, we have chosen genes which are expressed only in certain cell types of the immune system at specific developmental stages. Thus, in our approach to understand the molecular mechanisms underlying B and T lymphocyte development we have focused on the role of Ets related transcription factors in the regulation of B and T cell-specific genes. Due to the striking similarity of DNA binding sites recognized by different members of the Ets family it is difficult to determine which Ets related factor is the functionally relevant protein for a particular Ets binding site. We have, therefore, employed antibodies specific for individual members of the Ets family to evaluate which Ets related factor in B or T cell nuclear extracts interacts with Ets binding sites of different B and T cell-specific genes. Results from these studies suggest that each Ets binding site is recognized by specific members of the Ets family. Protein-protein interactions with other transcription factors appear to determine the specificity.
We have also isolated two novel members of the Ets gene family, NERF (New Ets Related Factor) and ERP (Ets Related Protein) which are differentially expressed in the immune system. Whereas ERP is highest related to ELK-1 and SAP-1, NERF is closer related to ELF-1. This difference is reflected in distinct DNA binding specificities of ERP and NERF. ERP interaction with DNA is inhibited by a carboxy-terminal negative regulatory domain indicating the role of putative posttranslational modifications and/or protein-protein interactions in the control of DNA binding of ERP. The involvement of different signal transduction pathways in the regulation of ERP and NERF will be discussed.

A1-250 ASSOCIATION OF TENSIN WITH TYROSINE-PHOSPHORYLATED P130: A POTENTIAL ROLE IN NUCLEUS-FOCAL CONTACTS COMMUNICATIONS, S.H. Lo^, W. K. Wong*, Y. Liu*, A. H. Bouton,# J. T., Parsons,# and L. B. Chen*, *Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115, # University of Virginia, Health Sciences Center, Charlottesville, VA 22908, ^Howard Hughes Medical Institute, University of Chicago, Chicago, IL 60637

Tensin is a phosphoprotein component of focal contacts that has a Src homology 2 domain and exhibits actin-capping activities. Using tensin-conjugated beads, we have identified in lysates of Rous sarcoma virus (RSV)-transformed fibroblasts a 130 kD tyrosine-phosphorylated protein that binds to tensin. This protein is recognized by a monoclonal antibody raised against a previously described tyrosine-phosphorylated nuclear protien called p130. In RSV-transformed rat fibroblasts, both p130 and tensin stably colocalize at focal contacts, consistent with the results of experiments with tensin-conjugated beads. In contrast, in exponentially growing, nontransformed fibroblasts, p130 is detected in the nucleus and tensin is located at focal contacts. In resting rat fibroblasts that are stimulated by PDGF for 5 minutes, p130 colocalizes with tensin at focal contacts, and this colocalization is transient. After 90 minutes, p130 is no longer detected at focal contacts and is only found in the nucleus. It is possible that this relocalization of p130, from the nucleus to the focal contacts and back to the nucleus, mediates communication between focal contacts and the nucleus.

A1-251 INSULIN INDUCED FORMATION OF RASGTP AND MITOGENIC RESPONSE DO NOT REQUIRE ACTIVE PI3KINASE: EVIDENCE FOR EARLY BIFURCATION OF MITOGENIC AND METABOLIC SIGNALING PATHWAYS, J.Antonie Maassen, D.Margriet Ouwens, Gerard C.M. van der Zon, Janette Dorrestijn, Leiden University, Medical Biochemistry, 2333AL Leiden, Netherlands

Insulin is a powerful stimulator of ^{3}H Thymidine incorporation in A14 cells, an NIH3T3 derived cell line overexpressing insulin receptors. In addition, insulin induces metabolic responses like glycogen synthesis and glucose uptake. Early signaling processes affected by insulin are activation of PI3kinase, predominantly through interaction with Tyr-phosphorylated IRS1, Shc phosphorylation and formation of the Shc-Grb2-SOS complex and RasGTP . We have examined the effect of wortmannin on mitogenic and metabolic signaling in relation to activation of signaling intermediates. Wortmannin inhibits PI3kinase with an IC50 of approximately 10nM and up to 1μM no effect on receptor autophosphorylation, IRS1 and Shc phosphorylation and formation of RasGTP was seen. No effect on mitogenicity was observed. These data indicate that PI3kinase does not contribute to activation of the ShcGrb/RasGTP pathway which mediates a mitogenic response. Wortmannin inhibits other responses like stimulation of glycogen synthesis and glucose uptake. We previously showed that insulin induced activation of Ras proceeds predominantly via Shc phosphorylation and interaction with Grb2-SOS rather than via IRS1 phosphorylation and interaction with Grb2-SOS and that the Ras pathway is unlikely to mediate the signal to glucose uptake. These data suggest the presence of distinct insulin activated pathways where mitogenic signaling proceeds from the insulin receptor→Shc-Grb2-SOS→Ras-GTP without involvement of PI3kinase, whereas insulin induced glycogen synthesis and glucose uptake proceeds from the insulin receptor → IRS1→ PI3kinase or via another wortmannin sensitive intermediate.

A1-252 An identification of protein in v-Src transformed fibroblasts whose tyrosine phosphorylation is regulated either by cell to substratum adhesion or by growth factor stimulation in the absence of cell adhesion. Hisataka Sabe*,' , Hitoshi Kimura*, Ryuji Yamaguchi* and Hidesaburo Hanafusa' , *Inst. for Virus Res. Kyoto University, Kyoto 606, Japan; 'Lab. of Molecular Oncology, The Rockefeller University, New York NY 10021-6399.

A variety types of cells including fibroblasts require their adhesions to the extracellular substratum (ECM) for their growth, differentiation and survival. Protein tyrosine phosphorylations accompany cell to substratum adhesions and are believed to be important for the regulation. On the other hand, cells transformed by various oncogenes can grow in the absence of cell adhesions. Such transformation, which leads to anchorage-independent growth, may have bypassed some of the signalings evoked by cell adhesions to the ECM.

Our current study adresses the role of the cell to ECM adhesions for the cell growth and survivability; in particular, how signals from the cell adhesions and from growth factor receptors interplay.

In this report, we examined cellular protein tyrosine phosphorylations during cell to substratum adhesion and during anchorage independent growth in 3Y1/ v-Src transformed cells. We found that tyrosine phosphorylations of most of the proteins in v-Src transformed cells were still regulated by the cell to substratum adhesion: protein tyrosine phosphorylation in v-Src cells can be categolized into two classses; those dependent on the cell adhesions and others that are not. Moreover, we found that a protein, whose tyrosine phosphorylation was regulated by the cell adhesion in the absence of serum, was also tyrosine phosphorylated by growth factor stimulation in the absence of the cell adhesions. This protein may be located at the converging point of signalings from adhesion and from growth factor stimulation. The identity and the possible function of this protein in signalings will be discussed. Similar analysis using normal fibroblasts will also be described.

A1-253 MULTIPLE PROLINE-RICH REGIONS OF GAP-ASSOCIATED PHOSPHOPROTEIN p62 BIND WITH DIFFERENT AFFINITIES TO THE SH3 DOMAINS OF FYN AND SRC, Terukatsu Sasaki, Masaho Ishino and Hiroko Sasaki, Dept. of Biochemistry, Cancer Research Institute, Sapporo Medical University School of Medicine, Sapporo 060, JAPAN

Several proteins of Jurkat cells were identified on SDS-PAGE gels by Coomassie Blue staining that bound specifically to affinity matrices made of five different Src homology region 3 (SH3) domains fused to glutathione S-transferase (GST). Purification of a major specific band of 70kDa with an affinity matrix of the SH3 domain of Fyn tyrosine kinase resulted in an identification of GAP-binding phosphoprotein p62 as a ligand to the Fyn and Src SH3 domains. Indeed, Src was co-precipitated with p62 from a lysate of a Rous sarcoma virus-transformed rat fibroblast line, which was not blocked by 40 mM phospho-L-tyrosine. Bacterially expressed GST fusion proteins, containing sequences encompassing each of the proline-rich putative SH3 binding sites of p62, bound to a subset of SH3 domains with different affinities. Phospholipase C-γ-SH3 bound strongly to the bacterially expressed p62 fusion proteins but not to cellular p62, implying a competitive in vivo association of different ligand proteins to a SH3 domain. Since p62 is highly phosphorylated on its tyrosine residues in stimulated cells, proteins that have the Src homology 2 domain as well as those with the SH3 domain will bind together to p62 in dynamic competition for their binding sites, suggesting a role of p62 as a foothold on which signal transduction proteins link together.

A1-254 IDENTIFICATION OF A SHC RELATED GENE: SLI Tracy M. Saxton[1], John O'Bryan[2], Ka-Man Venus Lai[1] and Tony Pawson[1]

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The p21^{Ras} oncoprotein is an important modulator of cell growth, differentiation and transformation. Recent studies have implicated both Grb-2 and Shc proteins as upstream regulators of Ras. Grb-2 and Shc belong to a emerging group of molecules commonly referred to as adaptor proteins. Adaptors lack catalytic activity and they primarily function in protein-protein associations by linking diverse signaling molecules through SH2 and SH3 mediated interactions. We are in the process of identifying other adaptor molecules that function in cell signaling pathways.

Through low-stringency hybridization with the human Shc cDNA, we have isolated a novel murine Shc-like gene, which we call *Sli*. The SH2 domain of Sli and Shc are 68% identical, with an overall identity between these two proteins of 52%. Biochemical analysis of Sli function is presently being undertaken to decipher the role of this adaptor protein in cell signaling.

A1-255 PROTEIN TYROSINE KINASES, PAXILLIN AND INTEGRIN SIGNALLING. M.D. Schaller* and J.T. Parsons#, *Dept. of Cell Biology and Anatomy, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7090 and #Dept. of Microbiology, University of Virginia School of Medicine, Charlottesville, VA 22908

pp125^{FAK}, a unique protein tyrosine kinase (PTK), colocalizes with integrins and paxillin within focal adhesions and can complex with Src-related PTKs via its autophosphorylation site. A number of stimuli, including engagement of integrins with ligand, induces the phosphorylation of both pp125^{FAK} and paxillin on tyrosine. We have set out to explore the mechanisms regulating pp125^{FAK}-dependent phosphorylation events *in vivo* and the effects of phosphorylation of paxillin on tyrosine. FAK^{397F}, a variant of pp125^{FAK} which is incapable of autophosphorylation, retains enzymatic activity but fails to bind to pp60^{src}. pp125^{FAK}, but not FAK^{397F}, induces the phosphorylation of paxillin on tyrosine *in vivo*. Furthermore, exogenously expressed pp125^{FAK} and pp60$^{c\text{-}src}$ form a complex in chicken embryo cells, the pp60$^{c\text{-}src}$ in complex with pp125^{FAK} is enzymatically active, and the two kinases act synergistically to induce the phosphorylation of paxillin. These observations suggest the autophosphorylated pp125^{FAK} may serve to recruit and activate a second PTK, eg pp60^{src}, which in turn is responsible for phosphorylating paxillin. We have identified several tyrosine residues within paxillin which become phosphorylated *in vivo* creating binding sites for the SH2 domain of crk. We propose that pp125^{FAK}-dependent phosphorylation of paxillin on tyrosine regulates the recruitment of signalling molecules to the cytoskeleton which likely triggers the generation of second messages and transmission of a signal to the cytoskeleton or into the cytoplasm.

A1-256 FOCAL ADHESION KINASE LINKS INTEGRIN-MEDIATED SIGNAL TRANSDUCTION TO THE RAS PATHWAY, David D. Schlaepfer, Steven K. Hanks#, Tony Hunter, and Peter van der Geer, Molecular Biology and Virology Laboratory, The Salk Institute, P.O. Box 85800 San Diego, CA 92186, #Department of Cell Biology, Vanderbilt University School of Medicine, Nashville, TN 37232.

Adhesive interactions between cells and extracellular matrix (ECM) proteins play important roles in the regulation of cellular growth and differentiation. The integrin family of receptors mediate cell adhesion to the ECM and can affect intracellular signaling pathways. Since most transmembrane integrin receptors contain short cytoplasmic domains which exhibit no enzymatic activity, cellular signals must be generated by associated proteins. Focal adhesion protein-tyrosine kinase (FAK) phosphorylation is increased upon integrin engagement. In this study, we show that adhesion of murine NIH3T3 fibroblast cells to fibronectin promotes the in vivo association of the protein-tyrosine kinase c-Src with FAK. In addition, after plating onto fibronectin-coated dishes, complexes of FAK and the adaptor protein GRB2 were detected in NIH3T3 cells by co-immunoprecipitation. In vitro binding studies showed NIH3T3 cell plating onto fibronectin promoted the Nck, PLCγ, p85 PI3 kinase, and Csk Src homology 2 (SH2) domain binding to FAK. In v-Src transformed 3T3 cells, the association of GRB2 and FAK was found to be independent of cell adhesion and mediated by the direct GRB2 SH2 domain binding to FAK. NIH3T3 cell plating onto fibronectin also resulted in mitogen-activated protein kinase stimulation. We show that FAK may mediate signal transduction of the $\alpha 5\beta 1$ integrin, the receptor specific for fibronectin. Our results indicate that fibronectin binding promotes the association between c-Src and FAK, and that the increased tyrosine phosphorylation of FAK creates binding sites for other SH2-containing proteins such as GRB2 which may link integrin engagement to the activation of the Ras signaling pathway.

A1-257 PDGF β RECEPTOR SIGNALING IS AFFECTED BY JUXTAMEMBRANE TYROSINE RESIDUES. John Paul Secrist and Andrius Kazlauskas, Department of Pediatrics, Division of Basic Sciences. National Jewish Center, Denver CO 80206

Juxtamembrane (JM) domains are increasingly being recognized as important regions in regulating signal transduction by growth factor receptors. In order to more fully define the signaling contribution of the JM domain of the PDGF β receptor (βPDGFR), we introduced tyrosine to phenylalanine mutations in this region (F579 and F581) and observed their effects on various components of βPDGFR signal relay.

Tyrosines 579 and 581 have previously been shown to mediate the specific binding of Shc and Src to the βPDGFR. In agreement, we found that coimmunoprecipitation of the βPDGFR with $pp60^{src}$ was drastically reduced by either mutation, with the F579 mutation showing a greater effect. In further examining the binding specificity of these sites, we discovered that in addition to $p60^{src}$, ras-GAP binding was also severely reduced by the F579 mutation. To determine whether these mutations affected the receptors catalytic activity, *in vitro* assays were performed and receptor autophosphorylation, as well as phosphorylation of a GST-PLCγ fusion protein were measured. The *in vitro* activity of the F579 and F581 mutants was consistently lower than wild-type (WT) receptor levels with a larger effect again seen with the F579 mutation.

To determine whether substitution of both sites simultaneously (F579/581) would produce more pronounced effects, the double mutation was introduced and expressed. The F579/581 mutant was much less capable of associating with effector proteins than were the single mutants or the WT receptor. This effect was explained by the finding that the double mutant was nearly devoid of ligand-stimulatable kinase activity. Interestingly, this mutant displayed WT levels of kinase activity when activated *in vitro* with ATP, indicating that the F579/581 mutation did not simply produce a kinase-inactive receptor, but rather inhibited an apparently necessary *in vivo* activation step. Whether this step involves $pp60^{src}$ remains to be determined.

These results indicate that 1) the Y579 phosphorylation site is important for binding multiple proteins, and 2) that the receptors kinase activity is somehow regulated by phosphorylation sites in the JM domain.

A1-258 OVEREXPRESSED SH2 AND SH3 DOMAINS OF CHICKEN C-YES CAN TRANSFORM LIVER AND LUNG CELLS IN TRANSGENIC MICE

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Src homology 2(SH2) and src homolgy 3(SH3) domains are conserved among various cytoplamic signaling proteins including non-receptor protein tyrosine kinases(PTK). These domains are thought to participate in the control of intracellular responses to growth factor stimulation. To determine whether overexpression of SH2 and SH3 domains can aberrate signal transduction pathway *in vivo*, we produced transgenic mice that carry amino-terminal 1.1 kb fragment of chicken c-*yes* containing amino-terminal unique region, SH2 and SH3 domains, under the transcriptional control of murine sarcoma virus long terminal repeat sequences. We found that mice from three different lines developed tumors in liver and/or lung. We concluded that cells in liver and lung can be transformed by aberration of signal transduction pathway due to overexpression of SH2 and SH3 domains.

A1-259 CLONING AND CHARACTERIZATION OF SIK, A NOVEL TYROSINE KINASE EXPRESSED IN THE SKIN AND GASTROINTESTINAL TRACT, Elena Yu. Siyanova, Valeri Vasioukhin, Michael S. Serfas, Marina Polonskaia, and Angela L. Tyner, Department of Genetics, University of Illinois College of Medicine, Chicago, Illinois 60612.

To identify tyrosine kinases that may be involved in the regulation of the regeneration of the mammalian intestinal epithelium, we amplified portions of the catalytic domains of protein kinases expressed in intestinal crypt cells, using the polymerase chain reaction technique with primers directed against two invariant amino acid sequence motifs found in all kinases. These fragments were cloned and a library of kinase catalytic domains was generated. Sequence analysis of unique clones resulted in the identification of the catalytic domains of several characterized tyrosine kinases, including lyn, hck, c-fgr, tec, JAK2, itk, and the putative receptor kinase ryk. Expression of these kinases has not previously been reported in the intestine. Clones encoding two novel catalytic domain sequences were also identified. One of these which we have named sik (for src-family intestinal kinase) shares highest homology with members of the src subfamily of tyrosine kinases. We have cloned a cDNA encoding this kinase. The sik cDNA encodes a 451 amino acid protein that shares highest homology with a human tyrosine kinase, FRK, and a Src-like kinase SRK1, isolated from the fresh water sponge *Spongilla lacustris*. We have determined that sik is expressed in epithelial tissues that have contact with the environment including the skin and linen of the alimentary canal, and using *in situ* hybridization we show that expression of sik mRNA is restricted to the cell layers immediately above the proliferative cell zone in these epithelia. The sik mRNA is first detected at day 15.5 gestation in the mouse embryo, where it is expressed in the newly forming granular layer of the skin. The striking tissue specific expression of sik has led to studies examining the transcriptional control of the sik gene. The promoter region of the sik gene was cloned and is currently under study. The tissues expressing sik are functionally dependent on their epithelial linings that are constantly regenerating, migrating, and differentiating. It is possible that the sik kinase plays a role in the regulation of one of these processes.

A1-260 A NOVEL PROTEIN TYROSINE PHOSPHATASE IN HUMAN MAMMARY EPITHELIAL CELLS,

Anna L. Smith[1], Philip J. Mitchell[1], Janet Shipley,[1] Mark Rogers[2], & Mark R. Crompton[1] ; [1]Section of Cell Biology and Experimental Pathology, Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey SM2 5NG; [2]Department of Cell Biology, The Wellcome Research Laboratories, Langley Court, South Eden Park Road, Beckenham, Kent BR3 3BS, UK

Tyrosine phosphorylation and dephosphorylation are critical in the control of cell differentiation, proliferation and transformation. We are interested in the reversible phosphorylation of tyrosyl residues with regard to the regulation of mitogenic signalling pathways in the normal human breast. Using the polymerase chain reaction we have identified a novel protein tyrosine phosphatase expressed in the normal human breast. Following the separation of human breast luminal and myoepithelial cells on the basis of surface antigen expression, we have shown that both cell types express the novel gene. Our data suggests that the expression of the gene may be down-regulated in the luminal epithelial cells, the cells involved in the majority of mammary carcinomas. Northern blot analysis indicates that the protein is encoded by a mRNA of greater than 9.5kb. Fluorescence in situ hybridisation has shown that this gene localises to the chromosomal region 1q41-42, a region prone to rearrangements. Efforts are now directed towards the further characterisation of this protein tyrosine phosphatase and this will be the subject of the poster.

A1-261 SPECIFICITY OF PROTEIN TYROSINE KINASES AND SH2 DOMAINS,

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The substrate specificity of several protein-tyrosine kinases was studied using an oriented degenerate peptide library. Most kinases selected substrates with acidic residues at positions -2 and -3 N-terminal to the tyrosine (Tyr). Cytosolic Tyr kinases preferentially phosphorylated peptides with Ile or Val at the -1 position while receptor family Tyr kinases selected for peptides with Glu at this position. In general cytosolic protein tyrosine kinases preferentially phosphorylated peptides that would subsequently bind to Group I SH2 domains. On the other hand, receptor tyrosine kinases preferred sequences that would be expected to bind Group III SH2 domains. Each receptor Tyr kinase selected a unique optimal motif. These results suggest that the binding sites of individual SH2 domains and protein Tyr kinases have converged upon overlapping selectivities in order to maintain specificity in downstream signaling.

A1-262 **THE BOVINE PAPILLOMAVIRUS E5 ONCOPROTEIN REQUIRES *TRANS*-GOLGI LOCALIZATION FOR CELLULAR TRANSFORMATION.**

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E5, the major transforming protein of bovine papillomavirus type 1, is a 44 amino-acid transmembrane polypeptide which is found predominantly in the Golgi apparatus. Transformation of fibroblasts by E5 is accompanied by activation of the platelet-derived growth factor receptor (PDGF-R) and E5 has been shown to exist in a complex with this receptor as well as the 16K membrane pore protein of the vacuolar proton pump. Binding of E5 to both PDGF-R and 16K is mediated by transmembrane interactions. E5 contains two distinct domains: a 30 amino-acid transmembrane domain and a 14 amino-acid hydrophilic domain containing two cysteine residues necessary for homodimer formation and biological activity.

To determine whether Golgi localization was essential for E5 function, we constructed an E5 mutant to be retained in the endoplasmic reticulum (ER). The ER retention signal, KDEL, was added to the C-terminus of E5 and the mutant protein assayed for cellular localization, transforming activity, and association with cell target proteins (PDGF-R and 16K). In addition, a defective ER retention signal (KDEL*) was also added to the C-terminus of E5. Both E5-KDEL and E5-KDEL* were expressed efficiently in COS cells, formed homodimers, and were stable in both C127 and NIH3T3 cell lines. However, while wild-type E5 and E5-KDEL* localized normally to the Golgi apparatus and were transformation-competent, E5-KDEL was retained in the ER and was transformation-defective. E5-KDEL could bind normally to both the PDGF-R (although it was predominantly the immature form) and 16K targets in transfected cells, indicating that the simple association of E5 with these targets was insufficient for cellular transformation. Surprisingly, all three E5 proteins had similar capabilities to activate both forms of the PDGF-R, suggesting that activation alone of this receptor is insufficient for E5-mediated transformation. E5 may, therefore, induce mitogenesis via additional or alternative signal transduction pathway.

A1-263 DECREASED pp60$^{c\text{-}src}$ ACTIVITY BY A C-*SRC* ANTISENSE EXPRESSION VECTOR INHIBITS HT-29 HUMAN COLON ADENOCARCINOMA CELL GROWTH AND TUMORIGENICITY.

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Increased specific activities of the protein tyrosine kinases pp60$^{c\text{-}src}$ and pp62$^{c\text{-}yes}$ are among the most consistent alterations in human colon tumors. To determine the role of pp60$^{c\text{-}src}$ activation on the aberrant growth of colon carcinoma cells, we synthesized unmodified and phosphorothioate-modified antisense oligonucleotides to the translation start site of c-*src*, and examined their effects on the growth of HT-29 human colon adenocarcinoma cells. In cells treated with antisense oligonucleotides, a dose-dependent decrease in pp60$^{c\text{-}src}$ kinase activity and protein level was observed, to a maximal concentration of 75μM. HT 29 cell cultures fed with 75μM of antisense oligonucleotide daily were growth inhibited by 50% after 3 days, as compared to control cultures and cultures fed "sense" oligonucleotides. In cells treated with c-*src* antisense oligonucleotides, a compensatory ***increase*** in pp62$^{c\text{-}yes}$ kinase activity was observed. Because of the instability of exogenously added oligonucleotides, c-*src* antisense and sense constructs were ligated into pcDNA1/Neo plasmids under the control of the CMV promoter, transfected into HT-29 colon tumor cells, and clones were selected in G418. One stably transfected antisense clone, termed A15, has been extensively characterized. Properties of this clone include: four-five fold decreased pp60$^{c\text{-}src}$ expression and activity; 3 fold decreased rate of proliferation, 3 fold decreased ability to form soft agar colonies, and ~2 fold increased pp62$^{c\text{-}yes}$ activity relative to parental HT 29 cells and clones derived from transfection with "sense" expression vectors. Further, at the level of light microscopy, clone A15 has a distinct morphological change from the parental cells. All the described phenotypes have been stable for greater than ten passages. These results demonstrate that the kinase activity of pp60$^{c\text{-}src}$ contributes to proliferation and tumorigenicity of colon adenocarcinoma cells and that the roles of pp60$^{c\text{-}src}$ and pp62$^{c\text{-}yes}$ are not completely redundant.

A1-264 INTERACTION OF SRC WITH RECEPTOR TYROSINE KINASES, David R. Stover and Nicholas B. Lydon, Ciba Pharmaceuticals Division, Ciba-Geigy Limited, CH-4002 Basel, Switzerland.

The epidermal growth factor receptor (EGFR) autophosphorylates tyrosine residues located in its carboxy-terminus; in vitro, three tyrosines are highly phosphorylated, while two others are phosphorylated to lesser extents. In the presence of the protein-tyrosine kinase, pp60^{c-src}, the minor sites were phosphorylated to the same extent as the major sites and five new tyrosines were phosphorylated to an equally high level. Three of these sites (Y893, Y939, and Y968) were found to be phosphorylated in a colorectal cell line (DLD-1) after EGF stimulation, while two (Y939 and Y968) were detected in a breast tumor cell line (MCF7) again only after EGF stimulation. The other two sites were not found to be phosphorylated in any *in vivo* situation. The sequences surrounding Y939 and Y968, match the reported consensus binding sequences for the SH2 domains of pp60^{c-src} (YXXI) and PI-3 kinase (p85) (YMXM), respectively. Both of these proteins were found to bind to pp60^{c-src}-phosphorylated EGFR with a 10-100-fold greater affinity than to autophosphorylated EGFR, demonstrating that pp60^{c-src} creates new sites for SH2 binding. Furthermore, csk-inactivated pp60^{c-src} was activated in the presence of pp60^{c-src}-phosphorylated EGFR, but not in the presence of autophosphorylated EGFR. Upon treatment of MCF7 or 3 colorectal carcinoma cell lines (WiDr, DLD-1, and LS174T) with EGF, EGFR coimmunoprecipitated with pp60^{c-src}. Similar interactions were observed with the EGFR-related protein, erbB2. These data demonstrate that pp60^{c-src} can phosphorylate receptor tyrosine kinases *in vivo*, creating binding sites for itself as well as other SH2-containing proteins, and may be activated in the process. Overexpression of pp60^{c-src}, EGFR, and/or erbB2 in breast and colorectal tumor cells suggests the potential involvement of these mechanisms in tumorigenesis.

A1-265 ROLE OF SOMATIC GENETIC ALTERATIONS OF THE cAMP PATHWAY IN THYROID HYPERFUNCTIONING TUMORIGENESIS Suárez, H.G.*, Russo, D.**, Wicker, R.*, Du Villard, J.A.*, Filetti, S.**, Caillou, B.***, and Schlumberger, M.***. *: IFC, Lab. de Génétique Moléculaire, 94801, Villejuif, France; **: Cattedra di Endocrinologia, Universita di Reggio Calabria, Cattanzaro, Italy; ***: IGR, 94805, Villejuif, France

It has been well established that in some cells such as thyrocytes, cAMP plays a key role as a second messenger of thyroid stimulating hormone (TSH), in both mitogenesis and differentiation. Tissue hyperplasia and hyperthyroidism are therefore expected to result when activation of the adenylyl cyclase (AC) cAMP cascade is deregulated. Hyperfunctioning thyroid tumors are benign neoplasms characterized by the presence of all differentiation markers, associated with increased and TSH independent proliferation and constitutively enhanced AC actitivity (ACA$^+$), with few or no response to stimulation by TSH, GTP or GTP-homologs (TSH$^-$).

We looked for eventual somatic genetic alterations in different regions of 2 genes participating in the cAMP pathway: the TSH-receptor (TSH-R) (extra-cellular region and loops II and III, C-terminal domains) and the $G\alpha_S$ gene (exons 8 and 9). Thirty seven human thyroid hyperfunctioning adenomas, and 7 differentiated carcinomas characterized for the ACA before and after TSH stimulation and showing a ACA$^+$ and TSH$^-$ phenotype, were studied. We used PCR followed by sequencing and/or hybridization with wild type or mutated synthetic probes, for detection of genetic alterations. The results showed the presence of mutations:

1) in codon 623 of the TSH-R gene in 3/37 hyperfunctioning adenomas and 2/7 differentiated carcinomas, producing a major change (Ala--->Ser) in loop III of the receptor;
2) in exon 8 (codon 201: Arg--->Cys, Ser, Gly, His or Pro) and exon 9 (codon 227: Gln--->His, Glu, Lys) of the $G\alpha_S$ gene, in 9/37 hyperfunctioning adenomas and 3/7 differentiated carcinomas.

The mutant TSH-R (TSH-R*) confers a constitutive activation of AC after transfection in chinese hamster cells.

Our data suggest that in human thyroid hyperfunctioning adenomas and differentiated carcinomas, with a ACA$^+$/TSH$^-$ phenotype:

1) in about 10% of the tumors, the G-protein-coupled TSH-R, may play the role of a proto-oncogene susceptible of constitutive activation by somatic mutations (TSH-R*) and 2) in about 30% of the tumors, a similar role may be played by the $G\alpha_S$ mutated gene (gsp oncogene).

A1-266 Genetic Interactions of *Src*, *Yes* and *Csk* and the Identification of Specific Substrates. S.M. Thomas, A. Imamoto, F.B. Gertler, P. Soriano. Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

Csk negatively regulates Src family kinases by phosphorylating a carboxy-terminal tyrosine residue. Mice homozygous for a disruption of this gene die between embryonic days 9.5 and 10.5 (E9.5 and E10.5) and show a number of abnormalities including neural tube defects (1,2). To assess the effects of *Csk* disruption on tyrosine phosphorylation, cell lines have been derived from wildtype, heterozygous, and mutant embryos. There is a significant increase in the phosphotyrosine levels of certain Src-family substrates in mutant cells as compared to the heterozygous or wild type cells. Specifically, paxillin, cortactin, p120, FAK, and tensin show an elevated level of tyrosine phosphorylation in the mutant cells, while AFAP (p110) shows little or no change. These results are consistent with the hypothesis that deregulation of Src family kinases and the subsequent phosphorylation of these proteins may contribute to the phenotype observed in *Csk*$^-$ mice.

To test this hypothesis crosses were made to generate animals deficient in Src. Analysis of the double mutants indicates that removal of either *Src* or *Yes* allows the embryos to develop further. Biochemical analysis of cell lines derived from these double and triple mutants has identified both Src- and Yes-specific substrates as well as shared substrates. Identification of the sequences required for this specificity is in progress. Consistent with the changes in phosphorylation are alterations in the subcellular localization of some of these proteins. Analysis of whole embryos is in progress.

1. Imamoto, A. and P. Soriano. Cell 73, 1117-1124.
2. Nada et al., Cell 73, 1125-1132.

A1-267 **Signal transduction in fibroblasts that lack the negative Ras regulator p120 RasGap.** Peter van der Geer, Mark Henkemeyer and Tony Pawson. Division of Molecular and Developmental Biology, Samuel Lunenfeld Research Institute, Mount Sinai Hospital 600 University Avenue, Toronto, Ontario M5G 1X5, Canada.

The Ras GTPase activating protein p120 was originally identified as a negative regulator of Ras. p120 RasGap stimulates the intrinsic Ras GTPase activity, thereby down regulating the steady state level of Ras-GTP in the cell. The catalytic domain of p120 RasGap is located at the C-terminus of the protein and is the only region that shows homology with other RasGaps such as NF-1 and Drosophila Gap1. The N-terminal half of p120 RasGap contains two SH2 domains separated by an SH3 domain. SH2 and SH3 domains are not present in other known RasGaps.

It has been suggested that p120 RasGap is not merely a negative regulator of Ras, but might also fulfill effector functions. This possibility was initially fuelled by the observation that RasGap interacts with the effector domain of Ras. A number of studies support this possibility.

To address the function of p120 RasGap we have generated cell lines from knock-out mice that lack p120 RasGap or NF-1 and are currently studying signal transduction in these cells. A progress report will be presented.

A1-268 INTRODUCTION OF A LOSS-OF-FUNCTION POINT MUTATION FROM THE SH3 REGION OF THE *CAENORHABDITIS ELEGANS sem*-5 GENE ACTIVATES THE TRANSFORMING ABILITY OF c-*abl* IN VIVO AND ABOLISHES BINDING OF PROLINE-RICH LIGANDS IN VITRO, R.A. Van Etten, J. Debnath, H. Zhou and J.M. Casasnovas, Center for Blood Research, Departments of Genetics and Pathology, Harvard Medical School, Boston, MA 02115
The Abl tyrosine kinase appears to be inhibited in the cell through its src homology 3 (SH3) region, such that deletions in SH3 or addition of Bcr sequences are necessary for Abl to transform cells. Recently, SH3 domains have been found to specifically bind to proteins containing proline-rich sequence motifs. We have introduced two point mutations from a highly conserved region of the SH3 region of the *sem-5* gene, identified from genetic screens in the nematode *Caenorhabditis elegans*, into the SH3 region of the murine type IV c-*abl* proto-oncogene. In *C. elegans*, both mutations confer decreased *sem-5* function. In c-*abl*, one of the mutations, P131L, activated the ability of *abl* to transform fibroblasts while the other, G128R, did not. The c-*abl* G128R mutant was not activated by addition of a second transforming point mutation in the tyrosine kinase domain. The c-Abl G128R mutant, like wild type c-Abl protein, was localized to the nucleus and the actin cytoskeleton and had normal tyrosine kinase activity *in vitro*, while the transforming c-Abl P131L protein was localized exclusively to cytoplasm and plasma membrane and exhibited somewhat decreased *in vitro* kinase activity. The wild type Abl SH3 domain bound *in vitro* to two target proteins containing proline-rich motifs with dissociation constants of 0.2 and 17 μM, measured by real-time biospecific interaction analysis utilizing surface plasmon resonance. In this assay, the G128R mutant bound to a proline-rich ligand with 50-fold lower affinity than the wild type SH3, while no binding was detected by the P131L mutant. Both point mutations completely abolished binding of the Abl SH3 domain to proline-rich target proteins in a filter-binding assay. These results suggest that the transforming activity of Abl is regulated *in vivo* by an inhibitor protein which associates with the SH3 domain via a proline-rich sequence.

A1-269 **In vivo association between p56 lck and MAP Kinase during IL-2-mediated B Lymphocyte proliferation.** Aimé **VAZQUEZ**, Marie-Thérese **AUFFREDOU** and Joelle **TAIEB**. INSERM U.131, 32 rue des Carnets, 92140 Clamart, FRANCE.

We previously reported that p56 lck expression is upregulated in human B lymphocytes upon mitogenic stimulation. In this report, we characterized the molecules associated in vivo with p56 lck in B cells stimulated with IL-2 for 72 hours. In vitro phosphorylation after p56 lck immunoprecipitation indicates that p56 lck is associated in vivo to the b chain of the IL-2 Receptor and to p42 MAP Kinase as well as a number of other proteins. Moreover, p56-associated MAP Kinase is tyrosine and threonine phosphorylated, suggesting that this MAP Kinase is activated. The association between p56 lck and MAP Kinase is related to S phase since prevention of DNA synthesis with aphidicolin also abrogates this molecular association. Taken together, these data suggest that MAP Kinase is probably directly involved through association with p56 lck, in the control of IL-2-mediated B lymphocyte DNA synthesis.

A1-270 RETROVIRAL-MEDIATED EXPRESSION CLONING OF A NOVEL ONCOGENE WITH STRUCTURAL SIMILARITIES TO THE CDC24 FAMILY OF GUANINE NUCLEOTIDE EXCHANGE FACTORS, Ian Whitehead, Heather Kirk, Robert Kay, Department of Medical Genetics, University of British Columbia and Terry Fox Laboratory, British Columbia Cancer Agency, Vancouver, British Columbia, Canada, V5Z 4E6
In order to identify cDNAs which can induce oncogenic transformation, a retroviral vector was used to transfer a library of cDNAs from the murine 32D hemopoietic cell line into NIH-3T3 fibroblasts. We have identified and recovered a provirus containing a 1.8 kb cDNA (designated TL18-9c1) whose expression causes strong morphological transformation of NIH-3T3 cells. The transforming cDNA contains a complete ORF which encodes a protein with a region of high sequence similarity to the Lbc oncogene. This region includes a domain which is characteristic of the Cdc24 family of guanine nucleotide exchange factors (GEF) in tandem with a pleckstrin homology (PH) domain. The TL18-9c1 encoded protein is distinguished from Lbc by a 150 amino acid N-terminal extension which contains a cysteine- and histidine-rich domain which is very similar to the diacylglycerol (DAG)-binding site found in Protein Kinase C. N-terminal and C-terminal deletion analysis revealed that intact PH and GEF domains are required for oncogenic activity. The TL18-9c1 gene is expressed in a variety of hemopoietic and non-hemopoietic cell lines as well as in brain, spleen and thymus tissue. The protein encoded by TL18-9c1 may be a member of a growing family of exchange factors whose function is to modulate the activity of Ras-like proteins in a developmental or cell-lineage specific manner.

A1-271 INDUCTION OF *PIM-1* PROTO-ONCOGENE EXPRESSION IN T LYMPHOCYTES BY ANTI-CD3 MONOCLONAL ANTIBODY IS DEPENDENT ON PROTEIN KINASE C AND INVOLVES BOTH TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL MECHANISMS, Denise Wingett, and Nancy S. Magnuson, Department of Microbiology, Washington State University, Pullman, WA 99164-4233
We have studied the expression of a delayed-early response gene, *pim-1* in human T cell responses to Ag receptor-generated signals. The *pim-1* proto-oncogene encodes a serine/threonine protein kinase that is expressed primarily in cells of hematopoietic lineage and is implicated in the intracellular signaling processes accompanying lymphocyte activation. In resting T cells, *pim-1* expression is typically low, however, a rapid increase in *pim-1* mRNA levels was observed after receptor crosslinking with anti-CD3 antibodies. We examined the linkage of *pim-1* expression to known signaling pathways generated through the T cell Ag receptor. *pim-1* mRNA was not substantially induced after elevation of intracellular free Ca^{2+}. In contrast, PMA, which directly activates protein kinase C, induced rapid *pim-1* expression. Further, the protein kinase C inhibitors, H-7 and staurosporine, blocked both basal and anti-receptor antibody-induced *pim-1* expression. Thus, T cell Ag receptor-linked *pim-1* expression is likely coupled to the protein kinases C component of transmembrane signaling. Because the activation of protein kinase C has been shown to activate c-Raf kinase activity, the involvement of c-Raf on *pim-1* expression was investigated using human T cells (Jurkat) stably transfected with an inducible c-Raf expression vector. Although the overexpression of c-Raf was shown to cause a substantial increase in IL-2 production, no discernible effects on *pim-1* expression were apparent. In addition, we examined transcriptional and post-transcriptional mechanisms involved in protein kinase C-mediated *pim-1* gene expression and observed that both transcriptional and post-transcriptional mechanisms are coordinately involved in the up-regulation of *pim-1* gene expression following activation of protein kinase C.

A1-272 AN INCOMPLETE PROGRAM OF CELLULAR TYROSINE PHOSPHORYLATIONS INDUCED BY KINASE-DEFECTIVE EGF RECEPTORS

Jacqueline D. Wright, Christoph W. M. Reuter and Michael J. Weber, Department of Pharmacology and Department of Microbiology and Cancer Center, University of Virginia, Charlottesville, VA 22908

Although signaling by the EGF receptor is thought to be dependent on receptor tyrosine kinase activity, several groups have recently shown that MAP kinase can be activated by receptors lacking kinase activity. To analyze the signaling pathways used by kinase-defective receptors, we examined in detail the tyrosine phosphorylations and enzymes of the MAP kinase pathway induced by kinase-defective EGF receptors. We found that ERK2 and ERK1 MAP kinases, as well as MEK1 and MEK2 were all clearly activated upon EGF-stimulation of mouse B82L cells expressing a kinase-defective EGF receptor mutant (K721M). Furthermore, SHC and ErbB2/c-neu became prominently tyrosine phosphorylated and SHC was associated with kinase-defective EGF receptors and Grb2. By contrast, kinase-defective receptors failed to induce detectable phosphorylations of GAP, p62, JAK1 or p91STAT1, all of which were robustly phosphorylated by wild-type receptors. These data demonstrate that kinase-defective receptors induce several protein tyrosine phosphorylations, but that these represent only a sub-set of those seen with wild-type receptors. This suggests that kinase-defective receptors activate a heterologous tyrosine kinase. Since kinase-defective EGF receptors induce substantial phosphorylation of ErbB2/c-neu, heterodimerization with and activation of ErbB2/c-neu is a possible mechanism by which kinase-defective receptors stimulate the MAP kinase pathway.

A1-274 C-erbB-2/*neu* GENE IN METASTASIS: FROM 3T3 CELLS TO HUMAN CANCERS

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The c-erbB-2/*neu* oncogene encodes a 185 kDa transmembrane protein with extensive sequence homology to EGF receptor. Amplification/overexpression of the c-erbB-2/*neu* gene has been shown to correlate with poor prognosis in different types of human cancers. We have recently provided direct experimental evidence that *neu* oncogene expression can enhance the metastatic potential in 3T3 cells by facilitating multiple steps in the metastatic cascade. We have also shown that the adenovirus E1A gene product suppressed *neu*-induced transformation and metastasis in 3T3 cells through repression of the *neu* gene expresssion. In addition, reexpression of p185 in *neu*+E1A cells can counteract the tumor-suppressing function of E1A but not the metastasis-suppressing function of E1A. We and others have previously reported high levels of expression of the c-*erb*B-2/*neu* gene in non small cell lung cancer (NSCLC) cell lines and primary tumors. We have also found that $p185^{neu}$ expression correlated with lymph node metastasis in the squamous cell carcinomas. To investigate the role of c-*erb*B-2/*neu* gene in human lung cancer metastasis, we introduced the human c-*erb*B-2/*neu* gene into the very low $p185^{neu}$ expressing H460 human NSCLC cells and established stable transfectants that expressed increased levels of $p185^{neu}$. Compared to the parental H460 cells, the $p185^{neu}$ overexpressing H460 transfectants produced significantly more pulmonary and extrapulmonary metastatic tumors in nude mice upon tail vein injection. Enhanced tumor metastatic potential *in vivo* was accompanied by an increase of invasiveness and migration *in vitro*, and secretion of basement membrane-degradative enzymes were also enhanced. Moreover, scanning electron microscopy revealed that the $p185^{neu}$ overexpressing H460 transfectants had significantly more microvilli and membrane protrusions than the parental cells which provided a clear structural feature for increased cell motility and invasion. In addition, the $p185^{neu}$ overexpressing H460 transfectants were shown to be more resistant to chemotherapeutic agents Adriamycin, CDDP, and VP-16. These findings support the notion that the c-*erb*B-2/*neu* oncogene play a criticle role in certain human malignancies including NSCLC. Therefore, more aggressive therapy might be beneficial to patients whose tumors express high levels of $p185^{neu}$ and hence may have a higher risk for metastasis and may be more resistant to chemotherapy.

A1-273 ESTROGEN MEDIATED SUPPRESSION OF EGFR EXPRESSION IN ESTROGEN RESPONSIVE BREAST CANCER CELLS

Ronit I. Yarden and Susan A. Chrysogelos, Lombardi Cancer Center, and the Department of Biochemistry and Molecular Biology, Georgetown University, Washington, DC 20007

In breast cancer, over-expression of epidermal growth factor receptor (EGFR; the cellular homolog of the *v*-erbB oncogene) is associated with poor prognosis, loss of estrogen receptor (ER) and failure on endocrine therapy. In culture, estrogen-responsive, ER-positive breast cancer cells MCF-7, T47D and BT474 express low amounts of EGFR mRNA and protein in the continuous presence of estrogen. Upon withdrawal of estrogen, EGFR mRNA (and protein) levels increased 3-7 fold, while cell growth was inhibited. A further 2-3 fold transient increase in EGFR expression was observed within 2 hours following addition of estrogen back to the culture. By 5 hours following estrogen addition, EGFR mRNA was down-regulated back to the level observed in cells maintained in estrogen depleted medium. Down-regulation of EGFR was inhibited in the presence of cycloheximide, indicating a labile protein involvement. Addition of the anti-estrogen, ICI 164,384, to estrogen depleted medium had no further effect on EGFR expression, whereas addition of the anti-estrogens, ICI 164,384 or OH-tamoxifen to cells growing in the presence of estrogen resulted in an increase in EGFR expression similar to that seen upon estrogen withdrawal. Our results show that in the presence of estrogen, ER-positive breast cancer cells possess mechanisms to suppress EGFR expression. Up-regulation of EGFR expression in ER positive breast cancer cells in response to estrogen depletion and growth inhibition could represent an attempt to rescue cell growth. To that end, we are studying the effect of estrogen withdrawal on EGFR phosphorylation and signalling. Long term culture of ER positive breast cancer cells in the absence of estrogen or in the presence of anti-estrogens can result in evolution of the ER negative phenotype. Up-regulation of EGFR may represent an alternative pathway for growth that can contribute to the uncontrolled growth of breast cancer cells and could be a model system to study the development of anti-hormone resistance in response to endocrine therapy.

Cancer: Chromosomes and Translocation; The Suppressors

A1-300 TRANSCRIPTIONAL CONTROL OF THE TUMOR INDUCING GENE Xmrk OF XIPHOPHORUS

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Melanoma formation in the teleost Xiphophorus is caused by a dominant genetic locus, Tu. This locus includes the Xmrk oncogene, which encodes a receptor tyrosine kinase. Tumor induction is suppressed in wild-type fish by a tumor suppressor locus, R. Molecular genetic analyses revealed that the Tu-locus emerged by nonhomologous recombination of the Xmrk proto-oncogene with a previously uncharacterized sequence, D. This event generated an additional copy of Xmrk with a new promoter. Since the oncogene transcript is found exclusively in mela-noma, where its level is positively correlated with the malignancy of the tumors, it appears that overexpression of the Xmrk oncogene is the primary cause for neoplastic transformation. It is suggestive that the newly acquired Xmrk promoter is a target for transcriptional regulation by R.
To identify the regions that are responsible for the tumor specific overexpression of the oncogenic copy of Xmrk, transfectional analyses were carried out with melanoma and nonmelanoma cell lines of Xiphophorus. We could show that a 700 bp promoter fragment confers strong transcriptional activation of a reporter gene in melanoma cells only. This activation is due to the presence of at least one proximal enhancer element. DNA-protein interaction studies revealed several protein binding sites within the promoter, most of which do not correlate with consensus binding sites of known transcription factors. To assess the role of the characterized enhancer and the corresponding transcription factors in the tumor specific activation of Xmrk in situ-footprinting and contact site analyses with partially purified proteins are performed.

A1-301 CONSTRUCTION OF A 450KB COSMID CONTIG FROM HUMAN CHROMOSOME REGION 3P21.3 AND ISOLATION OF CANDIDATE TUMOR SUPPRESSOR GENES FOR LUNG CANCER,

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The first consistent chromosomal change found in a lung cancer was a deletion in chromosome 3p13-24 in small cell lung cancer (SCLC). Subsequently, we and others found that changes in chromosome 3p also occur in non-small cell lung cancer (NSCLC) and other types of cancer, such as inherited and sporadic renal, sporadic breast, ovarian, cervical, testicular and head and neck cancers. Overlapping homozygous deletions in chromosomal region 3p21.3 of three SCLC cell lines, narrowed our study of the area thought to contain a putative tumor suppressor gene. A map of about 1.5Mb has been constructed by pulse field gel analysis and a cosmid contig assembled spanning the shortest region of overlap of 450kb. Fragments of the cosmids have been used by a variety of techniques to isolate at least ten cDNAs. These have been sequenced and put through the Genbank showing that all are new genes, although some have limited homology to previously identified genes. These clones are now being assayed for mutations in lung cancer.

A1-302 CO-OPERATING FACTORS IN EPSTEIN BARR VIRUS NUCLEAR ANTIGEN 1 INDUCED LYMPHOMAS IN TRANSGENIC MICE,

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Epstein Barr Virus (EBV) is associated with a number of human malignancies including endemic Burkitt's Lymphoma. Epstein Barr Nuclear Antigen 1 (EBNA-1) is a DNA binding protein which is essential for latent replication and episomal maintenance of the virus. *In vitro* experimentation has not revealed any oncogenic activity of EBNA-1. However, two different lines of EBNA-1 expressing transgenic mice succumb to B-cell lymphoma, demonstrating the oncogenic properties of the protein *in vivo*. (Wilson and Levine 1992, Curr.Topics.Micro.Immunology Vol 182 pp 375-383).
Characteristic of Burkitt's lymphoma is a translocation involving the *c-myc* gene locus and the immunoglobulin loci. The effect of this is to deregulate *c-myc* expression. We are using a number of strategies to look at the role of *c-myc* in the EBNA-1 induced lymphomas in the transgenic mice. We have crossed mice of both transgenic lines with mice transgenic for deregulated *c-myc* expression. In order to address the action of EBNA-1 on the process of chromosomal translocation, injection of pristane has been used to induce plasmacytomas harbouring *c-myc* translocations in EBNA-1 transgenic mice.
In addition we are investigating whether other factors also contribute to the lymphomas in these transgenic mice.

A1-303 REGULATION OF APOPTOSIS AND DIFFERENTIATION BY *p53* IN F-MuLV INDUCED ERYTHROLEUKEMIA CELL LINES.

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We have previously reported that during the induction of erythroleukemias by Friend Murine leukemia virus (F-MuLV) activation of *Fli-1*, a member of *ets* transcription factor family, is the first detectable genetic change. Mutations within the *p53* gene, an event that has been previously shown to be associated with the tumorigenic progression of Friend virus-induced erythroleukemias, are only detectable in erythropoitin- (Epo) dependent cell lines established from F-MuLV induced primary erythroleukemias. Furthermore, transplantation of these cell lines into mice results in the production of Epo-independent cell lines expressing endogenous Epo. In this study, we reintroduced a temperature sensitive mutant of *p53* (Val135) into CB3, a F-MuLV-induced erythroleukemia cell line lacking endogenous *p53*. The CB3 cells have also acquired a high level of bcl-2 expression and are deficient in hemoglobin synthesis due to the loss of NF-E2/p45, an erythroid specific transcription factor (1)*. When CB3 cells expressing the ts-*p53* are incubated at the permissive temperature of 32.5°C, they become growth arrested in the G_0/G_1 phase of cell cycle. However, resistance to the apoptotic effect of *p53* is observed when Epo was added to the growth medium. Furthermore, the ts-expressing CB3 cells were able to moderate the cell cycle arrest induced by *p53* and proliferate with a slower rate after being at the permissive temperature for several days. Surprisingly, the expression of globin genes was restored upon activation of *p53* at 32.5°C. These results suggest that *p53* can induce erythroid differentiation when apoptosis is blocked by bcl-2 and Epo. Furthermore, we have provided evidence that *p53* can enhance the transcription of globin genes in the absence of NF-E2.

*1. Lu, S., Rowan, S., Bani, M.R., Ben-David, Y. *Proc. Natl. Acad. Sci., USA,* Vol. 91, 1994. In press.

A1-304 Involvement of p53, ras oncogene and loss of chromosome #15 in human skin carcinogensis

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UV-induced skin cancer is one of the most rapidly increasing cancer types in man. However, the genetic basis for its mechanism is still insufficiently understood. So far, mutational inactivation of the p53 tumorsuppressor gene and oncogenic activation of the c-Ha-ras gene are the only two alterations studied in more detail.
We have established an in vitro carcinogenesis model starting with spontaneously immortalized human skin keratinocytes (HaCaT). These cells contain UV-characteristical mutations in both p53 alleles already at very early passages indicating that p53 inactivation was an initial event for immortalization. In addition the cells were monosomic for chromosomes 3p, 4p, and 9p and the role of these chromosomes as carriers of senescence genes is under investigation.
By introduction (transfection and transfer of a chromosome carrying the stably integrated ras oncogene) of the c-Ha-ras oncogene these immortal but nontumorigenic HaCaT cells could be transformed to benign and malignant tumorigenic variants. Since both cell types expressed similar amounts of mutated p21-val the oncogene per se did not determine the tumor phenotype. Moreover, using HaCaT cells of different passages (with different karyotypes) we could show that the genetic background of the recipient cell was a critical determinant for the resulting tumor phenotype and that malignancy correlated with loss of 1 to 2 copies of chromosome #15. This chromosome also proved to be involved in aberrations of human skin carcinomas and transfer of a normal copy of this chromosome into one of these lines resulted in a drastic delay of tumorigenicity. At present we test whether thrombospondin (15q22) may be responsible for this tumor delay. Thus, this study provides the first functional evidence for tumor suppressor activity on chromosome #15 and for its relevance to human skin carcinogensis.

A1-305 IDENTIFYING TARGET GENES TO THE *EWS/FLI* ONCOGENE USING REPRESENTATION DIFFERENCE ANALYSIS (RDA), Benjamin S. Braun, Richard Freiden, Christopher Denny, Department of Pediatrics, UCLA School of Medicine, Los Angeles, CA 90024.
EWS/FLI is a chimeric fusion gene that results from the 11;22 chromosomal translocation that is characteristically found in Ewing's sarcoma and PNET (primitive neuroectodermal tumor). EWS/FLI differs biologically from normal FLI-1: EWS/FLI transforms NIH 3T3 cells, normal FLI-1 does not. Biochemical evidence suggests that EWS/FLI acts as a aberrant transcription factor. RDA is an iterative process of subtractive hybridization followed by PCR amplification that was initially developed to isolate differences in pools of genomic fragments. We have adapted this method to identify cDNA fragments to genes that are differentially expressed between 3T3 cells containing EWS/FLI or FLI-1. RDA subtraction of EWS/FLI by FLI-1 yielded 6 cDNA fragments. Each of these fragments detected unique transcripts that were present in 3T3 cells expressing EWS/FLI but absent in 3T3 cells expressing normal FLI-1. The reciprocal RDA experiment (FLI-1 subtracted with EWS/FLI) yielded 2 cDNAs. As before, both of these fragments detected mRNAs present in FLI-1 3T3 cells but absent in EWS/FLI 3T3 cells. These preliminary experiments demonstrate the utility of RDA for isolating differentially expressed genes. In addition, these data suggest that at least in part, EWS/FLI and normal FLI-1 activate distinct sets of genes.

A1-306 CLONING AND CHARACTERIZATION OF THE MOUSE *RFP* (*RET* FINGER PROTEIN) PROTO-ONCOGENE, Tongyu Cao and Laurence D. Etkin, Department of Molecular Genetics, University of Texas, M.D. Anderson Cancer Center, Houston, TX 77030
An important question in biology is to understand the role of specific gene products in regulating embryogenesis and cellular differentiation. Many of the regulatory proteins possess specific motifs, such as the homeobox, basic helix-loop-helix structure, zinc finger, and leucine zipper. The human rfp protein has two novel zinc fingers (the RING finger and the B box) and a coiled-coil domain, and binds to the nuclear matrix and DNA. Interestingly, a fusion protein between these domains from rfp and the tyrosine kinase domain from c-ret had transforming activity. We are interested in characterizing the *rfp* gene, understanding the function of the conserved domains, and eventually determining the contribution of the rfp domains to the activation of the rfp-ret oncoprotein. We have cloned the mouse *rfp* cDNA, which shares a 98.4% homology with the human sequence. The *rfp* transcripts were detected in developing mouse embryos and differentiating sperm. By using the somatic cell hybrid system, we assigned the *rfp* to mouse chromosome 13 and human chromosome six. By using the yeast two-hybrid system, we showed that the rfp domains found in the rfp-ret oncoprotein formed homodimers. By making mutations in the RING finger, the B box zinc finger, and the coiled-coil domain, we demonstrated that both the B box zinc finger and the coiled-coil domain were required for proper dimerization. The involvement of the B box zinc finger in rfp dimerization is one of the few examples of zinc fingers being involved in protein-protein interaction. We believe that this property is important for the proper functions of rfp during development and differentiation, and is likely to be involved in establishing the oncogenicity of the rfp-ret fusion protein.

A1-307 SUBCELLULAR LOCALIZATION OF THE FES NON-RECEPTOR TYROSINE KINASE AND ITS POTENTIAL ROLE IN CYTOKINE SIGNAL TRANSDUCTION, Anne Carlson, Karen E. Yates and Judith C. Gasson, Departments of Medicine and Biological Chemistry, UCLA School of Medicine, Los Angeles, CA 90024
Fes, a member of the non-receptor tyrosine kinase family, is normally expressed exclusively in hematopoietic progenitors and terminally differentiated myeloid cells. Interestingly, myeloid leukemias as well as numerous other human malignancies also express c-*fes* messenger RNA. Stimulation by granulocyte-macrophage colony-stimulating factor, interleukin 3 (IL-3) or erythropoietin of TF-1 cells, a human erythroleukemic cell line, has been reported to increase Fes tyrosine kinase activity. We have obtained functional data supporting a role for c-Fes in IL-3 and erythropoietin signaling. Although the intracellular site of action of Fes remains unknown, both Fer and FerT (the two other members of the Fes family) have been localized to the nucleus. Our goal was therefore to identify the subcellular localization of Fes in myeloid cells and leukemic cell lines. Biochemical fractionation of neutrophils, TF-1 and HL-60 cells revealed that the Fes protein is localized to the nucleus and plasma membrane. The nuclear localization of Fes was confirmed by immunocytochemical staining of neutrophils. Localization of Fes to the nucleus suggests that one of its functions may be to act as a transcription factor. We investigated the ability of the Fes protein, expressed in transiently transfected COS cells, to bind to DNA-cellulose. The nuclear extract from these cells was applied to a DNA-cellulose column, and Western blot analysis demonstrated that Fes was present predominantly in the column flow-through, whereas an endogenous DNA-binding protein, the cAMP response element binding protein (CREB), bound the column and was eluted under high salt conditions. These results demonstrate that although Fes is present in the nucleus, it does not bind to DNA and may instead interact with other signal transduction proteins within the nucleus. We are currently performing deletion and site-directed mutagenesis of the human c-*fes* gene to identify its nuclear localization domain.

A1-308 RT/PCR DETECTION OF SIL-TAL-1 FUSION mRNA IN CHINESE T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (T-ALL).

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The TAL-1 gene is located on chromosome 1p32. In about 20% of T-cell acute lymphoblastic leukaemias (T-ALL), this gene is disrupted in its 5' portion by a site-specific 100 kb deletion and is fused with the 5' part of the SIL gene, to form SIL-TAL-1 chimeric gene. We have established a "nested" retrotranscriptase/ polymerase chain reaction (RT/PCR) technique which allows to detect the SIL-TAL-1 transcriptional expression. A chimeric mRNA was observed in 4 out of 17 T-ALL cases and has been shown to result from the fusion between the exon 1 of SIL and exon 3 of TAL. Sensitivity test showed that this RT/PCR procedure could detect one leukemic cell among 10^6 normal cells. A positive RT/PCR result was obtained in two cases during clinical remission, suggesting the presence of minimal residual disease (MRD). One patient developed clinical relapse 3 months after PCR positivity. Moreover, analysis of the Tal-1 gene rearrangement by DNA-based PCR in 4 patients with SIL-TAL-1 fusion revealed the type A (Tal^{d1}) rearrangement in all cases. Sequence analysis demonstrated the presence of N region and non-random "P" nucleotide as well as base deletions at the genomic SIL-TAL-1 joining site. Our data indicate that detection of TAL-1 gene abnormality is important for diagnosis and monitoring of MRD in a subset of T-ALL.

A1-309 EFFECTS OF DIMETHYL SULFOXIDE AND RETINOIC ACID ON INDUCING DIFFERENTIATION OF HUMAN OVARIAN CANEER CELL IN VITRO,

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Effects of 1.3% dimethyl sulgvxide (DMSO) and 10μM retinoic acid (RA) on inducing differentiation of COC_1 cell, a cell line derived from human ovarian cancer, was studied. Results of this experiment were as follows:

1. Proliferation of COC_1 cells was significantly inhibited by DMSO and RA at the inhibitory rates of 62.7% and 42.1% respectively after treatment for 5 days .
2. ^3H-TdR incorporation assay showed that DNA synthesis of the cells were significantly inhibited in both groups of DNSO and RA at the inhibitory rates of 60.4% and 37.9% respectirely.
3. Electron microscopy demonstrated that after treatment of the alls with both imducers there was a increase in the number of mitochondria, rough endoplasmic reticulum, microtubules and microfilamants in their cytoplasm while a great decrease in the quantity of free polyribosones was found there also.
4. A immunocytochemical technic revealed a positive staining of transforming growth factor β1 in cytoplasm and plasmic membran of COC_1 cells, but it became weakly positive or negative after treatment of the eells with both inducers. These results suggested that DMSO and RA had ability to change the phenotypic characteristic of COC_1 cell from malignant state to differentiated one. This model might be useful for studying mechanism of inducing differentiation of cancer cells.

A1-310 COAMPLIFICATION OF N-MYC AND CAD GENES AFTER TREATMENT OF REF52 CELLS WITH A LOW CONCENTRATION OF PALA OVERCOMES NONPERMISSIVITY FOR GENE AMPLIFICATION,

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The rat cell line REF52 is not permissive for amplification of the CAD or DHFR genes when selected with 3 x LD_{50} (30 μM) N-(phosphonacetyl)-L-aspartate (PALA) or 50 nM methotrexate (MTX), respectively (frequencies less than 10^{-8}). Pretreatment with a low concentration of PALA (1.0-1.5 x LD_{50}) for 2-3 days before selection with 30 μM PALA allows PALA-resistant colonies to arise at high frequency (10^{-4}), whereas comparable pretreatment with MTX has no effect on permissivity. Pretreatment with PALA followed by MTX selection does not permit DHFR gene amplification. FISH analysis of the PALA-resistant colonies shows that the CAD and N-myc genes, which lie close to each other on rat chromosome 6, are coamplified 2- to 10-fold. N-myc expression is undetectable in unselected REF52 cells, but the PALA-resistant clones express different levels of N-myc. Introduction of a construct constitutively expressing human N-myc into REF52 cells gives PALA-resistant colonies with amplified CAD genes at high frequency (10^{-4}). In a high concentration of PALA, REF52 cells are arrested quickly and effectively, but in a low concentration, some cells divide several times. We propose that starvation for the deoxypyrimidine triphosphate precursors of DNA in the presence of a low concentration of PALA may lead to DNA strand breaks and thus to amplification. Since CAD and N-myc are near each other, they are likely to be coamplified. Thus, in the same cell, increased expression of N-myc leads to permissivity and increased expression of CAD leads to PALA resistance.

A1-311 INTERACTION OF AN INTERFERON-INDUCIBLE PROTEIN WITH THE RETINOBLASTOMA PROTEIN

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Interferons (IFNs) are a family of cytokines with antiviral, immunomodulatory and cell growth regulatory activities. The mechanisms underlying growth inhibition by IFNs are not well established. In IFN-responsive cells, growth regulatory actions of the IFNs are thought to be mediated by IFN-inducible proteins. We have characterized an IFN-inducible 52kD nuclear phosphoprotein, p202, which contains two contiguous repeats of 200 amino acids. Constitutive overexpression of p202 in transfected cells resulted in growth inhibition. The amino acid sequence of p202 contains a motif, LXCXE, reported to be present in several proteins known to bind retinoblastoma protein (pRb).

Prompted by the notion that p202 may contribute to the growth inhibitory actions of IFNs by interacting with pRb, we tested whether the two proteins interact. Our studies revealed that: (i) p202 binds to pRb, both *in vitro* and *in vivo* (ii) the interaction involves direct interaction between the two proteins (iii) p202 binds to the underphosphorylated form of pRb (iv) two nonoverlapping regions in pRb, one in the N-terminal region and the other in the "pocket" region, bind to p202. Transfection of 202 cDNA which resulted in growth inhibition of cells was accompanied by hypophosphorylation of pRb.

A1-312 INTERACTION BETWEEN CONSERVED DOMAINS A AND B IN THE POCKET MOTIF OF RB REGULATES TRANSCRIPTIONAL REPRESSOR ACTIVITY.

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The retinoblastoma protein (Rb) is thought to regulate cellular proliferation by inhibiting expression of cell cycle genes. Initially, Rb was shown to bind to and inactivate members of the E2F family of transcription factors (referred to collectively as E2F), and more recently Rb has been shown to bind to a number of other transcription factors. Previously, we found that, when wild type Rb was expressed in Rb(-) cells, E2F sites switched from enhancers to silencers. These results suggested that the Rb-E2F complex is an active transcriptional repressor. We proposed that repression by the Rb-E2F complex is crucial for inhibiting expression of cell cycle genes that contain enhancers in addition to E2F sites.

Here, we demonstrate that Rb is an active transcriptional repressor. We present evidence that Rb is selectively recruited to promoters through high affinity interactions with E2F, and, that once is concentrated at the promoter through this interaction with E2F, it can interact simultaneously, through lower affinity interactions, with other Rb-binding transcription factors. This interaction with Rb appears to quench the activity of the transcription factors by blocking their interaction with the basal transcription complex. The ability of Rb to interact concurrently with E2F and other transcription factors is the reason for the repressor activity of the Rb-E2F complex.

We examine the region of Rb required for repressor activity. The "small pocket" of Rb (aa 379-792) is sufficient for full repressor activity when Rb is brought to the promoter in an E2F-independent fashion using the DNA binding region of either the yeast protein Gal4 or the bacterial protein Lex A. The Rb pocket is comprised of two domains A and B that are conserved both across species and in the other pocket proteins p107 and p130. A spacer sequence, which is not conserved, separates A and B. We found that the spacer is dispensable, but deletion of either A or B prevents repressor activity. Unexpectedly, we found that A and B have full repressor activity when they are on separate proteins, and we demonstrate both *in vivo* and *in vitro*, that an interaction between A and B drives formation of a pocket motif with both transcriptional repressor and growth suppressor activities. We propose that intramolecular interactions between A and B regulate pocket activity.

A1-313 NONRANDOM CYTOGENETIC CHANGES ACCOMPANY MALIGNANT PROGRESSION IN CLONAL LINES OF ABELSON VIRUS-INFECTED LYMPHOCYTES,

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Lymphoid cells transformed by v-*abl* or *BCR/ABL* oncogenes are, at first, poorly oncogenic, but progress to full transformation over time. While expression of the oncogene is necessary to initiate and maintain transformation, other mechanisms are required for full transformation. To determine whether tumor progression in *ABL*-oncogene-transformed lymphoid cells has a genetic basis, we examined whether malignant progression of transformed clones correlates with particular cytogenetic abnormalities. A modified *in vitro* bone marrow transformation model was used to obtain clonal A-MuLV-transformed pre-B cells that were poorly oncogenic. Multiple sublcones were derived from each clone and maintained over a marrow-derived stromal cell line for several weeks. Related A-MuLV-transformed subclones progressed asynchronously to full transformation. The data show that tumor progression can occur in the absence of detectable cytogenetic changes, but more importantly, that recurring cytogenetic abnormalities appear in highly malignant subclones. Three independent subclones showed deletion of a common region of chromosome 13. Other highly malignant cells carried a common breakpoint in the X chromosome or an additional chromosome 5. These results support the hypothesis that *ABL* oncogenes are sufficient for the initial transformation of cells, but that additional genetic events can drive tumor progression. Furthermore, diverse genetic mechanisms may be able to drive tumor progression in cells transformed with *ABL* oncogenes.

A1-314 CHARACTERIZATION OF NOVEL GENES *SYT* AND *SSX* INVOLVED IN THE t(X;18)(p11.2;q11.2) TRANSLOCATION FOUND IN HUMAN SYNOVIAL SARCOMA.

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Synovial sarcoma, which accounts for approximately 10% of soft tissue sarcomas, occur most commonly in young adults at the extremities in the vicinity of the large joints. Cytogenetic studies have shown that a high proportion of tumours contain a characteristic chromosomal translocation t(X;18)(p11.2;q11.2). By screening a synovial sarcoma cDNA library with a YAC that spans the X breakpoint we have identified a hybrid transcript that contains 5' sequences (designated *SYT*) mapping to chromosome 18 and 3' sequences (designated *SSX*) mapping to chromosome X. A *SYT* probe detected rearrangements in 28/32 synovial sarcomas.

Sequencing of cDNA clones shows that the normal *SYT* gene encodes a protein rich in glutamine, proline and glycine and indicates that in synovial sarcomas rearrangements of the *SYT* gene results in the formation of a SYT-SSX fusion protein. Both *SYT* and *SSX* failed to exhibit significant homology to known gene sequences. The SYT protein contains several regions that were candidate ligands for binding to SH3 and SH2 domains. Notable the C-terminal eight amino acids of SYT that were removed during the formation of SYT-SSX removes one of the consensus sequences for binding to the SH2 domain of the Grb2.

The sequencing of *SYT* and *SSX* has allowed the development of PCR-based techniques that detect the *SYT-SSX* hybrid transcript on a high proportion of synovial sarcomas. This observation provides a new method for detection of this translocation which may be of used in tumour diagnosis. We wish to thank the Cancer Research Campaing for funding this work.

A1-315 THE CANNABINOID RECEPTOR-2 GENE IS LOCATED IN A COMMON VIRUS INTEGRATION SITE IN TWO MOUSE LEUKEMIAS AND AT A BREAKPOINT INVOLVING CHROMOSOME 1p36 IN A HUMAN AML.

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Acute myeloblasic leukemia (AML) results from a neoplastic transformation of immature hematopoietic cells of the granulo/monocytic lineages. In human AML genes that contribute to the development of leukemia are usually localized at chromosomal breakpoints. In murine leukemia transforming genes frequently reside near common viral integration sites (VIS). From a retrovirus induced mouse IL-3 dependent AML we isolated eight VIS. One of those was identified as a common VIS since this locus had also been target for viral DNA integration in another IL-3 dependent murine AML cell line (NFS-78). Using an exontrapping system two genes were identified in this region, i.e. the alpha-L-Fucosidase (Fuc-a) and the Cannabinoid receptor-2 (Cb-2) genes. The latter one, that codes for a G-protein coupled receptor with a yet unknown function is located approximately 3 to 6 kb away from the two virus integrations. In healthy mice this gene is expressed in monocyte/macrophage populations and not in other cells of the hematopoietic lineages (Munroe et al., Nature 1993,vol 365,p61). Northern analysis and RT-PCR are currently carried out to investigate whether this gene is inappropriately expressed in NFS-107 and NFS-78 cells. Chromosomal mapping demonstrated that the Cb-2 as well as the Fuc-a genes are located at mouse chromosome 18 in a region that is homologous to human 1p36. FISH analysis revealed that the CB-2 gene is indeed located at human 1p36, a region that is frequently involved in chromosomal aberrations in human AML. Southern blotting analysis using DNA from a human AML with a complex transformation t(1,17,9,22) involving 1p36, revealed deletions in the CB-2 gene but not in the FUC-A gene. Currently, more cases with a 1p36 abnormality are being investigated. We conclude that the Cb-2 gene is located in a common virus integration site in two mouse leukemias and at the 1p36 breakpoint in a human AML and that this gene may be an oncogene that is involved in leukemic transformation of certain human and mouse AMLs.

A1-316 MOLECULAR CLONING OF A GENE FROM METASTATIC TUMORS INDUCED BY INSERTIONAL MUTAGENESIS, Deng, E.F., Bhatia, P., Mowat M., Greenberg, A.H., and Wright, J.A. Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, MB R3E 0V9

A ras/myc transformed 10T1/2 non-metastatic fibroma cell line was converted to a metastatic fibrosarcoma following infection by moloney murine leukemia virus p-LTR supF. Five clones were isolated from individual metastatic lung tumors. Southern hybridization of DNA from these tumors with a probe from the virus showed distinct patterns of viral integration suggesting that the clones were of independent origin and that metastatic conversion might have been induced through an integration event. We adopted an inverse PCR technique and cloned a genomic DNA fragment flanking the viral insertional site from one of the tumors. This clone, named 2br37, was shown to be preferentially expressed in normal mouse brain tissue by Northern hybridization. In order to determine whether this was transcribed in brain, we PCR amplified a mouse brain cDNA library with nested primers from this clone. Two PCR fragments were selected based on Southern hybridization with 2br37. After cloning and sequencing, these cDNA showed identical sequence with the original 2br37, as well as additional sequence. We have searched Genbank with the complete sequence of the new clones and failed to identify any known homologous gene. A potential polyadenylation signal AATAAA was found in the new clones, which may indicate that this clone is at 3' end of the gene. We are continuing to characterize the gene.

A1-317 DETECTION OF DELETION OF THE TUMOR SUPPRESSOR REGION ON 9P21 IN ACUTE LEUKEMIA BY FLUORESCENCE *IN SITU* HYBRIDIZATION TO INTERPHASE NUCLEI. M.H. Dreyling, O.I. Olopade, S.K. Bohlander. The University of Chicago, Dept. of Hematology/Oncology, Chicago, Il 60637.

Deletions involving chromosomal band 9p21, both microscopic and submicroscopic, have been detected in acute lymphoblastic leukemia and in various solid tumors indicating the presence of an important tumor suppressor gene in this region. Recently, the *CDKN2* gene, an inhibitor of the cyklin-dependent kinase CDK4, was found to be included frequently in the deletions of tumor cell lines, and it has been proposed as a candidate tumor suppressor gene. However, reports about the frequency of point mutations of *CDKN2* in patient material are contradictory. Therefore, it is not certain whether *CDKN2* is the only relevant tumor suppressor gene in this region. Interphase fluorescence *in situ* hybridization (FISH) is a powerful method to detect chromosomal rearrangements including submicroscopic deletions. In addition, FISH is able to detect chromosomal rearrangements in subpopulations of cells. We used a 200 kbp cosmid contig probe which encompassed the tumor suppressor region on 9p21 to analyze 9 leukemia cell lines (6 T-ALL, 1 preB-ALL,1 AML, 1 CML-BC), that had been well characterized by conventional cytogenetics and molecular techniques. Interphase FISH was able to diagnose accurately all deletions of the tumor suppressor region detected by molecular techniques including submicroscopic deletions. 6 of 9 cell lines showed a homozygous deletion of the complete cosmid contig. 1 cell line had a hemizygous deletion which is difficult to detect by molecular techniques. This cosmid contig will be a very reliable probe for interphase FISH of patient material to determine the frequency of 9p deletions in hematologic neoplasias.

A1-318 NUCLEAR LOCALIZATION OF THE t(8;21) AML PROTEIN, AML1/ETO, IN CELLS HAVING CYTOPLASMIC AML1, Paul F. Erickson, Misi Robinson, and Harry A. Drabkin, Department of Medicine, University of Colorado Health Sciences Center, Denver, CO 80262

The nuclear localization of some transcription factors, and hence their functional capability, is known to be influenced by signal transduction pathways. We have molecularly cloned and characterized the t(8;21) AML fusion transcript, *AML1/ETO*, (Erickson et al., 1992, Blood 80:1825; Erickson et al., 1994, Cancer Res. 54:1782). The t(8;21) is characteristically involved in FAB M2 acute myelogenous leukemia (AML) and is the single most common chromosomal translocation associated with AML. We have raised rabbit antisera to a peptides extending from amino acid residues 32 to 50 of AML1 and to residues 528 to 548 near the carboxy terminus of ETO. The former was selected to give antibodies specific to AML1 (this sequence not being present in other transcription factors of the PEBP2alpha family to which AML1 belongs). A third antisera was raised to a bacterially expressed protein corresponding to residues 1 to 220 of ETO, an epitope that lies between the other two in AML1/ETO. The subcellular localization of AML1, ETO, and AML1/ETO has been investigated in the HEL, HL 60, and Kasumi 1 leukemic cell lines, the last from a t(8;21) AML. To date, we have observed immunohistochemical staining with anti-ETO in the nucleus of HEL and Kasumi 1. Anti-AML1 stains the cytoplasm of HEL, the nucleus of HL 60, and both in Kasumi 1. Since the staining in Kasumi 1 could be to the chimeric AML1/ETO or to the individual proteins, we biochemically fractionated its cytoplasmic and nuclear proteins for Western blot analysis. Anti-ETO identified a polypeptide of about 100 kDa in the nucleus, but not in the cytoplasm. Anti-AML1 identified the apparently same 100 kDa nuclear polypeptide and, in addition, a 60 kDa cytoplasmic polypeptide. We interpret our aggregate data to indicate that AML1 is present in the cytoplasm of Kasumi 1 cells and that AML1/ETO is nuclear. Our current working hypothesis is that AML1 may have a cytoplasmic localization in less mature, multipotent cells (e.g., HEL), that it is subject to regulation leading to nuclear localization in more mature cells (e.g., HL 60), and that as a consequence of the t(8;21) the DNA binding portion of AML1 is prematurely transported to the nucleus by virtue of its chimerism with ETO. Investigations with these and additional cells types (e.g., leukocytes from bone marrow) will be carried out to test this hypothesis and to define the phosphorylation state of these proteins.

A1-319 IDENTIFICATION OF A MURINE *polo*-RELATED SERINE/THREONINE PROTEIN KINASE REQUIRED FOR CELL GROWTH, Carol Fode, Benny Motro, Shida Yousefi, Mike Heffernan and James Dennis, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Avenue, Toronto, ON M5G1X5.

We have identified a novel murine serine/threonine kinase, which we have called *sak*, which is most closely related to members of the *polo* subfamily of protein kinases. The *polo* subfamily includes the *Drosophila polo* protein, *Saccharomyces cerevisiae CDC5* kinase, as well as two murine proteins encoded by the *snk* and *plk* genes. The *snk* and *plk* encoded proteins have been implicated as regulators of cell proliferation based on their expression profiles, and the *cdc5* and *polo* mutant phenotypes indicate that these genes are required for the proper formation and function of mitotic and meiotic spindles. We have examined the expression profile of *sak* in adult tissues and during embryogenesis by Northern blot and RNA *in situ* hybridization and have shown a correlation between *sak* expression and mitotic and meiotic cell division. In addition, interfering with *sak* expression by transfection of antisense constructs into Chinese Hamster Ovary (CHO) cells inhibits cell growth as assessed by a colony formation assay. We have generated polyclonal antibodies to the Sak protein and are currently examining the expression and activity of this protein throughout the cell cycle. Identification of the phase of the cell cycle during which Sak functions may reveal a role for this protein in the regulation of microtubule dynamics during spindle formation, similar to the known function of the *polo* and *CDC5* kinases.

A1-320 TISSUE SPECIFIC TRANSCRIPTIONAL REGULATION OF THE EVI-1 PROTO-ONCOGENE,

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The *Evi-1* proto-oncogene encodes a 145kd nuclear Cys_2-His_2 zinc-finger protein containing two zinc finger domains which have distinct DNA recognition sequences. *Evi-1* shows a temporally and spatially restricted pattern of expression in murine embryonic development and is expressed predominantly in kidney, lung and developing oocytes in adult mice. These properties suggests a role for *evi-1* in developmental regulation, at the level of transcription.

To determine the molecular basis for tissue specificity we have analysed the promoter region for DNase 1 hypersensitive sites (DHSS). This analysis has identified a major DHSS site 2.5kb upstream of the transcription initiation site and minor sites around exon 1 of the gene in kidney and lung. Sequencing analysis has identified putative SP1, GATA and ets binding sites. Functional analysis of a 900bp restriction fragment containing the major hypersite region shows a 4 fold enhancement over basal activity of a heterologous minimal tk promoter in L929 cells.

Available kidney cell lines show little or no *evi-1* gene expression. Therefore, to facilitate an investigation of tissue specificity we have taken advantage of the $H2K^b$tsA58 transgenic mouse, containing a tsA58 thermolabile mutant of the SV40 large T antigen, to isolate conditionally immortalised kidney cells. Primary kidney cultures are currently being screened for *evi-1* expression.

Functional studies, footprinting and EMSA analysis are underway to characterise the relevant DNA binding proteins and to assess their functional significance with regard to tissue specific expression of the *evi-1* gene.

A1-321 COMPARATIVE GENOMIC HYBRIDIZATION AS A TOOL TO DETECT NOVEL AMPLIFIED REGIONS IN HUMAN SARCOMAS WITHOUT INVOLVEMENT OF MDM2.

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Comparative Genomic Hybridization has proved to be a powerful tool for detection of genomic imbalances in tumor cells. Gains or losses of chromosomal material can be detected in a single-step in situ hybridization procedure. Tumor DNA and normal DNAs are labeled with different fluorochromes, mixed in defined ratios, and hybridized to normal metaphase spreads. Subsequently, the intensities of the different fluorescent signals along the chromosome can be measured. A change in fluorescent ratio reflects the copy number changes within the original tumor. Besides, CGH allows precise chromosomal localization of the amplified or deleted sequences. We have used this technique to detect novel amplified regions in a large panel of human sarcomas of various histiological subtypes. These tumors were previously examined for amplification of 12q13-14 located oncogenes (MDM2, CDK4 and SAS), and such amplifications were detected in 10% of the tumors. CGH analysis revealed additional amplified regions, most frequently including 1q, 3q, 4q, 6p, 8q, 13q or 15q derived sequences. Interestingly, these amplica were detected also in subgroups without any detectable 12q13-14 amplifications, eg MFH. On the other hand, no gains of chromosomal material could be detected in myxoid liposarcomas with t(12:16), which is in keeping with the fact that this translocation seems to be the sole anomaly of these tumors. - Detailed molecular analysis is required to determine which genes are involved, and to predict which of the amplica may be the most significant.

A1-322 GENETICS OF TUMOR PROGRESSION IN B-CELL NEOPLASM: BCL-3 AT CHR 17q22 - TISSUE SPECIFICITY AND CLONING OF TRANSLOCATION BREAKPOINTS.

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Tumor progression is a multiple step event involving subsequently occurring genetic changes. In B-cell malignancies the t(14:18) translocation involving bcl-2 occurs in low grade B-cell lymphoma, but disease progression is often associated with the overgrowth of a subclone containing a second Burkitt translocation activating c-myc. A new locus bcl-3 at chr 17q22 was cloned by us from a translocation involving myc in a case of aggressive leukemia and clinical studies indicated the specific involvement of bcl-3 in transformed follicular lymphoma. To investigate the role of bcl-3 further we have now studied the expression of bcl-3 and chromosomal abnormalities involving bcl-3 have been cloned. A variety of tumor cell lines including about 35 hematopoietic and 50 solid tumor cell lines were examined for expression of bcl-3. Northern hybridization to the bcl-3 probe detected a 1.7 kb transcript in T-cell, B-cell, erythro-, myeloid and monocytic leukemia cell lines suggesting that the 1.7 kb message is specific for hematopoietic cells. Since the association of bcl-3 to tumor progression had been shown by us, molecular changes of bcl-3 in transformed follicular lymphoma were characterized For all cases rearrangement had occurred in the 3' of bcl-3. The breakpoint region for one of the cases with rearrangement involving bcl-3 was cloned. By screening a genomic library of tumor DNA with the bcl-3 probe clones representing the normal and the rearranged bcl-3 allele were obtained. Sequence analysis of the breakpoint region revealed CA, Alu and Sl repeats a finding consistent with the observed genetic instability of bcl-3. Thus, these data confirm that bcl-3 has specific action in hematopoietic tissue and is subject to genetic alterations based on its nucleotide sequence composition.

A1-323 EVIDENCE IMPLICATING THE TEL GENE AT 12P13 IN MULTIPLE MECHANISMS OF LEUKEMOGENESIS.

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Tel is a novel ets-like protein which contains a helix-loop-helix (HLH) domain and an ets DNA binding domain. Chromosomal translocations and deletions of the *tel* gene contribute significantly to the pathogenesis of human leukemias. Tel was first identified by virtue of the fusion of its HLH domain to the tyrosine kinase domain of the platelet-derived growth factor receptor beta (PDGFRß) in patients with chronic myelomonocytic leukemia and t(5;12) chromosomal translocations. A possible consequence of the fusion was dimerization of PDGFRß mediated by the Tel HLH domain, leading to constitutive receptor activation. In support of this hypothesis, we now demonstrate the constitutive phosphorylation and transforming potential of Tel-PDGFRß in the hematopoietic cell line BaF3. A second example of Tel fusion to a tyrosine kinase was also identified in a patient with acute undifferentiated leukemia and a complex karyotype including a t(9;12;14) translocation. We demonstrate that this translocation results in fusion of the Tel HLH domain to c-Abl. The Tel-Abl fusion is similar to both the Tel-PDGFRß and BCR-Abl fusions, but its role in leukemogenesis remains to be characterized. In contrast, a different mechanism of transformation is predicted by ribonuclease protection assays performed in a patient with acute lymphoblastic leukemia. In this patient, we have demonstrated aberrant expression of a fusion transcript encoding not the Tel HLH domain, but rather its DNA binding domain. This fusion resembles previously reported chimeric proteins involving ets DNA-binding domains, such as the EWS-Fli-1 fusion in Ewing's sarcoma. Finally, several patients have been identified in which one *tel* allele is disrupted by a chromosomal translocation, and the other allele is deleted. Although the functional consequences of these translocations have not been fully elucidated, it is possible that loss of Tel function may contribute to the pathogenesis of some leukemias. Chromosome band 12p13 abnormalities, translocations and deletions, are common in lymphoid and myeloid leukemias. The *tel* gene is involved in the majority of these rearrangements, and may be leukemogenic by more than one mechanism.

A1-324 ECTOPIC EXPRESSION OF PROTO-ONCOGENE RHOMBOTIN-2 CAUSES SELECTIVE PROLIFERATION OF $CD4^-CD8^-$ T CELL SUBSET AND THYMIC TUMORS IN MICE,

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The rhombotin-2 (RBTN-2/TTG-2) locus at chr 11p13 is the most frequent site of chromosomal translocation in childhood T-cell acute lymphoblastic leukemia (ALL). While expressed widely in normal tissues, RBTN-2 is not normally expressed in T-cells. This aberrant expression of RBTN-2 in T-cells is thought to contribute to leukemogenesis in T-cell ALL. To investigate the mechanism of action of RBTN-2 in leukemogenesis we constructed transgenic mice that express RBTN-2 in all tissues, including T-cells. Despite high expression of RBTN-2 in other tissues, these mice developed thymic tumors only with delayed onset of disease (range 37-71 weeks) confirming RBTN-2 as a proto-oncogene specific for T-cell ALL.

Immunophenotype analysis of lymphocytes isolated from transgenic mice prior to tumor development showed that the proportion of $CD4^-CD8^-$ lymphocytes was greatly increased in the thymus of transgenic mice (range 15-86%) compared with control mice (range 3-7%). No differences were seen between transgenic and control mice in the total number of thymocytes but T-cell receptor β-chain gene analysis showed that, in several preleukemic mice, thymocytes population represent monoclonal expansion. Presumably proliferating $CD4^-CD8^-$ clones retain the capability to differentiate into $CD4^+CD8^+$ and mature single positive ($CD3^+CD4^+$ or $CD3^+CD8^+$) T-cells. As determined by immunofluorescence, enforced expression of RBTN-2 in T-cells leads to nuclear localization, consistent with the notion that RBTN-2 acts as a transcriptional regulator.

Our results suggest that RBTN-2 causes selective proliferation of $CD4^-CD8^-$ T cells thereby creating a pool of T-cells, some of which may acquire subsequent changes required for tumorogenesis.

A1-325 A CELLULAR SYSTEM FOR STUDIES OF THE EFFECT OF p53 ON GENE EXPRESSION,

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The tumor suppressor gene p53 is closely involved in the control of cell proliferation. This function of p53 may be mediated by its capacity to positively or negatively regulate the transcription from cellular gene promoters. Indeed, p53 was shown to stimulate transcription from promoters containing p53 binding sites, or to down-regulate the activity of a large set of promoters in cotransfection assays. The latter capacity of p53 to repress promoter activity in many cases may be due to the high amount of p53 used for cotransfection, thus inducing a squelching of transcription factors from the promoters. We propose a cellular system to assess the influence of p53 on gene expression. We chose HeLa cells for stable transfection with a p53 antisense encoding plasmid, because these cells contain a very low level of free wild-type p53 and it is possible to efficiently eliminate this low amount by antisense RNA. This cellular system enabled us to look for genes that could be normally repressed by wild-type p53. The expression of fibronectin was found to be highly up-regulated in the p53 antisense HeLa clones, both at the protein and the mRNA levels. This observation provided an evidence that p53 may be involved, directly or indirectly, in the regulation of fibronectin gene expression *in vivo*. Finally, we used FN promoter-CAT constructs to show that the down-regulation was due to a direct effect of wt p53 on this promoter. Taken together, our data indicate that the effect of p53 on FN expression seems to be direct and to be physiologically relevant.

A1-326 Cytoplasmic pRb-Glucocorticoid Receptor Chimeric Proteins Affect Transcription by Modulating the Subcellular Localization of Transcription Factors: Evidence for Nuclear Co-Translocation of pRb with Transcription Factors

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We developed an inducible system to control the subcellular localization of pRb by fusing a mutant of RB1 with altered nuclear localization signal (NLS) (Zacksenhaus *et al.*, 1993 MCB 13:4588) to the c-terminal portion of the glucocorticoid receptor (GR). The nuclear localization of the chimerical protein, $pRb^{\Delta NLS}$-GR, is hormonally regulated, i.e. it is cytoplasmic in the absence of hormone and nuclear in its presence. Intriguingly, we found that in the absence of hormone cytoplasmic $pRb^{\Delta NLS}$-GR could efficiently suppress transcription of E2F/DP1-regulated promoters. The activity of the cytoplasmic $pRb^{\Delta NLS}$-GR was related to its ability to retain E2F/DP1 in the cytoplasm. These results suggest that one mechanism by which pRb regulates transcription is by sequestering transcription factors (TFs), and that the physical presence of pRb in vicinity of the basal transcription machinery is not critical for this activity. These observations reflect the strong affinity of pRb to bind TFs. We hypothesize a model in which pRb acts to preempt, rather than suppress, ongoing transcription. In this model, pRb interacts with a TF in the cytoplasm and the complex translocates efficiently to the nucleus. Then, the TF binds target DNA but is prevented from affecting transcription since its transcriptional activation domain is masked by pRb. As pRb becomes phosphorylated at the G1/S boundary, pRb is dissociated from the TF allowing transcriptional activation to occur.

A1-327 BIOACTIVITY OF AN NDF/ HEREGULIN SYNTHETIC PEPTIDE IN BREAST CANCER CELLS

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NDF/ heregulin has been reported to be a ligand of Her3 and Her4, which belong to the EGF receptor family.
We have synthesized a cyclic peptide analog(NDF5), which has a 52 amino acid sequence consisting of the EGF-like domain of NDFa2. The cyclic peptide stimulates the tyrosine phosphorylation of Her2, Her3 and Her4 in breast cancer cells while the linear form does not stimulate those receptors. The cyclic peptide competes with full length NDFa2(14-241) for binding to cells which express all these receptors, Her2, Her3 and Her4. The linear peptide does not compete under the same conditions. The results show that the peptide secondary structure is essential for bioactivity. NDF5 induced a morphologic change in some cultured breast cancer cells but not in the line MCF7. When this line was transfected with Her2, and subsequently treated with NDF5, a major morphological change occured. The high constitutive phosphorylation of tyrosine on Her2 in Her2/MCF7 decreased after several days of treatment with NDF5. The mechanism of this Her2 dephosphorylation with NDF treatment is currently under investigation. Thus, the cyclic peptide has been found to mimic certain biological activities of intact native NDF. These results suggest that the cyclic peptide NDF5 might be useful in clinical conditions involving regulation of cell growth.

A1-328 c-Ha-*ras Val 12* EXPRESSION MODIFIES THE CHROMATIN STRUCTURE.

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We show here that in mouse NIH 3T3 fibroblasts the copy-number and expression level of the activated c-Ha-*ras* Val 12 oncogene, which is often involved in the genesis of human malignancies, correlates with the degree of chromatin decondensation, as assessed by micrococcal nuclease (MNase) and DNase I digestions. MNase and DNase I analyses further revealed that the nucleosomal repeat lengths were different in the normal and *ras* oncogene-transformed cells, 162.3 bp and 178.1 bp, respectively. These chromatin changes were accompanied by alterations in the content of histone H1^{o}. Further, by using DNase I as a probe we discovered that serum stimulation of normal and transformed cells synchronized by serum starvation induces rapid reversible changes in the structure of bulk chromatin which may be linked to transcriptional activation. Our data thus indicate that cell transformation by *ras* is associated with specific changes in chromatin structure which make it more vulnerable, and whereby may play an important role during tumour progression in vivo.

A1-329 THE ONCOPROTEIN MDM2 ASSOCIATES WITH THE TATA-BINDING PROTEIN (TBP).

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Mdm2 oncogene amplification renders the mouse fibroblast NIH3T3 cell line tumorigenic. It's human homologue is amplified in tumors such as sarcomas.The oncoprotein binds and inhibits transactivation by wild type p53 . We show that mdm2 interacts with the TATA-Binding Protein (TBP). This interaction maps to the acidic domain of mdm2 (aa 220-273), a domain distinct from the p53 binding domain that we mapped to the N-terminus region of mdm2 (aa 38-118). The interaction requires the C-teminal conserved domain of TBP. Since a complex of the two purified proteins can be immunoprecipitated, they direct contact each other. Mdm2 is also able to interacts with TBP in the presence of TBP Associated Factors (TAFs). They also interact in vivo, in a cell line containing amplified mdm2 (NIH3T3DM), highlghting the importance of this interaction. This suggests that mdm2 has a role in addition to its negative role on p53 transactivation during the process of transformation, the direct control of gene expression.

A1-330 ONCOGENIC RAF-1 SUPPRESSES XENOPUS NEURAL DEVELOPMENT AND CAUSES CELL CYCLE ARREST IN EARLY EMBRYOS.

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We have previously demonstrated that a dominant negative Raf-1 (NAF) inhibited FGF signaling during mesoderm induction in the frog, *Xenopus laevis*.

Microinjection of RNA encoding a constituatively active mutant of Raf-1 (vRaf) resulted in a striking "butthead" phenotype after five days. Anterior pattern formation was disrupted, resulting in tadpoles with little or no head development. Posterior (tail) development was normal. The data further implicate a role for Raf in myogenesis, and suggest that vRaf mimics the action of activated FGF receptors.

In addition to the "butthead" phenotype induced by low dose vRaf RNA injection, high dose vRaf lead to a dramatic phenotype in the early embryo. In the four hours following fertilization, normal embryos undergo approximately 10 cycles of synchronous cell division without cell growth, resulting in blastula stage embryos containing homogeneously sized cells. vRaf injection lead to blastula embryos which contained abnormally large cells interspersed with normal size cells. The large cells were isolated and demonstrated to be cell cycle arrested at metaphase. At the biochemical level, vRaf lead to inappropriate reactivation of both MAP kinase and MAP kinase kinase (MEK). Microinjection of other serine/threonine kinases which did not lead to MAP kinase activation, did not cause cell cycle arrest. We propose that the action of the previously identified cytostatic factor (CSF) is specifically mediated by MAP kinase. These findings raise the possibility that in addition to G_o/G_1 entry, MAP kinase activation may be necessary for G_2/M transition during somatic cell divisions.

A1-331 THE cAMP-DEPENDENT PROTEIN KINASE INHIBITS DIFFERENTIATION OF PC12 CELLS BY NERVE GROWTH FACTOR, v-RAS AND V-RAF.

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Activation of the mitogen activated protein kinase (MAPK) signaling pathway and the proto-oncogene Raf is necessary, in PC12 cells, to effect a mitogenic response to epidermal growth factor (EGF). Interestingly, this same signaling pathway, including Raf activation, is also utilized in the differentiation of PC12 cells to a sympathetic neuron-like phenotype in response to nerve growth factor (NGF). It is unclear how these cells can utilize the same pathway in two opposite processes. Inhibition of this pathway, at the step of Raf activation, has been recently implicated as a mechanism by which cAMP, via the cAMP-dependent serine/threonine protein kinase (PKA), regulates mitogenesis. We report here that transfection of PC12 cells with a gene encoding the catalytic subunit of PKA inhibits NGF-stimulated differentiation and that treatment of PC12 cells with forskolin, to raise intracellular cAMP levels, inhibits NGF-stimulated Raf activation. Transfected PKA also inhibits the differentiation of PC12 cells by the oncogenes *v-ras* and *v-raf.* This latter effect was unexpected, if PKA is acting on Raf, since v-Raf activity was thought to be independent of cellular regulatory processes. These results suggest that regulation of the MAPK signaling pathway is conserved at the level of Raf activity in both mitogenesis and differentiation and that the physiological decision of a cell to divide or differentiate are determined by events subsequent to Raf.

A1-332 IDENTIFICATION OF A NOVEL PROTEIN SEQUENCE MOTIF: THE RAN GTPASE BINDING DOMAIN.

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RAN/TC4, a member of the RAS superfamily of GTPases, is the only known nuclear GTPase and appears to play a key role in the control of the cell cycle. Mutations in RAN that prevent it switching from the GTP- to the GDP-bound state cause cell cycle arrest at either the G1/S or G2/M check points. In intact cells the RAN GTPase is also required for RNA export from the nucleus, and may be involved in nuclear protein import. A deep understanding of RAN function requires the identification of regulatory factors and downstream effectors. We have sought for such targets by interaction cloning and have isolated clones encoding several RAN-binding proteins that interact specifically with the GTP-bound state of RAN. These genes constitute a novel family, which possesses a new, highly conserved protein sequence motif we have termed the **RAN-binding domain** (RanBD). The isolated domain will associate with RAN-GTP. We have constructed a library of randomly mutated RanBDs expressed as GST fusion proteins, and demonstrated that point mutations in conserved residues of the domain dramatically reduce the affinity of the domain for RAN. The RanBD stabilizes the GTP-bound state of RAN. While it has no intrinsic GAP activity, the RanBD behaves as a co-activator of Ran-GAP. A previously uncharacterized gene from the nemotode *C. elegans* possesses an open reading frame encoding two RAN-binding domains. Many members of the family of RAN-binding proteins are located in the cell nucleus. [*NIH grant GM50526*]

A1-333 INTERACTION OF JAK3 AND JAK1 TYROSINE KINASES WITH THE IL4 RECEPTOR COMPLEX.

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Hematopoietin receptors have recently been reported to utilize JAK tyrosine kinases as primary effector proteins. The current investigation was undertaken to identify and characterize IL4 receptor-associated JAK tyrosine kinases. In agreement with recent findings, JAK3 was the predominant tyrosine phosphorylated protein in human T-lymphocytes and myeloid TF-1 cells induced by recombinant human IL4. The tyrosine phosphorylation of JAK3 stimulated by IL4 was time and dose dependent, with peak phosphorylation levels reached at 5 min of stimulation with 100 nM hIL4 at 37°C, and half-maximal tyrosine phosphorylation levels at approximately 1-10 nM. Although JAK3 was the dominant inducibly phosphorylated protein, JAK1 was also phosphorylated but to a lesser extent, as has been observed in response to IL2. On the other hand, JAK2 and TYK2 were not detectably phosphorylated by IL4. JAK3 was associated with the activated IL4 receptor complex as determined by indirect binding to biotinylated IL4, possibly by binding to the common IL2Rγ chain, which also copurified with biotinylated IL4. Moreover, immunoprecipitation of IL2Rγ with a polyclonal antiserum directed to a COOH-terminal peptide sequence coprecipitated JAK3 and JAK1, but not JAK2 or TYK2, from lysates of IL4-stimulated cells, but not from unstimulated cells. Furthermore, anti-JAK immunoassays showed that IL4 stimulated cellular JAK3 and JAK1 activities, and JAK3 was catalytically active in biotin-IL4 receptor complexes. Further studies are directed to mapping the cytoplasmic domains of IL4 receptor components that are critical to JAK activation.

A1-334 TYROSINE PHOSPHORYLATION OF NCK--RELEVANT EVENT OR RED HERRING?

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NCK is a 47 kDa protein which consists almost entirely of three N-terminal SH3 domains and a C-terminal SH2 domain. Having no known catalytic function, NCK thereby falls into the class of so-called "adaptor molecules". When isolated from growing or growth factor-treated resting NIH3T3 cells, NCK is found to be phosphorylated on serine and tyrosine residues. Purified EGF and PDGF receptor preparations as well as baculovirus-produced Src are able to phosphorylate NCK *in vitro* on the same tryptic peptide that is found to be tyrosine-phosphorylated in intact cells. By a variety of techniques, including direct sequencing and site-directed mutagenesis, we have established that Y105 is the primary site of tyrosine phosphorylation in NCK. Y105 lies in a short stretch of amino acids located between the second and third SH3 domains; notably this sequence is not homologous to other SH2/SH3-containing proteins but rather is peculiar to NCK. The tryptic peptide containing Y105 also contains a second tyrosine, Y112, which is phosphorylated in intact cells to a lower stoichiometry than Y105. Does NCK's own SH2 domain bind this tryptic peptide when Y105 is phosphorylated? Our data thus far suggest that this is not the case. This and other attempts to show some biological significance of Y105 phosphorylation will be presented.

A1-335 TRANSCRIPTIONAL REGULATION OF THE HUMAN EGR-1 GENE BY PIXY321 IN A FACTOR-DEPENDENT MYELOID LEUKEMIC CELL LINE,

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The signal transduction pathways activated by PIXY321, a hybrid molecule consisting of GM-CSF and IL-3, are unknown. The biological activity of PIXY321 is greater than the sum of its component parts and is mediated by both the GM-CSF and IL-3 receptors in addition to a unique 100kD cell surface protein. Both GM-CSF and IL-3 rapidly and transiently induce the early response gene, egr-1, in a myeloid leukemic cell line, TF-1. This induction is independent of protein synthesis, and therefore, provides an ideal system for studying signal transduction in reverse, from the nucleus to the plasma membrane. TF-1 cells were transiently transfected with recombinant constructs containing regions of the egr-1 promoter including -600 to -56 nucleotides (nts) linked to the CAT reporter gene, and stimulated with PIXY321. Four-fold induction of CAT activity was observed with the -116 nucleotide (nt) construct which contains the cyclic AMP responsive element (CRE) and serum response element (SRE). A similar induction was observed with the -600 nt construct. This pattern differs from what we have previously observed with GM-CSF and IL-3. Transient transfections are in progress using site-directed and deletion mutants of the CRE and the SRE in the context of surrounding -116 nt promoter sequences. We have begun to identify PIXY321 responsive sequences, characterize the nuclear proteins interacting with these sequences, and determine the post-translational modification of nuclear factors in response to PIXY321. These studies will allow us to explain potentially unique properties of PIXY321 signalling pathways in comparison with GM-CSF and IL-3, thus contributing to the understanding of the regulation of myeloid cell proliferation in response to growth factors.

A1-336 ACTIVATION OF C-MYC EXPRESSION BY HERPES SIMPLEX VIRUS (HSV-1) INFECTION OR BY HSV-1 IMMEDIATE-EARLY (IE) GENE CO-TRANSFECTION, Robert L. Millette*, John Paulson*, Edward DesJardin**, and Shanli Ye*, Department of Biology, Portland State University, Portland, OR 97207* and The Ben May Institute, University of Chicago, Chicago, IL 60637**

We have shown previously that infection by HSV-1 strongly activates c-myc expression in transient assays. Using co-tranfection of HSV-1 IE genes we further demonstrated that this activation is due primarily to the action of the IE protein, ICP4, with ICP0 and 27 providing an additional (3.4-fold) synergystic stimulation.

To delineate the promoter sequences involved in HSV-1 transactivation we examined c-myc promoter deletions and point mutations. Only 108 bp of promoter flanking the major P2 start site ('+66-Myc-CAT', containing E2F and Sp1 sites) were found to be required for maximum transactivation. Deletion of the Sp1 ("CTI2") site had a relatively minor effect, whereas mutation of the E2F site strongly reduced c-myc activation by either HSV-1 superfinfection or IE gene cotransfection. We are investigating possible mechanisms by which ICP4 may activate c-myc through the E2F site. Further deletions into the first exon indicate that also this region plays a role in HSV-1 transactivation. We are presently investigating the role of a YY1 site and other protein binding sites found in this region.

To determine the effect of HSV-1 infection on endogenous c-myc expression, L cells containing integrated copies of wt (wild-type) and mutant +66-Myc-CAT plasmids were infected with HSV-1 and mRNA levels measured by RPA analyses. The wt c-myc mRNA levels were stimulated 14-fold by HSV-1 infection, whereas mutations of the E2F and Sp1 sites resulted in activation levels that were about 55% and 20% of wt levels, resp. We are now investigating the extent of cellular c-myc activation by HSV-1 using nuclear run-offs. These results may provide new insight into the mechanism of gene activation by HSV IE proteins, and the possible role of c-myc activation during the infectious cycle.

A1-337 **PURIFICATION AND CHARACTERIZATION OF A NOVEL CDK-LIKE KINASE FROM SEA STAR OOCYTES,** **Donna L. Morrison, and Steven L. Pelech, Kinetek Site, Biomedical Research Centre, University of British Columbia, Suite 500-520 West 6th Avenue, Vancouver, B.C., V5Z 1A1, Canada**

Cyclin-dependent kinases (Cdk's) are strongly implicated in cell cycle control, primarily at the onset of S and M phases. Using an anti-peptide antibody modelled after human Cdk5, a potentially novel 43 kDa protein kinase has been identified in sea star oocytes. However, screening with other antibodies modelled after cdk5 and other cyclin-dependent kinases, as well as MAP kinases failed to show cross-reactivity with this protein. Through immunoprecipitation studies, this protein was shown to undergo changes in phosphorylation state during oocyte maturation and fertilization. With the pattern of phosphorylation paralleling the regulation of cyclin-dependent kinases, this protein is tyrosine phosphorylated in immature and fertilized sea star oocytes, but becomes tyrosine dephosphorylated during oocyte maturation. This protein appears to be one of the major tyrosine phosphorylated proteins found in immature and fertilized oocytes. Myelin basic protein and histone H1 have been shown to be potential substrates. Using the cdk5-CT antibody as a probe, this p43 cdk-like kinase has been purified to near homogeneity using sequential chromatography over SP-Sepharose, hydroxylapatite, phenyl Sepharose, polylysine Agarose, MonoQ, and Superdex 200. Gel filtration analysis on Superose 12 indicated the enzyme to be a monomer of ~43 kDa. **[Supported by M.R.C. of Canada].**

A1-338 RAF-1 KINASE INTERACTS WITH 14-3-3 PROTEINS. Anthony J. Muslin*, Wendy J. Fantl, Jay A. Martin, Angus M. MacNicol, Richard W. Gross*, and Lewis T. Williams. Daiichi Research Center, CVRI, University of California, San Francisco, 94143. *Washington University, St. Louis, MO, 63110.

Raf-1 is a cytoplasmic protein kinase that participates in mitogenic signal transduction pathways. Activation of Raf-1 requires a direct interaction with the small guanine nucleotide binding protein Ras. In order to identify additional proteins involved in Raf-1 activation a yeast two-hybrid system was employed to screen a human HeLa cell cDNA library. A full length Raf-1/Gal4 fusion protein was employed as a probe. The majority of clones identified encoded two members of the 14-3-3 family of proteins. These proteins bound to the amino-terminal portion of Raf-1. Raf-1 and 14-3-3 protein associated with each other when expressed in Xenopus oocytes. In addition, expression of 14-3-3 protein in oocytes induced meiotic maturation that was blocked by co-expression of dominant-negative Raf-1. These results demonstrate that Raf-1 can physically associate with 14-3-3 proteins in a yeast reporter strain as well as in Xenopus oocytes.

A1-339 ΔFosB-MEDIATED STABILIZATION OF CYCLIN E AND CDK2 mRNA AT THE G_1-S TRANSITION IN RAT-1A CELLS EMERGED FROM G_0 STATE, Shinya Oda, Yusaku Nakabeppu and Mutsuo Sekiguchi, Department of Biochemistry, Medical Institute of Bioregulation, Kyushu University 69, Fukuoka 812, Japan. An immediate-early protein ΔFosB was expressed as a fusion protein with the ligand binding domain of the human estrogen receptor (ER) in Rat-1A cells. After estrogen treatment, ER-ΔFosB accumulates in the nucleus and triggers the cell to emerge from a quiescent state (G_0), transit G_1, initiate DNA replication, and ultimately undergo cell division, at least once (Nakabeppu, Y., S. Oda, and M. Sekiguchi, 1993. Mol. Cell. Biol. 13: 4157-4166). In this system, accumulation of approximately 1,000 molecules of ER- ΔFosB in each nucleus of quiescent Rat-1A cells is sufficient to trigger the transition from G_0 through S phase. To investigate the mechanism by which ΔFosB controls cell proliferation, we measured levels of mRNAs for cell cycle control proteins, cyclins and cyclin-dependent kinases. In serum-deprived quiescent Rat-1A cells, levels of mRNAs for cyclin E and Cdk2 are very low. Levels of the two mRNAs increase 7 to 14-fold prior to DNA replication in estrogen-treated Rat-1A cells that are producing ER-ΔFosB. Nuclear run-on analyses revealed that after estrogen administration there is only a slight increase in transcription of these genes. The mRNAs are fairly stable at the G_1-S transition in these cells, and a similar stabilization of *cdk2* mRNA was observed in serum-stimulated cells.

A1-340 ORNITHINE DECARBOXYLASE : AN ONCOGENE ?

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We have previously shown that ornithine decarboxylase (ODC), the key regulator of polyamine synthesis, shows a transient increase upon stimulation by growth factors, but becomes constitutively activated during cell transformation by different oncogenes, such as c-Ha-*ras*, *neu* and v-*src*. Recently, we have found that overexpression of human ODC in NIH3T3 and Rat-1 fibroblasts induces cell transformation (Auvinen, M., Paasinen, A., Andersson, L.C. and Hölttä, E. Nature 360, 355-358, 1992) and tumour formation in nude mice. Further, we have found that blocking of endogenous ODC with a specific inhibitor or antisense RNA expression abrogates transformation of rat fibroblasts by a temperature-sensitive v-*src* oncogene. These data indicate that the ODC gene is a proto-oncogene central for regulation of cell growth and transformation. The constitutive high expression of ODC did not appear to cause any alteration in the expression of the c-*myc*, c-*fos*, c-*jun* and *junB* proto-oncogenes. Unexpectedly, the ODC-transformed cells displayed an increased tyrosine phosphorylation of a cluster of 130 kDa proteins. We have identified one of the proteins as the previously described p130 substrate of pp60$^{v\text{-}src}$, the structure and function of which is still unknown. Further, we have studied the role of protein tyrosine phosphorylation in the ODC-induced cell transformation by exposing the cells to inhibitors of protein tyrosine kinases, like herbimycin A, genistein and tyrphostins. Treatment with the inhibitors reversed the phenotype of ODC-transformed cells to normal. Coincidently, the tyrosine phosphorylation of pp130 was markedly reduced, while the activity of ODC remained highly elevated. Our data implicate an important role for pp130 in signalling by ODC and imply one or more protein tyrosine kinases further downstream of ODC exerting a critical role in cell transformation.

A1-341 PROPERTIES OF THE CATALYTIC DOMAIN OF Ira2p, A *Saccharomyces cerevisiae* GTPase ACTIVATING PROTEIN

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The *S. cerevisiae* Ira2p protein is a GTPase activating protein (GAP) down regulating the activity of the products of the yeast *RAS1* and *RAS2* genes. The Ira2p catalytic domain of 383 aminoacids (aa: 1644-2026) was produced in *E. coli* as glutathione-S-transferase fusion protein (GST-Ira2p-383) and highly purified (>90 %) by affinity chromatography. The biochemical parameters of the stimulatory activity of Ira2p-383 on the Ras2p GTPase were characterized. The affinity of Ras2p for the GST-fused Ira2p-383 was ~10 μM and the maximal stimulation of the Ras2p GTPase more than 5,000 times. The activity of Ira2p-383 was strongly inhibited by monovalent and divalent salts and was very sensitive to the temperature. It was strictly specific for Ras2p, no stimulatory effect on human c-H-ras p21 GTPase being detectable. The simultaneous presence of the catalytic domains of Ira2p and the yeast GDP/GTP exchange factor Cdc25p made it possible to reconstitute *in vitro* a multi-round Ras2p GDP/GTP cycle. The comparison of the properties of the catalytic domain of Ira2p with those of the homologous domains of mammalian GAP and neurofibromin emphasized the existence of specific differences in the components of the GAP-like family.

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A1-342 DISTINGUISHABLE PROLIFERATIVE PATHWAYS ACTIVATE TRANSCRIPTION OF HUMAN CYCLIN D1 THROUGH DISTINCT PROMOTER REGIONS.

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The cyclin dependent kinases (Cdks) are a family of serine/threonine kinases that play a pivotal role in controlling progression through the cell-cycle. Cyclin D1 is the regulatory subunit of cyclin dependent kinases (Cdks) required for, and capable of shortening, the G_1 phase of the cell-cycle. Several different growth factors and oncogenes promote G_1 phase progression through sequential or parallel pathways. For example, the intracellular transmission of growth factor signals is thought to be mediated by sequential activation of protein kinases including the mitogen activated protein (MAP) kinases. These studies examined the role of the MAPK pathway in regulating CD1 transcription. Progression through the cell-cycle, as well as epidermal growth factor (EGF), induced cyclin D1 promoter activity and protein levels in stable and transient expression assays, concordant with the induction of cellular proliferation. Basal activity of the human cyclin D1 promoter was stimulated by overexpression of p21Ha-ras, mitogen activated protein kinase (p42^{MAPK}), c-jun, ATF-2 and c-Ets-2 proteins. Dominant negative mutants of p42^{MAPK}, p21Ha-ras, and c-Ets-2 proteins reduced basal cyclin D1 activity in normally cycling cells. Distinguishable regulatory elements of the cyclin D1 promoter were identified as the targets for activation by either p21ras/c-jun, or epidermal growth factor, p42^{MAPK},/Ets, and by growth factor-independent cell-cycle progression. Anti-sense ras, MAPKi and EtsLacZ reduced the induction of the cyclin D1 promoter by EGF 60-to 80%. The induction of the proximal -141 bp cyclin D1 promoter by p41^{MAPK}, was reduced or abolished by ets-lacZ, suggesting c-Ets-2 functions downstream of MAPK in the context of the proximal cyclin D1 promoter. The identification of distinguishable mitogenic pathways regulating cyclin D1 transcription provides a mechanism by which distinct proliferative pathways may integrate G_1 phase progression.

A1-343 <u>RAS</u> INDUCED PHOSPHORYLATION OF DROSOPHILA <u>JUN</u> MEDIATES PHOTORECEPTOR DETERMINATION,

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One of the central problems of neural development concerns the mechanisms that restrict and determine cell fate. In R7 photoreceptor differentiation of the Drosophila compound eye, the sevenless (sev) receptor tyrosine kinase (RTK) mediates ras activation, which governs the decision between neuronal and nonneuronal cell fates. In addition to sev, other RTKs activate the ras pathway which is also required for the determination of all other photoreceptors and of many additional cell types throughout Drosophila development. We have recently shown that Drosophila Jun (dm-Jun) is required downstream of the sevenless/ras signaling pathway for neuronal development in the Drosophila eye. Overexpression of wild-type or dominant negative forms of Jun in precursor cells can induce the differentiation of additional photoreceptors, or a dose-dependent loss of photoreceptors in the adult fly, respectively. Importantly, Jun mutants suppress the transformation of nonneuronal cone cells into R7 neurons elicited by constitutively active forms of sev, ras1, raf and rolled MAP kinase (rl^{sem}) (Bohmann et al., (1994) Cell, *78*: in press). Here we present a more detailled study on the function of dm-jun in the ras-signaling cascade. We found that activated form of ras and rl^{sem} strongly enhance the phosphorylation levels of three N-terminally located amino acids of dm-Jun during Drosophila development. Conversely, a fly line carrying a hypomorphic allele of rolled MAP kinase which suppresses the phenotype of activated ras, shows a reduction of the phosphorylation levels of dm-Jun. Consistently, expression of a point mutant of dm-jun that can no longer be phosphorylated during eye-development interferes with photoreceptor differentiation. These results suggest that the ras-dependent phosphorylation by a kinase with the characteristics of rolled is an important signal for photoreceptor differentiation.

A1-344 NET, A NEW *ETS* TRANSCRIPTION FACTOR THAT IS ACTIVATED BY RAS, Alexander Pintzas, Antoine Giovane , Michel Maira, Peter Sobieszczuk and Bohdan Wasylyk, CNRS-LGME, INSERM U-184, Institut de Chimie Biologique, Faculté de Médecine, Strasbourg, France.

Ras signalling is mediated in part by transcription factors that belong to the *ets* gene family. In order to identify downstream targets of the Ras signal transduction pathway, we have used Ras transformed mouse fibroblasts to isolate a new member of the *ets* gene family, *net*. Net has similarity of sequence in three regions with the *ets* factors Elk1 and Sap1, which are implicated in the serum response of the *fos* promoter. Net shares some properties with these proteins, including the ability to bind to *ets* DNA motifs through the Ets domain of the protein and form ternary complexes with the serum response factor SRF on the *fos* serum response element, SRE. However, the pattern of *net* RNA expression in adult mouse tissues is different. The effects of Net on transcription in a number of assays is negative, which is not true for Elk1. Strikingly, Ras, Src and Mos expression switch Net activity to positive. The study of Net should help to understand the interplay between Net and the other members of the Elk subfamily and their contribution to signal transduction through Ras to the nucleus.

A1-345 RAS MEMBRANE LOCALIZATION IS REQUIRED FOR GLUCOSE SIGNALING BUT NOT VIABILITY IN YEAST, Scott Powers[1], Sharmila Bhattacharya[2], Li Chen[1], and James Broach[2] - [1]Onyx Pharmaceuticals, Richmond CA 94806 [2]Department of Molecular Biology, Princeton University, Princeton NJ 08544

Like many Ras proteins, yeast Ras proteins are posttranslationally modified by both prenylation and palmitoylation. These modifications are required for targeting the protein to the cytoplasmic face of the plasma membrane. In mammalian cells, this localization appears to be essential for Ras's role in signal transduction, since signaling through the pathway relies of repartioning the Ras activating protein, Sos, and the Ras effector protein, Raf, to the membrane compartment at which Ras resides as a means of completing the signalling circuit. In contrast, we have found that the postranslational modification of prenylation is critical for yeast Ras biological activity but that membrane localization is not. Yeast Ras proteins that are prenylated but not palmitoylated are not localized to the plasma membrane but function in the cell almost as well as wild type protein. Such mutant proteins fail to induce a transient increase in intracellular cAMP concentration in response to glucose addition, but this deficiency does not yield a marked growth phenotype. These results suggest that the primary role of the farnesyl moiety on yeast Ras is not to insure membrane localization but rather to enhance productive protein-protein interaction between Ras and its downstream target, adenylyl cyclase.

A1-346 **RAF-1 SEQUENCES NECESSARY FOR RAS-RAF INTERACTION AND FOR SIGNAL TRANSDUCTION**, *Kevin Pumiglia, Yu-Hua Chow, John Fabian, Deborah Morrison, Stuart Decker and Richard Jove, Department of Microbiology and Immunology, and Comprehensive Cancer Center, University of Michigan Medical School, Ann Arbor MI 48109.*

Interaction of Ras with the N-terminal half of Raf-1 is postulated to regulate Raf-1 protein kinase and signaling activities. To understand better the molecular interactions between Ras and Raf-1 and regulation of the Raf-1 kinase, a series of deletions was introduced into the N-terminus of full-length, functional Raf-1 kinase and expressed in the baculovirus/Sf9 cell system. An 80 amino acid sequence in Raf-1 between positions 53-132 was found to confer the ability to bind Ras protein *in vitro* and *in vivo*. Deletion of residues 53-132 abolished Raf-1 kinase activation by Ras in insect cells, indicating that activation of the Raf-1 kinase by Ras requires the capacity to interact physically with Ras. In contrast, deletion of this Ras binding site did not diminish activation of Raf-1 kinase by Src, implying that Src and Ras can activate Raf-1 through independent mechanisms. Mutations affecting the zinc finger of Raf-1 did not abolish the ability of Raf-1 to bind Ras or be stimulated by Ras. Consistent with these results, deletion of residues 53-152, but not mutations in the zinc finger motif, abrogated the ability of kinase-inactive, dominant negative Raf-1 to block Ras-mediated signaling in *Xenopus* oocytes. Together, these results provide evidence that the direct physical interaction of Ras with Raf-1 amino acids 53-152 is required for activation of the Raf-1 kinase and signaling activities by Ras but not by Src. Furthermore, the adjacent zinc finger motif in Raf-1 is not essential either for interaction with Ras or for activation of the Raf-1 kinase. Experiments are currently underway to delineate further the requirements of Ras-mediated activation and to identify other potential regulators of Raf-1.

A1-347 DIFFERENTIAL EFFECTS OF THE LEUKEMOGENIC FUSION PROTEINS V-ABL AND BCR-ABL IN ACTIVATION OF MYC AND RAS RESPONSIVE PROMOTER ELEMENTS. Arthur B. Raitano, Robert Mignacca, Kathy Sakamoto and Charles L. Sawyers, Departments of Medicine and Pediatrics, Hematology-Oncology, University of California Los Angeles, CA 90024.

V-ABL and BCR-ABL are oncogenic fusion proteins responsible for murine and human leukemia. Both proteins activate tyrosine kinase signaling pathways which lead to transcription from nuclear target genes. Both proteins require the cytoplasmic signaling molecule RAS and the nuclear transcription factor MYC for cellular transformation. To characterize the signals from v-ABL and BCR-ABL to the nucleus, we have investigated the effects of these oncogenes on RAS and MYC responsive DNA elements in transient transcription assays. In NIH3T3 cells v-ABL, but not BCR-ABL, activated transcriptional reporters containing either a RAS responsive ETS/AP-1 element or 3 repeats of a TPA response element. V-ABL also activated transcription from a reporter containing 4 copies of MYC DNA binding sites, whereas BCR-ABL did not. Similar differential activation results between v-ABL and BCR-ABL were obtained using 600 bp of the promoter for the early growth response gene EGR-1. These results correlate with the ability of v-ABL, but not BCR-ABL, to transform NIH3T3 cells. Because both proteins readily transform bone marrow cells, we compared their ability to activate EGR-1 transcription in this cell type. In contrast to the results in fibroblasts, v-ABL and BCR-ABL activated the EGR-1 promoter to similar levels in the hematopoietic cell line MLA. These findings suggest that the lack of BCR-ABL transforming activity in NIH3T3 fibroblasts may be due to defective signaling to the nucleus.

A1-348 ARACHIDONATE-INDUCED C-JUN EXPRESSION IN STROMAL CELLS STIMULATED BY IL-1 AND TNF: EVIDENCE FOR A TYROSINE KINASE DEPENDENT PATHWAY.
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We have previously demonstrated that GM-CSF gene expression induced by IL-1 plus TNF in the murine stromal cell line, +/+-1.LDA 11, involves activation of phospholipase A_2 (PLA_2). Increased expression of the transcriptional factor, *c-jun*, which is induced in response to the release of arachidonic acid by IL-1 plus TNF- activated PLA_2 may be a key event in the activation of GM-CSF gene expression (*Rizzo et al, Exptl. Hemat. 22:87, 1994*). The present study was undertaken to explore potential mechanisms by which arachidonate induces expression of *c-jun* in +/+-1.LDA 11 cells. Depletion of cellular protein kinase C by pretreatment with TPA (400nM) abolished *c-jun* expression induced by TPA but had no influence on arachidonate-induced expression of the transcriptional factor. In addition, inhibition of PKC by 1 μM calphostin C abrogated TPA - induced *c-jun* expression but had no effect on expression of *c-jun* induced by exogenous arachidonic acid. In contrast, pretreatment of stromal cells with the tyrosine kinase inhibitor genistein decreased, in a time and dose dependent manner, *c-jun* expression induced by 50 μM arachidonic acid. Genistein similarly inhibited *c-jun* expression induced by IL-1 plus TNF. Exposure of stromal cells to arachidonic acid induced a 2.2-fold increase in intracellular tyrosine kinase activity, determined by phosphorylation of the synthetic peptide raytide in the presence of ATP-^{32}P and cellular extract. IL-1 and TNF induced a 1.8 and 2.7-fold increase of intracellular tyrosine kinase activity, respectively. These results are consistent with the hypothesis that IL1 plus TNF induced activation of *c-jun* is mediated by upstream activation of an arachidonate-dependent protein tyrosine kinase. This enzyme potentially exerts its influence in a PKC - independent pathway to transduce information from plasma membrane growth factor receptors to the nucleus, where tyrosine phosphorylated intermediates regulate key processes involved in the regulation of gene transcription.

A1-349 EXPRESSION CLONING OF cDNAs WHICH STIMULATE PDGF B/c-*SIS* PROTO-ONCOGENE EXPRESSION,
Donald F. Robinson, Carsten-Peter Carstens, and William E. Fahl, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706
The c-*sis* proto-oncogene, which encodes the platelet-derived growth factor B-chain (PDGF B), is transcriptionally activated in many human cancers, establishing growth stimulatory autocrine and/or paracrine growth circuits. We have recently identified a *cis*-acting element, the *SIS* proximal element (SPE), which is essential for PDGF B/c-*sis* transcription. Preliminary data indicate that the SPE is bound by at least one previously uncharacterized transcription factor which acts to enable transcription from the PDGF B/c-*sis* promoter. The human hematopoietic stem cell line K-562 has been shown to undergo megakaryocytic differentiation, expressing mRNAs of both PDGF A and B-chains, upon treatment with nanomolar concentrations of TPA. Because of this differentiation-dependent activation of *c-sis* transcription, K-562 cells provide a unique system in which to study the mechanisms by which PDGF expression is regulated. In order to understand the regulated expression of the c-*sis* proto-oncogene, and its contribution to oncogenic transformation, it is necessary to identify genes, the products of which act upstream in the signaling cascade which leads to PDGF B/c-*sis* expression. We have devised an expression cloning strategy to isolate cDNAs whose products participate at any stage in the differentiation pathway leading to PDGF B/ c-*sis* activation in K-562 cells. Splicing by Overlap Extension PCR was used to construct a recombinant PDGF B/c-*sis* promoter (shown to faithfully reproduce TPA induced transcription of PDGF B/c-*sis* in K-562 cells) linked to CD4 transmembrane protein cDNA engineered to contain a carboxyl terminal truncation. Also within the expression library vector are the Epstein-Barr Virus (EBV) origin of replication-P allowing extra-chromosomal replication in K-562 cells expressing EBV nuclear antigen-1, and the CMVie promoter driving expression of juxtaposed library cDNAs. Expression of a K-562 cDNA protein product that stimulates expression from the PDGF B/c-*sis* promoter results in CD4 expression on the host cell surface providing a rapid means to select positive cells harboring PDGF B/c-sis activating clones.
Here we present our results on the construction and screening of an expression library of TPA-inducible cDNAs from K-562 cells.

A1-350 PHOSPHATIDYL INOSITOL 3' KINASE AS AN EFFECTOR OF RAS.
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Phospatidyl inositol 3' kinase (PI3K) is a lipid kinase that phosphorylates phosphoinositides at the 3' position. It is activated in response to a multitude of growth factors, but its function is not yet known, although a role in membrane ruffling has been suggested. The Ras GTPases are at a critical crossroad in the signalling pathways regulating cell growth and diffentiation, downstream of tyrosine kinases and upstream of the Raf ser/thr kinases. We have shown , both in vitro and in vivo, that Ras can directly regulate PI3K, providing a point of divergence in the signalling cascade downstream of Ras. Our latest results will be discussed.

A1-351 D-TYPE CYCLINS RESCUE MITOGENIC SIGNALING BY A CSF-1 RECEPTOR DEFECTIVE IN *MYC* INDUCTION.
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Mouse NIH-3T3 fibroblasts expressing a mutant colony-stimulating factor-1 receptor (CSF-1R 809F) defective in inducing c-*myc*, but not c-*fos*, *c-jun*, and *jun*B, do not proliferate in serum-free medium containing purified recombinant colony-stimulating factor-1 (CSF-1) but are rendered fully responsive to CSF-1 after c-*myc* is ectopically expressed. Transduction of either of three D-type cyclin genes, but not cyclin D mutants defective in their binding to the retinoblastoma protein (pRB), rescued the mitogenicity of cells bearing the mutant CSF-1 receptor without restoring c-*myc* induction. Microinjection of antibodies to cyclin D1 into either c-*myc*- or cyclin D1-rescued cells prevented their entry into S phase, indicating that the action of c-*myc* during G1 phase normally requires cyclin D function. Antisense oligonucleotides to c-*myc* blocked CSF-1 induced S phase entry in cells expressing wild-type CSF-1 receptors but were without effect in cyclin D1-rescued cells expressing CSF-1R 809F. Therefore, D-type cyclins act downstream of c-*myc* during the mitogenic response to CSF-1 and can bypass those c-*myc* functions that are normally required for G1 progression.

A1-352 GENISTEIN AND STAUROSPORINE BLOCK PROLACTIN-INDUCED TYROSINE PHOSPHORYLATION OF SHC WITHOUT INHIBITING JAK2: EVIDENCE FOR SHC PHOSPHORYLATION BY A DOWNSTREAM TYROSINE KINASE.

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We and others have previously shown that prolactin (PRL) induces rapid activation of receptor-associated JAK2 in the PRL-dependent T-cell line Nb2. Moreover, coupling of PRL receptors (PRLRs) to Raf-1 activation has been reported, suggesting that the Ras-Raf-1 pathway at least partially mediates the proliferative signal. One potential receptor-proximal component of this signaling pathway is Shc, an SH2-domain protein which upon tyrosine phosphorylation couples growth factor receptors to Ras via Grb2/Sos. Here, we show that PRL indeed induced tyrosine phosphorylation of Shc and its association to Grb2. However, the phosphorylation kinetics of Shc was distinctly slower than that observed for JAK2, PRLR and a 78 kDa JAK2-associated protein temporarily designated p78. Shc was phosphorylated maximally at 15 min of PRL stimulation, as opposed to 1-3 min for JAK2, PRLR and p78. Several tyrosine kinase inhibitors, including genistein, staurosporine, lavendustin, tyrphostin and geldanamycin, failed to suppress PRL-induced JAK2 autophosphorylation in Nb2 cells, despite preincubation of cells with inhibitors for up to 60 min at high concentrations before exposure to PRL. However, of these inhibitors staurosporine and genistein completely blocked Shc tyrosine phosphorylation, suggesting that Shc is primarily phosphorylated by one or more tyrosine kinases acting downstream of JAK2. On the other hand, PRL-induced tyrosine phosphorylation of p78 was insensitive to all inhibitors tested, indicating that p78 may be a substrate of JAK2. Ongoing studies seek to identify p78 and the tyrosine kinase(s) responsible for Shc phosphorylation.

A1-353 THE SIGNALING PATHWAY COUPLING EPIDERMAL GROWTH FACTOR RECEPTORS TO ACTIVATION OF RAS,

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Epidermal growth factor (EGF) treatment causes autophosphorylation of the epidermal growth factor receptor (EGFR) leading to increased guanine nucleotide exchange factor (GEF; Sos) activity and enhanced formation of p21*ras*-GTP. The connection of the EGFR to p21*ras* activation can occur through binding of Grb2•Sos complexes to the EGFR or through the adaptor protein Shc via EGFR•Shc•Grb2•Sos multimeric complexes. Therefore, we investigated the relative contributions of the EGFR•Grb2•Sos or EGFR•Shc•Grb2•Sos pathways in mediating EGF induced GEF (Sos) activation of p21ras leading to DNA synthesis in Rat1 fibroblasts and NR6 cells overexpressing wild-type human EGFRs. EGF treatment led to rapid tyrosine phosphorylation of Shc. Although phosphorylated EGFR can bind to both Shc and Grb2, the predominant linkage was observed between EGFR and Shc. Similarly, quantitatively more Grb2 was associated with Shc than with EGFR after EGF stimulation. Immunoprecipitation of Shc from EGF-stimulated cells removed almost all EGFR-associated Grb2. In addition, a much greater amount of Grb2 was precipitated by anti-Shc antibody even when the samples were first immunoprecipitated by anti-EGFR antibody. EGF increased by 2.3-fold the ability of membrane fractions to enhance *ras* guanine nucleotide dissociation. Immunodepletion of Shc proteins from membrane fractions of EGF-stimulated cells removed 93% of the *ras* GEF activity, whereas precipitation of EGFR had only a small effect on *ras* GEF activity. Furthermore, to examine the functional role of Shc, we have conducted single cell microinjection studies. Microinjection of anti-Shc antibody or Shc SH2 GST fusion proteins into living Rat1 fibroblasts and NR6 cells overexpressing human EGFRs inhibited EGF induced DNA synthesis by about 80%. Taken together, these data indicate that coupling of Shc provides the major pathway linking activated EGFRs to Grb2•Sos and stimulation of the p21*ras* pathway leading to cell cycle progression.

A1-354 INHIBITION OF CELL GROWTH BY TGFß1 IS ASSOCIATED WITH INHIBITION OF THE IMMEDIATE EARLY GENE N-MYC IN BALB/MK CELLS,

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N-myc was originally identified as an amplified gene in neuroblastoma cells that was found to be structurally similar to the c-myc proto-oncogene. During early development N-myc is expressed in a variety of proliferating epithelial tissues in the embryo including skin, and the expression of N-myc has been shown to be essential for normal development. We have shown that the non-transformed cell line Balb/MK cells, originally derived from mouse skin keratinocytes, expresses N-myc mRNA at levels that are at least 40-fold higher than in primary keratinocytes isolated from newborn mice. N-myc is present at low levels in quiescent MK cells, and the pattern of expression following mitogenic stimulation is biphasic beginning with a rapid spike. A second wave of expression is seen beginning in mid G1 and continuing through early S phase. The rapid spike in expression following mitogenic stimulation does not require new protein synthesis, thus N-myc behaves as an immediate early gene. TGFß1 rapidly inhibits N-myc mRNA expression in both synchronized and unsynchronized rapidly proliferating MK cells, providing further evidence that N-myc may be a target of TGFß1 in normal developing epithelial tissues. We have also shown that TGFß1 does not significantly alter the mRNA levels of Max, Mad, or B-myc in MK cells, while L-myc expression is not detected. Studies are currently in progress to determine if the inhibition of N-myc expression by TGFß1 is due to transcriptional repression, as has been reported with c-myc, and whether N-myc expression is required for MK cell proliferation.

A1-355 STUDIES ON THE ACTIVATION OF THE MAPKINASE PATHWAY BY PROTEIN KINASE C

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Treatment of mammalian cells with the phorbol ester TPA leads to rapid activation of p42 MAPK, measurable by mobility shift of the kinase on polyacrylamide gels and in vitro kinase assays. This effect is believed to be mediated by PKC that phosphorylates and activates Raf which is responsible for activating MAPK via MEK.

Coexpression studies (Sf9 cells) and reconstitution studies in vitro have failed to provide a role for the direct action of PKC on Raf in the activation of its MEK kinase activity. It is thus likely that other mechanisms are responsible for PKC control of Raf activity. In order to substantiate this conclusion and to dissect the nature of the control processes involved, further studies have been carried out in mammalian cells involving specific PKC isotype expression and analysis of TPA-induced MAPKinase activation. The in vivo and in vitro studies will be presented.

A1-356 **IDENTIFICATION OF TRANSCRIPTION FACTORS INTERACTING WITH C-ETS-1.**
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Several lines of evidence suggest that the c-ETS-1-protein is regulated by an internal repression mechanism, to which domains both upstream and downstream of the DNA binding domain contribute. The domain encoded by exon VII of the chicken c-ETS-1p^{68} appears to be important in this mechanism since its deletion relieves repression and a natural splice variant exists that is devoid of this exon. To identify potential interaction partners of c-ETS-1 we have performed an interaction screen in *S.cerevisiae*. We used a transactivation defective deletion construct of c-ETS-1 (consisting of exon VII and the DNA binding domain) bound to ets binding sites as a "bait". From a screen of an avian fibroblast cDNA library we have identified three transcription factors, two of which belong to the b-HLH-zip family and which were isolated twice as independent clones. In transient transactivation assays in avian fibroblasts these clones synergize with c-*ets*-1. The third clone encodes a new member of the AP-1 family and inhibits c-*ets*-1 transactivation. Furthermore it is expressed very specifically in monocytic cells of the chicken hematopoietic system. We are currently investigating whether expression of this clone in E26 transformed multipotent progenitor cells can also relieve the differentiation block imposed by the v-*ets* oncogene by inducing their differentiation towards monocytes.

A1-357 *IN SITU* HYBRIDISATION ANALYSIS OF THE C-*MYB* PROTO-ONCOGENE RELATED GENES A-*MYB* AND B-*MYB* IN THE MOUSE,
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The protooncogene c-*myb* encodes a transcriptional activator whose function is restricted to the precursor cells of all hematopoietic lineages. Two*myb*-related genes, referred to as A-*myb* and B-*myb*, have been identified recently and it has been suggested that these *myb*-related genes might perform c-*myb* like functions outside of the hematopoietic system. To better understand the function of these *myb*-related genes, we have investigated their expression during embryogenesis and in adult mouse tissues using *in situ* hybridisation analysis. During embryogenesis A-*myb* expression is predominantly restricted to several regions of the developing central nervous system (CNS) where it coincides with the presence of proliferating immature neuronal precursor cells and to the urogenital ridge. In the adult mouse, A-*myb* is expressed during the early stages of sperm cell differentiation and in B-lymphocytes located in germinal centers of the spleen. In contrast to A-*myb* the B-*myb* gene is broadly expressed during all stages of embryonic development, where its expression is strongly correlated to still undifferentiated, proliferating regions. B-*myb* is also expressed in many adult tissues but not in organs containing mostly non-proliferating cells, such as liver and brain. Taken together, these results suggest a link between B-*myb* expression and proliferation (and/or differentiation) of many, if not all cell types and a comparable role also for A-*myb* but restricted to neurogenic, spermatogenic and B-lymphoid cells .

A1-358 C-MYB TRANSACTIVATION AND FINE TUNING OF THE PR264 / SC35 SPLICING FACTOR EXPRESSION IN HEMATOPOIETIC CELLS.
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We are interested in identifying c-myb target genes whose products are involved in the control of proliferation and differentiation processes in hematopoietic cells. We have previously reported that the expression of a subset of c-myb mRNAs requires the intermolecular recombination of coding sequences (ET and c-myb) localized on different chromosomes both in chicken and human. In the two species, we have established that the ET locus is submitted to bidirectional transcription and that the antisense mRNAs encode the PR264/SC35 essential splicing factor. Since it has been reported that PR264/SC35 regulates alternative splicing in a concentration-dependent way, we have analyzed the mechanisms involved in the control of the PR264 expression. Our results indicate that the transcription of the PR264 gene is modulated both in vitro and in vivo by different c-myb products, showing thereby that it constitutes a new target of the c-myb proto-oncogene. We have also established that the PR264/SC35 splicing factor is encoded by several mRNAs which only differ in their 3' untranslated sequences and exhibit different stabilities. Furthermore, in situ hybridization experiments performed on hematopoietic cell lines show that both ET and PR264 mRNAs are expressed in the same cell. In order to determine the contribution of the bidirectional transcription to the regulation of the PR264 expression, we have undertaken the characterization of the sense mRNA species containing ET sequences. Results concerning the structure of the ET messengers and their relationship with PR264 antisense mRNAs will also be presented.

Taken together, our observations strongly suggest that the PR264 expression is regulated in a tight and subtle way by i) the transactivation of the PR264/SC35 promoter by c-myb proteins ii) the sequence of the PR264 mRNA 3' untranslated region and iii) the bidirectional transcription of the ET locus which could lead to either the formation of unstable RNA-RNA duplexes or to transcriptional attenuation.

In connection with the control of c-myb expression in hematopoietic cells the potential role of the PR264/SC35 alternative splicing factor in the processing of genes specifically involved in hematopoiesis will be discussed.

A1-359 **Identification of RalGDS as a Putative Effector Molecule of R-ras, H-ras, K-ras and Rap**
Marcel Spaargaren*, Frank McCormick, and James R. Bischoff; ONYX Pharmaceuticals, Richmond CA, USA. *Present address: Laboratory for Physiological Chemistry, Utrecht University, Utrecht, the Netherlands.
R-ras is a member of the *ras*-family of small GTPases with 55% sequence identity to H-ras. We have recently shown that R-ras associates with the apoptosis suppresssing proto-oncogene product Bcl-2, suggesting a role for R-ras in the regulation of apoptosis. To identify other proteins that bind to R-ras we performed a yeast two-hybrid cDNA library screen. Several clones were obtained encoding the C-terminal region of the exchange factor for Ral (RalGDS). This R-ras-binding domain of RalGDS (RalGDS-RBD) is distinct from the conserved catalytic exchange factor regions. Using the two-hybrid system, RalGDS-RBD was also shown to interact with H-ras, K-ras, and Rap, and with active but not with inactive point mutants of the Ras-like GTPases. Moreover, using purified proteins we demonstrate the direct GTP-dependent interaction of the Ras-like GTPases with RalGDS-RBD and full length RalGDS *in vitro*. Furthermore, we show that RalGDS-RBD and the Ras-binding domain of Raf-1 compete for binding to the Ras-like GTPases. These data indicate that RalGDS is a putative effector molecule for R-ras, H-ras, K-ras, and Rap, thereby supporting the notion that these Ras-like GTPases do not solely exert their activity by modulation of the activity of the Raf-1 kinase, and suggesting the involvement of RalGDS and Ral in their downstream signaling. We are currently investigating the biological function of RalGDS and Ral with respect to signal transduction by these Ras-like GTPases.
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A1-360 ACTIVATION OF MAP KINASE BY TPA: DIFFERENTIAL SIGNALLING BY PROTEIN KINASE C-ISOENZYMES VIA C-RAF.

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Short term treatment of mammalian cells with a tumor promoting phorbol ester (PMA/TPA) can trigger an oncogenic signalling pathway by activating the c-Raf kinase and the MAP kinases ERK1 and ERK2. We have investigated the mechanism of TPA activation of MAP kinase and we have shown that this activation proceeds via the protein kinases c-Raf and MEK in the presence of classic PKC isoenzymes α, β or γ (1).

Overexpression of the novel PKC-δ or PKC-ε isoenzymes in mammalian cells leads to growth arrest or to oncogenic transformation, respectively (2, 3, 4). We present evidence that these PKC enzymes differ significantly in their signalling mechanism from the classic PKCs as well as in their interaction with the Raf protein kinase.

(1) Marquardt, B. et al., *Oncogene* 9, (in press)
(2) Watanabe, T. et al., *Proc. Natl. Acad. Sci. USA*, **89**, 10259-10163 (1992)
(3) Cacace, A.M. et al., *Oncogene* 8, 2095-2104, (1993)
(4) Mischak, H. et al., *J. Biol. Chem.* **268**, 6090-6096, (1993)

A1-361 **INTRACELLULAR EXPRESSION OF A SINGLE CHAIN DERIVATIVE OF A c-Raf SPECIFIC MONOCLONAL ANTIBODY.** Elisabeth J. Stöcklin, Manuela Wissler, Winfried Wels and Bernd Groner, Institute for Experimental Cancer Research, Tumor Biology Center, Freiburg, Germany.

The c-Raf serine/threonine protein kinase plays a central role in mitogenic signal transduction pathways. c-Raf-1 is a protein of 74 kDa. It is activated by mitogenic agents like growth factors, serum and phorbol esters. It links the receptor tyrosine kinases and c-ras to the cytoplasmic protein kinase cascade. Interference with the function of the c-raf-1 kinase could yield insights into the signal transduction pathways and could possibly serve as a cytostatic strategy. We have used the intracellular expression of a single chain antibody (scFv) directed against c-Raf-1 to achieve this aim. The scFvs were derived from the mRNA of hybridoma cells expressing the monoclonal antibodies PBBJ and URP26-3S by a combination of RT and PCR. They are composed of the heavy and light chain variable domains connected by a flexible peptide linker. Both scFvs were expressed in bacteria and purified by affinity chromatography. The purified scFv bind to the raf protein in vitro. The scFv cDNA was introduced into NIH3T3 cells under the control of the inducible MMTV-LTR. Quiescent cells were stimulated with PDGF and the phosphorylation of c-raf-1 was found to be inhibited in scFv expressing cells. The influence of scFv expression on the c-Raf-1 kinase activity, MEK, MAPK phosphorylation and activity and c-fos gene induction were measured.

A1-362 **THE TISSUE DISTRIBUTION OF A NOVEL GENE DIFFERENTIALLY EXPRESSED IN ESOPHAGEAL CANCER** Hiroyuki Suzuki, John M. Abraham, and Stephen J. Meltzer, University of Maryland / Veterans Affairs Hospitals, Baltimore, MD 21201

In order to gain insight into the mechanism of esophageal tumorigenesis, genes differentially expressed in esophageal normal versus tumor messenger RNA were cloned. The location of one novel differentially expressed gene, named *JMA-1*, was determined by the *in situ* hybridization technique. An anti-sense RNA probe transcribed from this novel gene sequence was labeled with digoxigenin-11-dUTP and then hybridized to messenger RNA within a paraffin embedded section. We confirmed the gene was expressed only in esophageal, oral and cervical epithelium, but not near the most basal layers. Fluorescence *in situ* hybridization (FISH) refined the mapping of this gene to 1q21-22. Portions of its predicted protein (MW=18,162Da) sequence are highly homologous to the *jun*/AP-1 transcription factor. It also contains a unique and provocative 8-amino-acid repeating unit suggesting a highly organized structure. These results suggest that *JMA-1* is associated with epithelial differentiation in the esophagus. We are currently screening a genomic library to isolate the promotor of this gene. This should eventually lead to analysis of the activator and/or repressor factors that regulate the dramatic decrease in expression of this gene in the malignant state.

A1-363 REGULATION OF HUMAN DNA-DEPENDENT PROTEIN KINASE, Hirobumi Teraoka, Fumiaki Watanabe, Kinji Tsukada and Masayuki Nagasawa*, Department of Pathological Biochemistry, Medical Research Institute, Tokyo Medical and Dental University, Tokyo 101, Japan; *National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206

DNA-dependent protein kinase (DNA-PK) composed of a catalytic component (p350) and a DNA end-binding component, Ku protein (p70/p80), catalyzes phosphorylation of a variety of nuclear proteins including p53, SV40 T antigen, Sp1, Myc and TFIIB. However, little is known about regulation of expression of DNA-PK and control mechanism of the enzyme activity.

We have investigated regulation of human DNA-PK and obtained following results: (i) In resting human peripheral lymphocytes, no significant activity of DNA-PK was detectable, but drastic appearance of the activity was observed 48-72 h after stimulation with PHA, a T-cell specific mitogen. Cyclosporin A reduced the DNA-PK activity as well as T-cell proliferation. In contrast, the activity level of DNA-PK in Raji Burkitt's lymphoma cells arrested at Go/G1 by treatment with 1.5% DMSO for 4 days was 2-fold higher than that in actively growing cells, and further increased after release from DMSO, suggesting that regulation of DNA-PK is anomalous in Raji cells; (ii) HMG1, HMG2 and the DNA-binding domain enhanced autophosphorylation of p350, DNA-PK activity and DNA-binding ability of DNA-PK. Non-histone chromosomal proteins HMG 1 and 2 may contribute to phosphorylation of DNA replication/transcription factors by DNA-PK on chromosomal DNA; (iii) When DNA-PK with or without DNA was incubated with PKC, casein kinase II, cdk2/cyclin A and MAP kinase in phosphorylation reaction mixtures, no additional phosphorylation of p350 over the autophosphorylation was observed.

A1-364 ACTIVATION OF THE MAP KINASE CASCADE CORRELATES WITH v-mos TRANSFORMATION IN BOTH mos-RESISTANT REVERTANTS AND ts-TRANSFORMED CELLS, L. Z. Topol[1], M. Marx[2], G. Calothy[2] and D. G. Blair[1], [1]Laboratory of Molecular Oncology, National Cancer Institute, Frederick, MD 21702-1201; [2]Institut Curie-Biologie, Centre Universitaire, Orsay, France.

We have characterized MAP kinase cascade activation in response to transformation by the v-mos oncogene. Transformation of immortalized rat embryo fibroblasts by v-mos constitutively activates both MAP kinase and MAP kinase kinase (MKK), while cells transformed by a ts isolate of Mo-MuSV (6m2ts110) show elevated levels of MAP kinase activity only at the permissive temperature. Following a shift of ts transformed cells from the non-permissive to the permissive temperature, MAP kinase and MKK are activated concurrently with the reappearance of active mos kinase. We also examined c-raf-1 phosphorylation and its activity in vitro in ts-transformed cells following the temperature shift. C-raf-1 activity slightly increased in parallel with v-mos activity with similar kinetics, but a mobility shift, indicative of increased phosphorylation, was detected only 18 hrs after the temperature transition, showing that c-raf-1 mobility was not correlated with c-raf-1 activity. We have also isolated a transformation-resistant revertant of mos transformed cells which are resistant to transformation by v-mos or v-raf, but not v-ras. In revertant cells the kinase cascade is not activated by v-mos or v-raf, but is activated by v-ras or by serum stimulation to levels seen in ras-transformed or serum-stimulated control cells. Interestingly, in the latter cells c-raf-1 enzymatic activity is much higher than in v-mos-transformed cells. Revertant cells express elevated levels of protein phosphatases in comparison with parental v-mos-transformed cells. In vivo treatment with okadaic acid (OA), a potent protein phosphatase inhibitor, leads to an increase in MKK-1 and MAP kinase activity in revertant, but not normal cells. The results support the hypothesis that mos acts through the MAP kinase cascade (MKK-1 and ERK-2) to induce cell transformation, and that blocking v-mos activation of that cascade (possibly because of increased levels of phosphatase) prevents transformation.

A1-365 TWO DIFFERIANTIALLY EXPRESSED TRANSCRIPTS OF MOUSE mxi1 ENCODE DRAMATICALLY DIFFERENT ANTI-myc ACTIVITES, Richard Torres, Nicole Schreiber-Agus, Lynda Chin, Ken Chen, and Ronald DePinho. Department of Microbiology and Immunology, Albert Einstein College of Medicine. Bronx, NY 10461

Members of the myc family of nuclear proto-oncogenes (c-, N, L-myc) have been implicated in the control of normal growth and differentiation, apoptotic cell death, and cellular transformation. Accumulating evidence affords the view that these nuclear proteins function as sequence-specific transcription factors dimerized with another transcription factor, Max. The recent isolation and characterization of two additional Max-associated proteins, Mad and Mxi1, has expanded the view of how activities of the Myc family may be modulated. Biochemical properties of the highly homologous Mad and Mxi1 proteins have led to a model for their regulation of Myc activity in which Mad and Mxi1 compete with Myc for both Max and common target sequences. *In addition Mad and Mxi1 dramatically* suppress Myc transformation activity in the rat embryo fibroblast (REF) cooperation assay.

In this study we have identified two different cDNA forms of mouse mxi1 that arise from a single genomic locus and exhibit distincts patterns of developmental stage-specific expression. The presence of a 36 amino acid NH2-terminal extension in the putative Mxi-1 protein encoded by one of the two forms augments dramatically its capacity to suppress myc-induced transformation and allows for association with a murine homolg of the yeast transcriptional repressor Sin3. As such, the antagonistic role of Mxi1 may be executed not only through its competition with Myc for Max and common targets but also through its association/recruitment of Sin3 to such targets.

A1-366 THE ANTI-TUMOUR COMPOUND FOSTRIECIN CAUSES PREMATURE CHROMOSOME CONDENSATION WITHOUT CDK1 ACTIVITY IN HUMAN CELLS AND INHIBITS CDK1 ACTIVITY AND MATURATION IN SEA STAR OOCYTES. Christopher Tudan and Steven Pelech, Kinetek Site, Biomedical Research Centre, University of British Columbia, Suite 500 - 520 West 6th Avenue, Vancouver, B.C., V5Z 1A1, Canada.

Fostriecin is an anti-tumour drug under phase I clinical studies. Exposure to non-synchronized or synchronized A549, Jurkat and U937 cells to 375 μM fostriecin for two hours caused them to enter mitosis prematurely, as determined by chromosome condensation. Fostriecin induced entry into mitosis in cells arrested in S phase with aphidicolin, with typical S phase premature condensed chromosome (PCC) morphology. Fostriecin also induced normal chromosome condensation morphology and premature mitosis in cells arrested in late G2 phase with VM-26, delaying VM-26 associated chromosome decondensation. Cells arrested in S and G2 phases that underwent PCC also showed no significant increase in histone H1 phosphorylation which is usually associated with chromosome condensation and the onset of mitosis. While cyclic dependent kinase-1 (CDK-1, *cdc2*) was indirectly inhibited by fostriecin, MAP kinase was activated. Temperature-sensitive FT210 cells have a reduced CDK-1 activity and are unable to enter mitosis at the restrictive temperature. Treatment of FT210 cells at the restrictive temperature caused a 10-fold increase into mitosis with no chromosome condensation associated histone H1 phosphorylation, despite the repressed CDK-1 activity. These observations suggest that the induction of mitotic events by fostriecin does not require CDK-1 activity and histone H1 phosphorylation. Fostriecin inhibited activiation of CDK-1 and the meiotic maturation of sea star oocytes in response to 1-methyladenine.These effects are surprising as fostriecin accelerates mitosis in somatic cells, but appears to inhibit meiosis in oocytes. (Supported by M.R.C. of Canada).

A1-367 STABILIZATION OF MURINE IkB BY DELETION MUTAGENESIS, Daniel J. Van Antwerp and Inder M. Verma, Molecular Biology and Virology Lab, The Salk Institute, 10010 N. Torrey Pines Rd., La Jolla, CA. 92037

The Rel/NFkB family of transcription factors activate a wide variety of inflammatory and immune response genes. IkB, an inhibitor protein, regulates NFkB activity by retaining it in the cytoplasm. Upon signaling, IkB is quickly degraded allowing nuclear transport of NFkB. The rapid proteolysis of IkB can be reconstituted when overexpressing the protein by transient transfection in 293 cells. Using this system, we have found that a 70 amino acid deletion in the carboxy terminus leads to a 2-3 fold stabilization of IkB. This region includes a candidate PEST sequence. We are currently narrowing down the residues required for instability. Stable mutants of IkB are also being tested for their ability to respond to signaling.

A1-368 ACTIVATION OF STAT PROTEINS BY GROWTH FACTORS. Marie-Luce Vignais, Henry B. Sadowski and Michael Z. Gilman, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 11724.

We are interested in the mechanism of activation of Stat1 and Stat3 proteins by polypeptide growth factors. Stat proteins bind to the c-*sis* inducible element (SIE) in the c-fos promoter. They are activated by tyrosine phosphorylation in response to EGF or PDGF. Phosphorylation of Stat1 at tyr701 is required for dimerization, activation of DNA-binding, and nuclear translocation. The SH2 domain of Stat1 is required for all these functions. Stat1 is also activated by interferons and a variety of other cytokines. Unlike Stat1, Stat3 is preferentially activated by growth factors and certain cytokines, but not by interferons. Stat1 and Stat3 can bind to the SIE *in vitro* either as homo- or heterodimers, giving three distinct DNA-protein complexes in mobility-shift assay (SIF A, B and C). Activation of Stat1 by the interferon receptors requires a family of non-receptor tyrosine kinases including JAK1, JAK2 and Tyk2. These kinases are clearly involved in the action of other cytokine receptors as well. It is not clear how the Stat proteins are activated by growth factor receptors with intrinsic protein tyrosine kinase activity. We have taken several approaches to address this question. A panel of PDGF receptor mutants were tested to determine what elements in the receptor are required for Stat activation. While receptors with a mutation in the ATP-binding site (kinase negative) do not support Stat activation, most of the known autophosphorylation sites are not required. One site, Y579, is absolutely required for Stat activation, as well as for receptor/src-family kinase association. We are determining if Y579 is a site for recruitment of Stats to the receptor and whether src-family kinases are involved in Stat activation. We have observed tyrosine phosphorylation of JAKs in PDGF-treated cells. To address the role of these kinases in Stat activation by PDGF, we have stably expressed functional PDGF receptors in cell lines that are defective for different JAK family members and assessed Stat activation by PDGF.

A1-369 PHOSPHORYLATION OF PHOSPHATASE-1 ISOFORMS IN MITOTIC HeLa CELLS. Emma Villa-Moruzzi and Franca Puntoni, Department of Biomedicine, University of Pisa, 56126, Pisa, Italy

Protein phosphorylation is probably the major regulatory mechanism in eukaryotic cells. It is now established that protein phosphatases play a critical role in the regulation of cell growth and differentiation and in oncogenic mechanisms. Protein phosphatase PP1 is purified as inactive complex consisting of a 37kDa catalytic subunit and inhibitor-2 (I2). Such complex is activated "in vitro" through phosphorylation of I2 at Thr 72 by F_A/GSK3. Recently we reported that also the mitotic kinase cdc2 phosphorylates I2 and activates PP1 "in vitro". When PP1 was phosphorylated by either cdc2 or GSK3, the same two phospho-peptides were obtained from I2 by two-dimensional tryptic maps. Phosphoaminoacid analysis showed that the peptides contained essentially P-Thr. It is likely that also cdc2 phosphorylates Thr 72.

Additionally, we found that cdc2 (but not F_A/GSK3) phosphorylates also the catalytic subunit of PP1 in the trypsin-sensitive, C-terminal region. Recently different catalytic subunit isoforms (α, γ1, γ2, δ) were discovered by cDNA cloning. These have high sequence homologies and differ mainly in their C-terminal regions. Using antibody directed against their C-terminal peptides, we found that in the inactive PP1 complex purified from muscle cytosol, three different isoforms (α, γ1, δ) were present. "In vitro" cdc2 phosphorylates all isoforms on a conserved Thr residue (Thr 320 in α, Berndt and coll., 1994). In agreement with the fact that the isoforms differ in the C-terminal regions, their tryptic phosphopeptides were different.

Since cdc2 is activated at mitosis we immunoprecipitated the PP1 isoforms and I2 from "in vivo" [32]Pi-labelled HeLa cells. We found that in mitotic (nocodazole-blocked) cells the immunoprecipitated α and γ1 isoforms were phosphorylated. Phosphorylation was mainly on Ser for the α form, and mainly on Thr for the γ1. δ was phosphorylated already in control cells on both Ser and Thr, but Thr phosphorylation increased at mitosis. However, the phosphatase activity was not different in immunoprecipitates obtained from mitotic and control cells. Also I2 was phosphorylated in mitotic cells only, on both Ser and Thr residue.

Our data indicate that the PP1 phosphorylation is increased at M-phase and might involve both cdc2 and other mitotically-activated Ser-kinases.

A1-370 USE OF TEMPERATURE-SENSITIVE (TS) MUTANTS OF THE v-REL ONCOPROTEIN TO STUDY APOPTOSIS IN CHICKEN SPLEEN CELLS, David White & Thomas Gilmore, Biology Department, Boston University, Boston, MA 02215

The v-Rel nuclear oncoprotein is a transcription factor encoded by the avian Rev-T retrovirus that can transform and immortalize chicken spleen cells in vitro. We have constructed two ts v-Rel mutants (v-R273H and v-G37E) that are ts for transformation and DNA binding. When ts v-Rel-transformed chicken spleen cells are shifted to the nonpermissive temperature, the cells rapidly undergo apoptosis, as characterized by condensed chromatin and the formation of "DNA ladders". Many proteins, including v-Rel, Bcl-2, c-Myc, Rb and p53, are quite stable in these cells while undergoing apoptosis. However, the v-Rel-associated protein p40 (IκB-α) is specifically degraded when ts v-Rel-transformed cells are shifted to the nonpermissive temperature. In cells transformed by ts v-Rel mutant v-R273H, p40 is completely degraded, similar to what is observed in other cells in which Rel complexes are induced to enter the nucleus. In cells transformed by ts v-Rel mutant v-G37E, p40 is cleaved to an intermediate form, which is found in a detergent-insoluble fraction and is missing approximately 3 kDa from the N terminus. These results suggest that v-Rel stabilizes p40 in transformed cells and that changes in the structure of a Rel/NF-κB protein can initiate proteolysis of an associated IκB protein. Furthermore, they indicate that v-Rel blocks a normal pathway of programmed cell death as part of its transforming and immortalizing process. We are currently attempting to understand the molecular basis for v-Rel-mediated inhibition of apoptosis.

A1-371 MUTATIONAL ANALYSIS OF THE FOSB C-TERMINAL ACTIVATION DOMAIN DEFINES RESIDUES CRITICAL FOR TRANSFORMATION AND TRANSCRIPTIONAL ACTIVATION. R. Wisdom and S. Qu. Dept. of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37232.

Transformation by AP-1 proteins is believed to be the result of transcriptional activation of a set of target genes. Consistent with this hypothesis, previous mutational analysis of the FosB protein, a transforming member of the Fos gene family, has demonstrated that transforming activity by this protein requires a functionally intact DNA binding domain and a proline-rich C-terminal activation domain (amino acids 225-338). To begin to define the mechansim by which the C-terminal activation domain functions, we have generated an extensive collection of mutants in this region and characterized their ability to activate reporter genes when fused to the GAL4 DNA binding domain. These mutations have also been engineered back into the FosB gene and examined for their effect on transforming activity. This analysis permits the following conclusions: 1) many regions can be deleted without a major effect on the ability of this region to direct trancriptional activation, but deletions of amino acids 256-275 and 288-305 result in a severe loss of activity; 2) the overall net acidic nature of this domain is important, but no single acidic residue is critical; 3) we have generated four independent point mutants which show less than 5% wild type activity, three of which involve substitutions of proline to alanine; and 4) all mutants that show impaired transcriptional activation also show impaired transforming activity when introduced back into the native FosB protein, supporting the hypothesis that transformation is the result of transcriptional activation. These results are consistent with the idea that this region is involved in protein-protein interactions. Therefore, we have used recombinant proteins corresponding to either the wild type or inactive point mutants to search for proteins that interact with this region in an activity dependent manner.

A1-372 IDENTIFICATION AND CHARACTERIZATION OF TWO RELATED PROTEINS, E3B35 AND E3B78, THAT BIND TO THE SH3 DOMAIN OF *eps* 8,

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We have previously isolated a substrate protein, *eps* 8, for the epidermal growth factor receptor (EGFR) tyrosine kinase and shown that its overexpression in fibroblasts and hematopoietic cells enhances EGF responsiveness of the target cells. Sequence analysis of *eps* 8 revealed the presence of a SH3 domain. Microinjection of bacterially expressed SH3 domain of *eps* 8 in *Xenopus laevis* oocytes induced maturation and also enhanced insulin-induced maturation. In far-western analysis, the SH3 domain of *eps* 8 specifically bound several proteins from cellular lysates from different cell lines. To understand further the function of the SH3 domain in *eps* 8, we used the bacterially expressed SH3 domain to screen a M426 expression cDNA library and isolated two related cDNA clones, e3B35 and e3B78. Bacterially expressed e3B35 and e3B78 proteins bound specifically to *eps* 8 *in vitro*. The carboxyl termini of these two proteins consist of a proline-rich region which is followed by a SH3 domain. The predicted amino acid sequences of these two cDNA clones were also found to have sequence similarity to xlan4, a *Xenopus laevis* gene that is highly expressed in the oocyte.

A1-373 Interferons block protein kinase C-dependent, but not independent, activation of Raf-1 and MAPK and mitogenesis in NIH 3T3 cells

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Interferons (IFNs) exert anti-proliferative effects on many cell types. The underlying molecular mechanism, however, is unclear. One possibility is that IFNs block growth factor-induced mitogenic signaling, which involves activation of Ras/Raf-1/MEK/MAPK. We have tested this hypothesis using HER14 cells (NIH 3T3 cell expressing both PDGF and EGF receptors) as a model system. Our studies showed that IFNs (-α/β and -γ) blocked the PDGF- and phorbol ester-, but not EGF-, stimulated DAN synthesis and cell proliferation. While the ligand-stimulated receptor tyrosine phosphorylation and interaction with downstream signaling molecules, such as GRB2, were not affected, IFNs specifically blocked PDGF- and phorbol ester-, but not EGF-, stimulated activation of Raf-1, MAP kinases and tyrosine phosphorylation of an unidentified 34-kDa protein. This inhibition could be detected as early as 5 minute after IFN treatments and was insensitive to cycloheximide, indicating that de novo protein synthesis is not required. The IFN-induced inhibition acted upstream of Raf-1 kinase and downstream of DAG/phorbol ester, suggesting that protein kinase C (PKC) is the potential primary target. Consistently, downregulation of PKC by chronic PMA treatment or inhibition of PKC by H7 and staurosporine blocked PDGF- and PMA-, but not EGF-, induced signaling and DNA synthesis. Moreover, anti-sense oligo of PKCd eliminated production of PKCδ protein and specifically blocked PDGF-, but not EGF-, stimulated mitogenesis in these cells. Thus, these studies have elucidated a major difference in the early events of EGF- and PDGF-stimulated signal transduction and, more importantly, revealed a novel mechanism by which IFNs may execute their anti-proliferative function.

A1-374 FUNCTIONAL ROLE AND MECHANISM OF HEMATOPOIETIC CELL PHOSPHATASE (HCP) IN REGULATING THE SIGNALING PATHWAY OF THE ERYTHROPOIETIN RECEPTOR.

Taolin Yi[1]*, Jingli Zhang[1], Karim Berrada[1], James N. Ihle[2], Huaoyuan Jiao[1], [1]Department of Cancer Biology, The Cleveland Clinic Foundation Research Institute, Cleveland, OH 44195; and [2]Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, TN 38105.

Erythropoiesis is regulated primarily by erythropoietin (Epo), that binds to specific receptor (EpoR) on erythroid progenitor cells and induces a mitogenic response. The EpoR is a transmembrane protein composed of a extracellular, transmembrane and cytoplasmic regions. Ligand binding to The EpoR induces tyrosine phosphorylation in responsive cells and this ability is required for a mitogenic response. One of the substrates of tyrosine phosphorylation is the receptor. The carboxyl region of the EpoR's cytoplasmic domain is required for EpoR phosphorylation and has been shown to negatively affect the response to Epo both *in vivo* and in cell lines. Hematopoietic cell phosphatase (HCP) has also been hypothesized to negatively regulate erythropoiesis, based on the hypersensitivity of erythroid lineage cells in motheaten mice which genetically lack HCP. Our studies demonstrate that HCP binds the tyrosine phosphorylated EpoR via the amino terminal src-homology 2 (SH2) domain of HCP. Using a series of phosphotyrosine containing peptides, HCP binding sites in the cytoplasmic domain of EpoR are identified. To define the functional significance and mechanism of HCP interaction with EpoR, we have further examined the effect of overexpressing HCP mutants lacking each of its three functional domains on the tyrosine phosphorylation and proliferation induced by Epo stimulation. Our results support the concept that, following Epo stimulation, phosphorylation of the EpoR provides a docking site for HCP in the receptor complex. Recruitment of HCP to the complex and its subsequent dephosphorylation of substrates may be important to mitigate the ligand induced mitogenic response.

A1-375 ENHANCED EXPRESSION OF A PROTEIN TYROSINE PHOSPHATASE IN CELLS EXPRESSING p210 BCR/ABL

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Tyrosine phosphorylation plays a central role in the regulation of cellular growth, differentiation and neoplastic transformation. These mechanisms are controlled by the competing activities of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs).

Chronic Myelogenous Leukemia (CML) is a malignancy in which >95% of the leukemic myeloid cells display a reciprocal chromosomal translocation t(9;22), referred to as the Philadelphia chromosome (Ph^1). The molecular consequence of the Ph^1 chromosome is that the c-abl oncogene located on chromosome 9, encoding a PTK, translocates into a specific breakpoint cluster region (bcr) on chromosome 22. This results in a chimeric bcr-abl transcript, which encodes the p210bcr-abl fusion protein. Expression of the p210bcr-abl PTK has been implicated in the pathogenesis of CML. Although the p210 bcr-abl PTK has been studied extensively, little is known about the PTPase(s) that act upon p210 bcr-abl directly or upon its substrates.

In the present study we compared the levels of expression of a panel of receptor-like and cytoplasmic PTPases in a human myeloid cell line (MO7) in the presence and absence of expression of p210 bcr-abl. We have found that expression of one particular PTP, the cytoplasmic enzyme PTP1B, is enhanced as a consequence of production of p210bcr-abl. We have found that these changes are specific for PTP1B and are not observed in the closely related homologue TCPTP, which displays 75% identity to PTP1B in its catalytic domain. We have also found that PTP1B levels are enhanced coincident with expression of p210bcr-abl in a number of other cell lines including the mouse myeloid cell line 32D and Rat-1 fibroblasts. PTP1B is also enhanced in Ph^+ B-Lymphoid cells derived from a patient and immortalized with EBV, relative to Ph^- cells from the same patient. Furthermore these changes in PTP1B expression were induced specifically by p210bcr-abl and were not seen in cells expressing the src or v-abl PTKs or myc. Currently, we are testing the hypothesis that the enhanced expression of PTP1B may represent a compensatory response by the cell to the elevated PTK activity of p210bcr-abl. Experiments are underway to examine the effect of PTP1B on transformation induced by p210bcr-abl.

The New Frontiers; Summary and Future Perspectives

A1-400 THE INTERACTION OF TRANSFORMING GROWTH FACTOR α AND C-MYC IN MOUSE MAMMARY GLAND TUMORIGENESIS **Laufey Thora Amundadottir[1], Michael D. Johnson[1], Gilbert H. Smith[2], Glenn Merlino[3] and Robert B. Dickson[1]**; Lombardi Cancer Center, Georgetown University, Washington D.C. 20007[1], Laboratories of Tumor Immunology and Biology[2], and Molecular Biology[3], NCI, NIH, Bethesda, MD 20892. Transforming Growth Factor α (TGFα) binds to and activates the Epidermal Growth Factor Receptor (EGFR). Expression of TGFα is most predominantly found in transformed cell lines and tumors of epithelial origin, including breast tumors. The c-*myc* proto-oncogene is found amplified in about 30% of breast cancer. Both c-*myc* and TGFα are known to be induced by ovarian hormones in breast cancer. In various cell types *in vitro*, overexpression of c-*myc* results in increased responsiveness to the effects of mitogenic growth factors, including TGFα.

We are exploring the interaction of Myc and TGFα *in vivo* in mouse mammary gland tumorigenesis. We mated a transgenic mouse strain heterozygous for TGFα (MT100) to a strain heterozygous for Myc (MMTV-c-*myc*) to yield double transgenic offspring for TGFα and Myc. All (20/20) TGFα/Myc animals developed multiple mammary tumors at a mean age of 66 days. No single transgenic TGFα virgin mice or wild type mice have developed tumors, but single transgenic Myc virgin females developed adenocarcinomas of the mammary gland after a long latency time of 9 - 12 months. An interesting finding was that female and male double transgenic animals develop mammary gland tumors with identical latency and frequency, suggesting the tumors could be estrogen independent. All tumors are classified as adenocarcinomas type A and B that are locally invasive and have been established in nude mice. Of other organs that co-express TGFα and Myc in double transgenic mice, salivary glands show abnormalities ranging from ductule hyperplasia to adenocarcinomas. Salivary glands of single transgenic animals showed minimal ductule hyperplasia (TGFα mice) or no abnormalities (Myc mice and wild type mice). In summary, TGFα and c-*myc* are powerful, synergistic-acting genes in breast tumorigenesis. Data will also be presented on cyclin overexpression in the tumors and on characterization of epithelial cell lines from TGFα/Myc, TGFα and Myc tumors.

A1-401 INDUCTION OF CYCLIN D1 OVEREXPRESSION BY ACTIVATED *RAS*, Jorge Filmus[1], Ana I. Robles[2], Wen Shi[1], Michael J. Wong[1], Lucas L. Colombo[1] and Claudio J. Conti[2], [1]Div. Cancer Research, Sunnybrook Health Science Centre, 2075 Bayview Avenue, Toronto, Ont, Canada M4N 3M5 and [2]Dept. Carcinogenesis, University of Texas, M.D. Anderson Cancer Center, Science Park, Research Div., Smithville, TX.

Activated *ras* genes are known to alter control of cell proliferation. This is consistent with the fact that *ras* proteins are a key component of the biochemical pathway triggered by ligand-bound cell surface receptors that are tyrosine kinases. Although an important part of the *ras* signalling pathway has been recently uncovered, the molecular target(s) that mediates the effects of *ras* on cell cycle control remains unknown. Cyclins and cyclin-dependent kinases are key molecules in the control of cell cycle. Cyclin D1, in particular, is a critical target for proliferative signals in G_1 and it has been shown that ectopic overexpression of this cyclin can significantly alter cell cycle regulation. Here we report that activated *ras* induces significant overexpression of cyclin D1 in epithelial cells derived from normal rat intestine and mouse mammary gland. A definitive causal role for activated *ras* in this overexpression is demonstrated by using intestinal cells transfected with an inducible *ras* expression vector. Treatment of the *ras*-transformed intestinal clones with anti-sense cyclin D1 oligonucleotides reduces their rate of cell proliferation indicating that the increment in cyclin D1 expression induced by activated *ras* is instrumental in the higher rate of cell proliferation conferred by the *ras* oncogene to the IEC cells. Based on these results we propose that, at least in certain cell types, cyclin D1 can be one of the mediators of the transforming action of activated *ras*.

A1-402 TARGETED INHIBITION OF TUMOR CELL GROWTH BY RECOMBINANT HEREGULIN-TOXIN FUSION PROTEINS, Bernd Groner, Wolfgang Dengler, Winfried Wels, Roland Imber, Elisabeth Stöcklin, and Margit Jeschke, Institute for Experimental Cancer Research, Tumor Biology Center, Breisacher Str. 117; D-79106 Freiburg, Germany and Friedrich Miescher Institute, Schwarzwaldallee 215, P.O.Box 2543, CH-4002 Basel, Switzerland

Fusion of functional domains of proteins by in vitro recombination of gene fragments can be used to generate novel anti-tumor agents. The combination of tumor cell recognition functions and toxic functions result in cytotoxic molecules with a high specificity for tumor cells. Human adenocarcinomas are frequently characterized by overexpression of members of the epidermal growth factor (EGF) receptor family (ErbB-1, 2, 3 and 4), when compared to normal cells. These tumors are particularly suited for the treatment with recombinant toxins. The human heregulins (HRG) and their rat counterparts (neu differentiation factor, NDF) have been identified as ligands for these receptors. Two chimeric heregulin-toxin fusions consisting of the EGF-like receptor recognition domain of the heregulin isoforms HRGα and HRGβ_1, and the domains II and III of the Pseudomonas exotoxin A (ETA) were constructed. HRGβ_1-ETA is highly cytotoxic for the mammary carcinoma cell lines SK-BR-3 and MDA-MB-453. These cells were killed with an IC_{50} between 3 to 6 ng/ml. HRGα-ETA was 50-fold less active. The killing activity of the recombinant toxins correlated with the expression levels of ErbB-3 and/or ErbB-4 in the cell lines studied. High expression of erbB-2 is not sufficient to confer sensitivity towards the HRG-ETAs. The growth of MAXF 1162 cells, a human breast carcinoma xenograft, was retarded in mice treated with 0.2 or 0.4 mg/kg/day of HRGβ_1-ETA. Higher levels of HRGβ_1-ETA (0.5 mg/kg/day) administration resulted in acute hemorrhagic necrosis of the liver.

A1-403 OVEREXPRESSION OF E2F-1 IN TRANSGENIC MICE LEADS TO SEVERE THROMBOCYTOPENIA, Chantale T. Guy, Wen Zhou and Murray O. Robinson, Amgen, Inc., Thousand Oaks, CA 91320.

We have recently demonstrated that overexpression of SV40 T antigen under a tissue specific promoter in transgenic mice interferes with the ability of megakaryocytes to form platelets. Ten of seventeen transgenic lines generated exhibited low platelet levels, each line displaying a distinct, heritable level of thrombocytopenia. At six to twelve months of age, these animals also develop megakaryocytic neoplasia. Interestingly, the platelet level in these affected animals could be further reduced by the inactivation of one copy of the Rb gene. These experiments have shown that T antigen expression can interfere with terminal differentiation in this system possibly through its interaction with Rb or other related proteins. Cell cycle promoting transcription factor E2F-1 is known to be inhibited by Rb. Because E2F-1 has also been shown to form specific complexes with Rb or Rb related protein such as p107, we were particularly interested in assessing its role in megakaryocyte differentiation. We have generated a number of transgenic lines that overexpress the human E2F-1. All animals analyzed so far presented severe thrombocytopenia. Megakaryocytes from these mice are both increased in numbers and morphologically abnormal. The affected cells appear to be blocked in differentiation at the immature type 1 stage. In addition, within a line, the degree of platelet reduction correlated directly with transgene zygosity and level of expression of E2F-1. We are currently characterizing in more detail the molecular effects of E2F expression on the differentiation of transgenic megakaryocytes.

A1-404 p28 ^{Ick1} / p27 ^{Kip1}, A CELL CYCLE REGULATED INHIBITOR OF CYCLIN DEPENDENT KINASES,
L. Hengst and S. I. Reed, Dept. of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037
Cell cycle progression through G1 and from G1 to S phase involves the accumulation of G1 cyclins and their association with distinct Cdk subunits. Cyclin accumulation and Cdk binding do not constitute the only levels of Cdk regulation. Additional modes of regulation of the Cdk associated kinase activities include positive and negative phosphorylation events and various inhibitory proteins.
In the course of investigating the mechanism of the lovastatin induced G1 arrest in HeLa cells, we found high levels of inactive cyclinE/Cdk2 and cyclinA/Cdk2 complexes, suggesting the presence of a Cdk inhibitor. Using lovastatin arrested HeLa cells we identified a 28 kD protein, p28^{Ick1} (Inhibitor of Cyclin dependent Kinase), that binds to and inhibits the kinase activities of pre-formed Cdk/cyclin complexes. Boiling releases the heat stable 28 kD protein from protein complexes of 150-200 kDa, and no monomeric inhibitory activity was detected in extracts from arrested HeLa cells.
p28^{Ick1} inhibitory activity was also detected in growing HeLa cells, the activity fluctuates during the cell cycle and reaches maximal levels during the G1-phase. Similarly, elutriated human erythroleukemia-derived HL60 cells show a cell cycle dependent, heat stable inhibitory activity which reaches maximal levels in G1 cells.
Inhibitory activity can be coprecipitated with Cdk2, Cdc2 or Cdk4 immune complexes from lovastatin arrested cells . Gel-purified p28^{Ick1} protein can also inhibit cyclin A-, B-, D- and E-kinase complexes *in vitro*.
p28^{Ick1} was purified using a protocol involving size chromatography of the inhibitor containing complexes, boiling to release the inhibitor and subsequent chromatography of the monomeric protein. Peptides of this protein were sequenced and the cDNA encoding p28^{Ick1} was cloned. The obtained sequences are identical to the human sequences of p27^{Kip1}.

A1-405 EXOGENOUS MUTANT P53 GENE INDUCED HIGH LEVEL OF SPONTANEOUS TRANSFORMATION AND ABNORMALITY OF P53 PROTEINS IN RAT 6 FIBROBLAST CELL LINE,
W.L. Wendy Hsiao*, David Spodick, and Hung Fan, Dept. of Molecular Biology and Biochemistry, University of California, Irvine, CA 92717. * Dept. of Biology, Hong Kong University of Science and Technology, Kowloon, Hong Kong.
The Rat 6 embryo fibroblast cell line is poorly competent for expressing the transformed phenotype induced by an activated human H-*ras* oncogene. The transformation efficiency of Rat 6 cells can be greatly enhanced by addition of tumor promoters, various factors and chemicals in the growth medium after introducing the H-*ras* gene into the cells (Hsiao et al., 1986, 1987 & 1990). These findings suggested that there was host factor(s) in Rat 6 cells limiting the transforming ability of H-*ras*. Unlike some of the immortal cell lines, Rat 6 cells exhibit very low level of cellular p53. Mutant p53 gene encoding for a temperature sensitive protein was transduced into Rat 6 cells through a retroviral vector. The vectored cells expressed high levels of p53-specific mRNA and high levels of a *gag*-p53 fusion protein. Immunofluorescent stain of p53-overexpressing cells showed that the *gag*-p53 protein behaved in a temperature sensitive manner. Although expression of this *gag*-p53 had no apparent initial effect on Rat 6 cells, a high incidence of spontaneous transformation was observed in long term cultures. When the levels of p53 protein were compared between the p53-vectored Rat 6 cell lines and the isolated spontaneous transformants, a striking result was observed. Despite high levels of p53 mRNAs detected in all the transformants, the endogenous and the *gag*-p53 protein was not detectable by Western blot analysis and immunoprecipitation using either the p240 or p246 monoclonal antibodies. The result from the pulse-chase experiment indicated that both the endogenous and the exogenous p53 proteins in the transformants were either extremely labile or the proteins were never translated. *In vitro* translation assay indicated that both R6#13-8 and transformed R6#13-8 clone-T2 produced translatable p53 mRNA for *in vitro* translation. The failure of detection p53 proteins was observed in 11 out 12 transformants. Comparisons between the exceptional clone and the rest of the transformants are underway to elucidate the cause for the loss p53 protein in the transformants. We will also explore the possible relationship between the loss of p53- protein and the occurrence of spontaneous foci in these p53-expressing cells.

A1-406 KINASES IN THE YOLK SAC HEMATOPOIETIC STEM CELLS.
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Yolk sac is the first site of hematopoiesis during mammalian embryonic development. It is an ideal place to study initiation of hematopoiesis and formation of hematopoietic stem cells. Our previous efforts have been focused on identification and characterization of yolk sac hematopoietic stem cells. These primitive stem cells have capacity to give rise to various lineages including T cells, B cells, erythroid and myeloid cells, and they differ from fetal liver and bone marrow stem cells in response to cytokines and growth factors.
However, little is known how the yolk sac hematopoietic stem cells are regulated at molecular level. It has been shown that stromal and stem cell interaction is crucial in stem cell growth and differentiation, and receptor-ligand interaction is key players in such processes.
We used degenerate primers biased to receptor kinases to survey the profiles of kinases expressed in yolk sac stem cells at different developmental stages. After, screening over 1000 colonies, we found a collection of kinases in the enriched yolk sac stem cells. The spectrum of kinases in day 8 yolk sac stem cells differs from that of day 11 yolk sac stem cells, specifically, a kinase sharing a high percentage of homology to a yeast SNF and Tie2 was not present in day 11 yolk sac stem cells. The abundance of kinases in enriched yolk sac stem cells also differs. The most abundant kinases in day 8 yolk sac stem cells are FLT4 and c-KIT, while the most abundant one in day 11 yolk sac stem cells is c-FMS. Among the collection of kinases, we found a mouse homologue of human SPRK/MLK3, which is a newly found family, consisting of an SH3 domain, two putative leucine zipper domains and proline rich c-terminal. Using a normalized library, we found a new gene named YSK1 sharing some homology to FLK2. This study may provide some clues as to which kinases are most likely involved in yolk sac stem cell growth and differentiation and such information will prove useful for the further elucidation of the regulatory mechanism of in hematopoietic stem cells development.

A1-407 THE CHIMERIC PLZF-RARα FUSION PROTEIN OF t(11;17) ASSOCIATED ACUTE PROMYELOCYTIC LEUKEMIA IS AN ABERRANT RAR THAT INTERFERES WITH WILD TYPE RARα FUNCTION,
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The chromosomal rearrangement t(11;17) is associated with acute promyelocytic leukemia which unlike the more typical t(15;17) APL, fails to respond to treatment with All-*trans* Retinoic Acid (ATRA). In this syndrome, the PLZF gene on chromosome 11 is fused to the retinoic acid receptorα (RARα) to yield two reciprocal fusion proteins. We characterized the nature of the wild-type PLZF protein and its fusion proteins with RAR. PLZF gene produces an 81 kDa phosphoprotein. Confocal microscopy revealed nuclear expression of PLZF to be confined to small punctate domains that appear different from the POD domains of PML protein. The PLZF-RARα protein is also localized exclusively to the nucleus, while the reciprocal RARα-PLZF protein was found in the nucleus and the cytoplasm. PLZF-RARα could bind a retinoic acid responsive element (RARE) as a homodimer or as a heterodimer with the retinoid X receptor. The PLZF-RARα protein could also act as ATRA responsive transcription factor, but was less potent than the wild-type protein and had altered functional affinity for ATRA. Co-expression of the wild-type RARα and the PLZF-RARα or PML-RARα fusion protein antagonized the function of the wild-type RARα. Inhibition of the RARα function by PLZF-RARα was partially relieved by overexpression of RXR. This suggests two mechanisms of inhibition of RARα by PLZF-RARα-1) Competition for binding to the RARE and 2) Competition for limiting amounts of RXR. Consistant with the second mechanism, PLZF-RARα expression also inhibited Vitamin D_3 Receptor function. Since RXR is a preferred dimerization partner for thyroid hormone receptor, vitamin D receptor and RARs, its sequestration by PLZF-RARα can disrupt multiple pathways pertinent to cell maturation. In this regard, all of the properties of PLZF-RARα are similar to the PML-RARα fusion protein of t(15;17), yet the t(11;17) syndrome fails to respond to ATRA therapy. Thus disruption of the function of the PLZF protein may also play an important role in the mechnism of leukemogenesis.

A1-408 THE ROLE OF RB IN THE LENS- A GENETIC COMPLEMENTATION APPROACH, Nanette J. Liegeois, James W. Horner and Ronald A. DePinho, Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY 10461

The regional compartmentalization of growth, differentiation and apoptosis in the developing lens provides an ideal system to examine more closely the genetic control of these processes *in vivo*. These experimental merits were exploited recently to demonstrate that the *Rb*-deficient state is assoicated with unchecked proliferation, impaired expression of differentiation markers, and inappropriate apoptosis in lens fiber cells. This developmental system was also used to show that apoptosis in *Rb*-deficient lenses is dependent on *p53*, because embryos doubly null for *Rb* and *p53* show a nearly complete suppression of apoptosis. In an effort to fully exploit the power of the lens system and overcome limitations inherent to established gene targeting techniques, we have developed a genetic complementation approach which makes use of fertile mouse strains that harbor a mutation that results in failure of lens formation. Microinjection of ES cells into blastocysts from these strains generates chimeras possessing lenses that are consistently derived from the ES cells. We demonstrate that the microinjection of normal ES cells gives rise to normal, fully-formed lenses and that similar experiments using homozygous null *Rb* ES cells (a gift from Tyler Jacks, MIT) recapitulates the sequelae observed in the classical *Rb* knockout. Moreover, the chimerism permits survival of the organism to much later stages of development, permiting more extensive developmental genetic analysis. This new blastocyst complementation approach has general applicability to the study of any gene(s) involved in growth, differentiation and death. It provides several key advantages over established methods, including (i) the functional analysis of genes whose inactivation results in very early embryonic lethality and (ii) the rapid nature and low cost of experiments that obviate the need for germline transmission. The potential applications of this genetic approach will be presented in detail.

A1-409 A METHYL CPG BINDING PROTEIN MECP2 SELECTIVELY SUPPRESSES THE TRANSCRIPTION OF METHYLATED GENE AND ACTIVATES NONMETHYLATED GENE, +Xinhua Lin, +Lijuan Gu, *Adrian P. Bird and +Thomas F. Deuel, +Division of Hematology, Jewish Hospital at Washington University Medical Center, St. Louis, MO 63110 and *Institute of Cell and Molecular Biology, University of Edinburgh, Edinburgh EH9 3JR, Scotland.

Mammalian DNA is heavily methylated at cytosine residues within the CpG sequences. Methylation of DNA is increasingly recognized as important in mammalian development and imprinting and abnormal patterns of methylation appear to be important in neoplastic transformation. Because DNA methylation suppresses transcription of methylated genes, we wished to investigate the mechanism of how DNA methylation influences transcription of methylated genes. Using the PDGF A-chain gene as a model, we previously showed that the promoter activity of the PDGF A-chain gene methylated *in vitro* is inhibited and that suppression is mediated by methyl CpG binding protein(s). We now demonstrate that a methyl CpG binding protein (MeCP2, Cell 69:905-914, 1992) strongly suppresses transcription of HpaII methylated PDGF A-chain gene transiently expressed in Hela cells. A methyl CpG binding domain has been identified that is essential for the suppressive activity of transcription of the methylated PDGF A-chain gene. We compared constructs with different numbers of methylated CpG binding sites and found that inhibition of methylated promoter activity is essentially equal whether 21 methyl CpG sites are methylated or only 2 methyl CpG sites are methylated. These results suggest possible roles of DNA methylation in the regulation of the PDGF A-chain gene that potentially may be important in normal and abnormal cell growth.

A1-410 TRANSCRIPTIONAL REGULATION BY THE TUMOR SUPPRESSOR p53 PROTEIN: FUNCTIONAL INTERACTIONS AMONG MULTIPLE REGULATORY DOMAINS, Young-Sun Lin, Institute of Biomedical Sciences, Academia Sinica, Taipei 11529, Taiwan

The tumor suppressor p53 protein possesses activities of typical eukaryotic transcriptional activators: p53 binds to specific DNA sequences and stimulates transcription of the target genes. By a series of deletion and domain-swapping studies, here we report that (i) p53 has two auxiliary domains which have little effect on the DNA-binding activity of its core domain but are capable of modulating its transactivation activity in a target-site-dependent manner; (ii) p53 contains two cell-specific transcriptional inhibitory domains, I1 and I2, which are active in Saos-2 osteosarcoma cells and HeLa cells but not in HepG2 and Hep3B hepatoma cells; (iii) I1 inhibits the activity of several structurally different activating regions; and (iv) the p53 activating region is actually composed of two cooperative activation sub-domains and is strong enough to squelch the transactivation activity of the HSV VP16 activating region. These results demonstrate that the apparent transcriptional activity of p53 is determined by collaborations among its regulatory domains, its target sites and the cellular environment.

A1-411 **Cloning and Characterization of TGF-β Receptor Interacting Proteins**
Fang Liu, Jeffrey L. Wrana, Frances Weis-Garcia and Joan Massagué, Cell Biology and Genetics Program and Howard Hughes Medical Institute, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

TGF-β signals growth inhibition through a heteromeric receptor complex consisting of two transmembrane serine/threonine kinases, known as receptors I (TβR-I) and II (TβR-II). TGF-β binds directly to TβR-II which is a constitutively active kinase, and recruits TβR-I into the heteromeric complex. TβR-I is then activated through phosphorylation by TβR-II and propagates the signal to downstream substrates. To identify the targets of TβR-I, we used its cytoplasmic domain to screen for interacting proteins using the yeast two hybrid system. This assay detected strong interactions with the rapamycin/FK506-binding protein FKBP12 and a putative kinase, designated as TAK-1 (TβR-I Associated Kinase-1). We show that in both yeast and mammalian cells FKBP12 associates with TβR-I but not TβR-II, and this interaction requires the TβR-I domain that is phosphorylated by TβR-II. Furthermore, rapamycin can inhibit cell proliferation in cells defective in either TβR-II or the ligand binding function of TβR-I, but is much less effective in cells defective in the kinase activity of TβR-I. These observaitons suggest that rapamycin may inhibit cell proliferation through a FKBP12/TβR-I complex. Functional characterization of TAK-1 is in progress.

A1-412 MODULATION OF BREAST FIBROBLAST AND CARCINOMA CELL INTERACTIONS BY DEXTRAN DERIVATIVES. J.F. Liu, L. Adam, R.Y-Bagheri, M. Crépin. I.O.C.M.H., 129 route de Stalingrad, Université de Paris Nord, 93000 Bobigny, France.

Interactions between the human breast carcinoma cell line MCF7ras and the fibroblasts from normal breast tissue (MG3) and from a post-radiation fibrosis with recurrent breast carcinoma (FPR7) were investigated by co-culturing cells in separate chambers on both sides of a microporous membrane. The cell proliferation rate is assessed by ^{3}H-thymidine incorporation and cell counting.
MCF7ras proliferation increased (50%) in the presence of each two fibroblasts. MG3 and FPR7 showed a 125% and 120% growth rate increase respectively when cocultured with MCF7ras. This co-stimulation did not depend on the types of fibroblasts (normal or tumor).
Dextran derivatives are known to reduce the proliferation rate of breast carcinoma cells by modulating growth factor-receptor interactions. The additon of carboxy-methyl benzylamid dextran 7 (CMDB7) is found to block the co-stimulation observed in the co-culture model. Moreover, the influence of the CMDB7 on the epithelial cell-fibroblast interactions depended on the origin of the fibroblasts.
These data indicate paracrine interactions cause the increasing growth of carcinoma cells and fibroblasts and that CMDB7 is able to reverse this stimulatory effect. These suggest that CMDB7 can inhibit the breast cancer evolution by blocking the paracrine pathway, possibly by inhibiting growth factor actions.

A1-413 ANALYSIS OF THE FUSION GENE GENERATED BY CHROMOSOME 16 INVERSION IN LEUKEMIA, P. Liu, A. Hajra, P. Gregory, C. Wijmenga, T. Blake, and F. Collins, LGT, NCHGR, NIH, Bethesda, MD 20892
Acute myeloid leukemia subtype M4Eo is characterized by bone marrow eosinophilia and a chromosome 16 inversion, which is often the only cytogenetic abnormality seen in this subtype. Using positional cloning, we have cloned both the p and q arm breakpoints of the inversion and demonstrated that a chimeric transcript is consistently produced by the chromosome rearrangement. The two fusion partners are the transcription factor CBFβ gene on the q arm and smooth muscle myosin heavy chain gene on the p arm.
We have made a full-length cDNA construct of this fusion gene and used it in a variety of in vitro and in vivo studies. EMSA studies show that the fusion protein is still capable of associating with the CBFα subunit and forming protein-DNA complexes with the appropriate DNA sequence. Transient transfection experiments reveal differential effects of the fusion gene on enhancers containing the cognate DNA sequence. Transfection of the fusion gene into 3T3 cells results in a transformed phenotype; the ability of the fusion protein to both bind to CBFα and form myosin-like filaments appears necessary for its transforming properties. Studying leukemic cells at the RNA and protein levels demonstrates predominantly nuclear distribution of the fusion protein.

A1-414 **SELECTIVE INHIBITION OF THE PLATELET-DERIVED GROWTH FACTOR SIGNAL TRANSDUCTION PATHWAY BY A NOVEL TYROSINE PROTEIN KINASE INHIBITOR OF THE 2-PHENYLAMINO PYRIMIDINE CLASS.** Nicholas B. Lydon, Jürg Zimmermann, Helmut Mett, Thomas Meyer, Marcel Müller, Urs Regenass and Elisabeth Buchdunger, Ciba Pharmaceuticals Division, Oncology Research Department, Ciba-Geigy Limited, CH-4002 Basel, Switzerland.
The platelet-derived growth factor (PDGF) receptor is a member of the transmembrane growth factor receptor proteins with intrinsic tyrosine protein kinase activity. Its ligand, PDGF, is a key mitogen for mesenchymal cells and a potent chemoattractant for arterial smooth muscle cells, fibroblasts, neutrophils and monocytes. PDGF is thought to play an role in various neoplastic diseases and non-malignant proliferative diseases such as fibrosis, atherosclerosis and restenosis. We describe a potent tyrosine protein kinase inhibitor (CGP 53716) which shows selectivity for the PDGF receptor *in vitro* and in the cell. The compound shows selectivity for inhibition of PDGF-mediated events such as PDGF receptor autophosphorylation, cellular tyrosine phosphorylation and c-*fos* mRNA induction in response to PDGF stimulation of intact cells. In contrast, ligand-induced autophosphorylation of the epidermal growth factor (EGF) receptor, insulin receptor and the insulin-like growth factor I receptor, as well as c-*fos* mRNA expression induced by EGF, fibroblast growth factor and phorbol esters were insensitive to inhibition by CGP 53716. In antiproliferative assays, the compound was approximately 30-fold more potent in inhibiting PDGF-mediated growth of v-*sis* transformed BALB/c 3T3 cells relative to inhibition of EGF-dependent BALB/MK cells, interleukin-3-dependent FDC-P1 cells and the T24 bladder carcinoma line. When tested *in vivo* using highly tumorigenic v-*sis* and human c-*sis* transformed BALB/c 3T3 cells, CGP 53716 showed antitumor activity at well tolerated doses. In contrast, CGP 53716 did not show antitumor activity against xenografts of the A431 tumor, which overexpresses the EGF receptor. These findings suggest, that CGP 53716 may have therapeutic potential for the treatment of diseases which involve abnormal cellular proliferation induced by PDGF receptor activation.

A1-415 MYC AND GENOMIC ALTERATIONS AT THE DHFR LOCUS, Sabine Mai[1], Annika Jalava[2], Joan Hanley-Hyde[3]. [1]Basel Institute for Immunology, Grenzacherstr. 487, CH-4005 Basel, Switzerland, [2]Center for Biotechnology, P.O.Box 123, SF-20521 Turku, Finland, [3]Dept. of Molecular Biology MB-7, The Scripps Research Institute, La Jolla, California 92037, USA.
The dihydrofolate reductase gene (DHFR) is a c-Myc target gene (Mai and Jalava. 1994. *NAR*. 22: 2264-2273.). Upon induced overexpression of a transfected c-Myc cDNA, the protein complexes formed with DHFR E-box motifs are enhanced *in vitro*. Subsequently, the DHFR gene is amplified *in vivo* (Mai. 1994. *Gene*. In press). Furthermore, DHFR amplicons may be up- or downregulated with experimentally modulated c-Myc expression. To investigate genomic alterations at the DHFR locus, we have used fluorescent *in situ* hybridization of metaphase chromosomes and analysed cells overexpressing c-Myc in an inducible or constitutive way. In lymphoid and non-lymphoid cells from hamster, mouse, and human we have observed not only an increase in DHFR gene copy numbers at the original locus, but also DHFR signals as part of extrachromosomal elements and at new chromosomal locations. We discuss molecular mechanisms and consequences of unscheduled DNA replication at the dihydrofolate reductase (DHFR) locus under conditions of experimentally induced or constitutive c-Myc overexpression.

A1-416 THE CDK-ACTIVATING KINASE CAK CONTAINS A NOVEL CYCLIN (CYCLIN H) AND IS REGULATED DURING THE G0/G1 TRANSITION. Tomi P. Mäkelä and Robert A. Weinberg, Whitehead Insitute for Biomedical Research, Cambridge, MA 02142

The eukaryotic cell cycle is regulated by the sequential activation of cyclin-dependent kinases (CDKs). CDK activation is dependent on cyclin binding and phosphorylation of a conserved threonine (T 161 in cdc2) mediated by the CDK-activating kinase CAK. Recently a CDK-related kinase MO15 was identified as the catalytic subunit of CAK. We have identified a novel human cyclin (cyclin H) as a MO15-associated protein using a yeast two-hybrid screen. In cotransfection experiments cyclin H enhances the kinase activity of MO15 toward cdk2/cyclin A.. MO15 and cyclin H can be detected in physical complexes in vivo; this complex contains a third polypeptide of 32 kD. Anti-MO15 immunoprecipitates containing all three polypeptides have a strong kinase activity toward cdk2. This kinase activity is strongly induced during a G0/G1 transition in serum-stimulated HaCat keratinocytes. We are presently studying the mechanisms underlying this induction as well as trying to identify the signal transduction pathway leading to the activation of CAK.

A1-417 THE NOVEL TUMOR SUPPRESSOR p190 GTPase BLOCKS ONCOGENIC RAS SIGNALLING.

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Oncogenic mutations of RAS are found in 30 % of human carcinomas. A rapid disruption of actin stress fibers (cables) is an early event associated with RAS-induced malignant transformation. Interestingly the N-terminal SH2-SH3 domain of a RAS GTPase activating protein (GAP) called GAP1 also causes a rapid disruption of actin cables, and is weakly but significantly oncogenic. These observations indicate that GAP1 is a downstream target of oncogenic RAS. Furthermore, a RAS-related G protein called RHO is required for the organization of actin cables.

Thus, we have been exploring candidate tumor suppressors among the proteins which are involved in both RAS and RHO signal transduction pathways. Here we provide the first evidence indicating that the N-terminal GTPase domain of p190 acts as a tumor suppressor and blocks oncogenic RAS signalling.

p190 is one of the major Tyr-phosphorylated proteins that bind SH2 domain of GAP1. It contains two functional domains: a RHO GAP domain at its C-terminus and a GTPase domain at its N-terminus. We found that either a dominant negative mutant of its GTPase domain (GDP-bound form) or an anti-sense p190 DNA can transform normal NIH/3T3 cells, and that the wild-type GTPase domain of 250 amino acids alone can suppress RAS-induced malignancy.

These findings suggest that p190 acts as an antagonist of RAS by either sequestering GAP1 which serves as an effector of RAS, or binding (and activating) a downstream effector of p190 GTPase, probably a novel GAP, that specifically activates the p190 GTPase.

A1-418 A NEW TRANSACTIVATION DOMAIN PRESENT IN E12, E47, E2-2, AND HEB IS FUNCTIONALLY CONSERVED IN MAMMALIAN CELLS AND *Saccharomyces cerevisiae*, Mark E. Massari and Cornelis Murre, Department of Biology, University of California, San Diego, La Jolla, CA 92093-0116

The *E2A* gene, which encodes the helix-loop-helix (HLH) proteins E12 and E47, is involved in two acute lymphoblastic leukemias (ALL). In pro-B ALL, the E2A gene is translocated to a gene, designated *HLF*, located on chromosome 17. This translocation event replaces the E2A HLH domain with that of a leucine zipper DNA binding and dimerization domain. In pre-B ALL, involving a t(1;19) translocation, the E2A HLH domain is replaced with a homeodomain derived from the *Pbx1* gene. The presence of the E2A N-terminus in two fusion proteins, resulting from two different translocations raises the question: What is the role of the E2A domain in pro-B and in pre-B ALL?

We have identified a highly conserved stretch of amino acids, designated the HADI domain, which is present in the extreme N-terminus of a class of HLH proteins, including E12, E47, HEB, E2-2, and a *Xenopus laevis* HLH protein closely related to E12. We show that the HADI domain contains a highly conserved potential α-helix that is crucial for its transactivation properties. Specifically, amino acid substitutions that disrupt the putative helix abolish the transactivation ability of a GAL4-HADI fusion protein. Similarly, amino acid substitutions in the conserved helix of the E12 HADI domain and a second conserved helix present in a previously characterized E12 activation domain, have a dramatic impact on full-length E12 mediated transactivation. These data suggest that transactivation of this class of HLH proteins is mediated by two strikingly conserved helices, present in two distinct activation domains. Remarkably, when wild-type and mutant proteins are expressed in mammalian cells and *Saccharomyces cerevisiae*, identical patterns of transactivation are observed, suggesting that the target molecule is conserved between yeast and mammals.

A1-419 REGULATION OF THE EXPRESSION OF THE Cdk INHIBITOR p21 IN SENESCENCE OF RAT EMBRYO FIBROBLASTS, Georges-Raoul Mazars, Andy Powell, Jorge Burns, Anita Kobrna and Parmjit Jat, Ludwig Institute for Cancer Research, Riding House Street, London W1P 8BT

Normal rodent fibroblasts cultured *in vitro* undergo a limited number of divisions before entering a senescent phase in which they can be maintained for long periods but can not be induced to divide. In rodent fibroblasts, this loss of proliferative potential can be overcome by expression of any member of a family of viral and cellular immortalising genes such as the SV40 large T antigen (LT Ag). We have further shown that Rat Embryo Fibroblasts (REFs) immortalised with the thermolabile LT Ag derived from *tsA58* grow continuously at the permissive temperature but rapidly growth arrest upon shift up to the nonpermissive temperature where the LT Ag is inactivated. Analysis of these cell lines suggested that this loss of proliferative potential mimicks senescence.

Recently it has been suggested that the p21 gene is upregulated in senescence of human fibroblasts. To further evaluate our cell lines as a model for studying senescence, we have isolated the rat p21 cDNA. Northern blot analysis has shown that the p21 mRNA is specifically upregulated about 10 fold within 12-24 hours of transfer to the nonpermissive temperature. We have carried out a detailed time course analysis for shift up-shift down conditions to better understand p21 mRNA expression. Analysis of RNA prepared from early proliferating REFs and late passage REFs also shows upregulation. However, the p21 mRNA was detectable even in the early passaged REFs in contrast to our conditional cell lines where it becomes only detectable upon transfer to the nonpermissive temperature. These findings are consistent with the idea that senescence in a primary culture is an asynchronous process and that our conditional cell lines represent a system where senescence occurs in a synchronous manner.

We have also previously shown that the loss of proliferative potential can be overcome by introduction of SV40 LT Ag mutants identifying several regions that are critical for growth defect complementation. Experiments are now underway to determine whether the p21 upregulation correlates or not with a particular LT Ag region.

We present rat p21 cDNA sequence which reveals a perfectly conserved middle part of amino terminus region at amino acid level with human sequence suggesting an important function. Furthermore, we overexpress rat p21 as a GST fusion protein in bacteria and present its binding properties with cellular proteins.

A1-420 CONTROL OF CELL CYCLE PROGRESSION BY CYCLIN-DEPENDENT KINASE INHIBITORS: $p21^{CIP1}$ AND $p16^{INK4}$. René H. Medema and Robert A. Weinberg, Whitehead Institute for Biomedical Research, Cambridge, MA 02142, USA.

Timed activation of a specific complex of a cyclin-dependent kinase (cdk) with its cognate cyclin is thought to provide the basis for the cell cycle clock. This activation is the net result of several events: association of the cdk with a cyclin, phosphorylation of the cdk on a conserved threonine residue (Thr 160 in cdk2), and possibly dephosphorylation of a conserved serine (Ser14 in cdk2) and tyrosine (Tyr15 in cdk2) residue. Furthermore, several inhibitors of these cyclin/cdk complexes have recently been identified, adding yet another level of regulation to cyclin/cdk activation. One of these inhibitors, $p21^{CIP1}$, can inhibit a large variety of cyclin/cdk complexes, displaying its highest activity toward complexes of cdks with G1 cyclins, whereas another, $p16^{INK4}$, seems to specifically inhibit the kinase activity of cyclinD/cdk4 complexes. Overexpression of either $p21^{CIP1}$ or $p16^{INK4}$ in a transient transfection assay causes a dramatic G0/G1 arrest in a human osteosarcoma cell line (U2OS). However, only overexpression of $p21^{CIP1}$, but not $p16^{INK4}$, can cause an arrest in a distinct human osteosarcoma cell line (SAOS-2) which lacks functional retinoblastoma protein (pRb). This would suggest that the cyclin D/cdk4 complexes function solely to overcome the negative growth regulation imposed by pRb. We are in the process of extending these findings to mouse embryo fibroblasts derived from Rb nullizygous embryos and wild type embryos. In addition, we are trying to obtain cell lines carrying inducible constructs of these inhibitors to study at which point in the cell cycle the arrest occurs upon induction of each separate inhibitor.

A1-421 EFFECTS OF PHOSPHATASE TYPE 1 GENE DISRUPTION ON HUMAN p53 BIOCHEMICAL PROPERTIES IN *S. pombe*, Gavin J. Milczarek and G. Tim Bowden, Department of Molecular and Cellular Biology, University of Arizona, Tucson, AZ 85721. While examples of protein kinases involved in oncogenic processes are prevalent, the role of protein phosphatases (pp's) in carcinogenesis is comparatively unknown. Accordingly, the role of pp's in the regulation of p53 activity *in-vivo* has yet to be established. Thus, our objective has been to assay both mutant and wildtype human p53 for differences in growth inhibiting and biochemical properties in a genetic background containing phosphatase gene knock-outs. Due to the difficulty of genetic manipulation in mammalian models, we have commenced these studies in the yeast *S. pombe*, a system in which over-expression of human p53 has been shown to produce a wildtype specific growth-restricted phenotype. In addition, aminoacid sequencing analysis has shown that mammalian and *pombe* phosphatases are quite similar (greater than 75% identical), and tryptic peptide analysis in *pombe* and mammalian cells produce similar p53 phosphopeptide maps . Our initial results indicate that hybrid strains containing inducible mutant human p53 and a disrupted Dis2 allele (the major protein phosphatase type 1 activity in *pombe*) has very poor growth compared to the parental strains and strains in which wildtype p53 is expressed. We are following-up on these observations by focusing on potential alterations in the biochemistry of p53 in this system. Phosphorylation assays for p53 +/- dis2 are currently in progress. Additionally, DNA binding, oligomerization, and protein conformation assays also are underway.

This work was supported by Grants-in-Aid of Research from the Flinn Genetics Foundation and Sigma Xi, The Scientific Research Society.

A1-422 Ras-dependent signals leading to JNK activation are channeled through MEKK and not Raf. Audrey Minden, Anning Lin, Martin McMahon, Carol Lange-Carter, Benoit Derijard, Roger J. Davis, Gary L. Johnson, and Michael Karin. Department of Pharmacology. University of California, San Diego. La Jolla, CA 92093

Two new members of the mitogen activated protein (MAP) kinase group, JNK1 and JNK2 were recently identified by their ability to phosphorylate the c-Jun activation domain and potentiate its activity. Like the ERK1 and 2 MAP kinases, JNK activity is stimulated in response to growth factors and oncogenic Ha-Ras. The pattern of JNK activation differs from that of ERK activation however, suggesting that JNKs are activated by a different signaling cascade than the one involved in ERK activation. The activation of JNKs by Ha-Ras suggests that at least one signaling pathway leading to their activation is Ras-dependent. We have examined the roles of Ha-Ras and Ras activated protein kinases in activation of JNK in response to growth factors including epidermal growth factor (EGF) and nerve growth factor (NGF). We have found that activation of JNK by growth factors is dependent on normal Ha-Ras function. Activated Ras was previously shown to activate the serine/threonine kinase Raf-1 and more recently, has also been shown to be essential for activation of another serine/threonine kinase, MEKK1. Although Raf-1 is a strong activator of ERK1 and 2, it is an inefficient activator of the JNK pathway. On the other hand, MEKK1 is a potent activator of JNK and the effect of MEKK on JNK activity is more pronounced than its effect on ERK activity. These studies suggest that MEKK and Raf-1 define an important divergence point in Ras signaling. While the downstream effect of MEKK activation is channeled mostly toward JNK activation, the signal transmitted by Raf-1 leads only to ERK activation.

A1-423 SIGNALING REQUIREMENTS FOR ACTIVATION OF CDK4 AND CDK6 AND PHOSPHORYLATION OF $p107^{Rb}$ IN COMPETENT HUMAN T CELLS, Jaime F. Modiano, Joanne Domenico, Joseph J. Lucas, and Erwin W. Gelfand, Division of Basic Sciences, Department of Pediatrics, National Jewish Center For Immunology and Respiratory Medicine, 1400 Jackson Street, Denver, CO 80206.

Competent T cells are those that exit G_0, but stop their passage through the cell cycle at a defined point before entry into S phase (i.e., before the R point). These cells have been termed competent due to the fact that the addition of a second signal such as interleukin-2 (IL-2) will allow the cells to progress into S phase and undergo cell division. Induction of competence by a brief stimulation (20 minutes) with PDB and ionomycin, or by suboptimal concentrations of phytohemagglutinin (PHA), stimulated accumulation of CDK4 and CDK6 proteins that was IL-2-independent and cyclosporin resistant. Both enzymes had Rb kinase activity in vitro, and the maintenance of the competent state was paralleled by $p107^{Rb}$ phosphorylation in vivo. This IL-2-independent phase of CDK4 expression and $p107^{Rb}$ phosphorylation stimulated by PHA was markedly reduced in the presence of calcium chelators, despite the fact that these agents did not inhibit the Rb kinase activity of CDK4 directly in vitro. In contrast, neither protein kinase C inhibitors nor protein tyrosine kinase inhibitors affected the phosphorylation of $p107^{Rb}$ noticeably. These results indicate that triggering of the antigen receptor stimulates the expression and activation of CDK4 (and CDK6) leading to $p107^{Rb}$ phosphorylation, and that these events are dependent on calcium mobilization. The data also suggest that the induction and maintenance of competence in normal human T cells may be dependent on maintaining $p107^{Rb}$ in its phosphorylated state.

A1-424 EVOLUTIONARY CONSERVATION OF BCR KINASE ACTIVITY Randolph N. Mohr[1], Yoshiro Maru[3], Daniel E.H. Afar[1] and Owen N. Witte[1,2] Department of Microbiology and Molecular Genetics[1] and Howard Hughes Medical Institute[2], University of California-Los Angeles, CA 90024. The Institute of Medical Science, University of Tokyo, Japan.[3]

The BCR-ABL oncogene is the product of a chromosomal translocation fusing 5' BCR sequences to 3' ABL. The fusion protein has a deregulated tyrosine kinase activity resulting in cellular transformation. The 5' BCR sequences have been shown to be critical for transformation activity. It's essential features include an oligomerization domain and a GRB-2 binding site. The full length cDNA of human BCR has a unique ser/thr kinase activity in the first exon, a carboxy terminal rho-GAP activity and central DBL homologous and PH domains. Sequence comparison of human, mouse and chicken BCR reveals a striking degree of homology. The DBL and GAP domains are 95% conserved across all three species at the amino acid level. The amino terminal domain is less homologous with several insertions in the chicken sequence. However, the GRB-2 binding site and CYS332, the cysteine essential for kinase activity, are both retained in mouse and chicken BCR. Biochemical analysis reveals the unique kinase activity is conserved in all three species.

A1-425 REGULATION OF PROGRESSION THROUGH THE G2/M BOUNDARY OF THE CELL CYCLE BY THE CREM GENE PRODUCT, ICER. Carlos A. Molina and Paolo Sassone-Corsi, Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, U184 de l'INSERM, Faculté de Medecine, 11, rue Humann, 67085 Strasbourg, France
The CREM gene encodes a novel CRE binding factor termed ICER (Inducible cAMP Early Repressor). This factor consists of essentially only a DNA-binding domain (DBD) and functions as a powerful repressor of cAMP-induced transcription. ICER is generated by the use of an alternative intronic promoter which contains four CREs in tandem and which confers cell-specific cAMP inducibility. Importantly, ICER binds to its own promoter and represses its own production constituting a negative autoregulatory control mechanism. Interestingly, ICER is expressed at high levels predominantly in tissues of neuroendocrine origin. Here we report for the first time rhythmic expression of ICER during the cell cycle of the pituitary corticotroph, AtT20 cells. cAMP induces DNA synthesis in AtT20 cells, however, prolonged induction arrests the cells at the G2/M stage of the cell cycle. This arrest is due to expression of ICER since its ectopic expression augments dramatically the number of cells arrested in G2/M. In cells overexpressing ICER, cyclin A expression is deregulated, which suggests a possible mechanism to explain the G2/M arrest. Finally, we show that ICER rhythmic expression during the cell cycle may be explained in part by the previously documented negative autoregulation of ICER. These results place ICER in a pivotal position as a pacemaker in the neuroendocrine cell cycle.

A1-426 MYC-LIKE NOVEL bHLH PROTEINS BIND TO THE G-BOX IN THE PROMOTER OF BEAN SEED STORAGE PROTEIN ß-PHASEOLIN GENE, **Norimoto Murai and Yasushi Kawagoe, Plant Path. & Crop Phys., Louisiana State Univ. and LSU Agr. Ctr, Baton Rouge, LA 70803-1720**
Phaseolin is the predominant seed storage protein of common bean (*Phaseolus vulgaris*). Phaseolin expression is developmentally regulated mainly at the transcription level. DNA/protein binding assays indicated that three CANNTG (E-box) motifs were bound by a DNA binding protein CAN. Substitution mutation of the CACGTG (G-box) reduced the -295 promoter activity by 75 % in transient gene expression assay. In addition, the mutation analyses demonstrated that the G-box acts synergistically with a second CAN binding site CACCTG. These results suggest that CAN and the three E-box motifs play a major role in transcriptional regulation of the β-phaseolin gene. To understand the molecular nature of CAN, we isolated three cDNAs from a bean seed cDNA expression library, based on binding activity of proteins expressed in *E. coli* to an oligonucleotides probe containing phaseolin G-box sequence. Sequence analyses demonstrated that these cDNA clones represent two different genes and share an almost identical sequence for a basic region/helix-loop-helix (bHLH) domain. The bean bHLH domain belongs to that of the oncogene *myc* family and are most closely related to the maize *R* gene. Maize *R* gene family and *Antirrhinum del*, both of which are regulatory genes in anthocyanin synthesis, are only bHLH genes that have been isolated from plants. Genetic distance among the two bean bHLH genes, a maize *R*, and *Antirrhinum del* suggested that the bean bHLH genes are not a *del* counterpart in bean. We suggest that the bean bHLH genes represent a novel gene family of plant transcription factor.

A1-427 ROLES FOR Rb AND p53 IN DIFFERENTIATION OF THE OCULAR LENS, Paul A. Overbeek, Janet Butel and Larry Fromm, Departments of Cell Biology and Molecular Virology, Baylor College of Medicine, Houston, TX 77030
The ocular lens provides an excellent system for studies of cell cycle control during terminal differentiation. In the lens, differentiation of the epithelial cells into post-mitotic fiber cells is spatially specified and is accompanied by distinctive changes in cellular architecture. In order to examine the role of the retinoblastoma protein (pRb) in the differentiation process, viral proteins from SV40 (truncated T antigen) and human papillovavirus (E7) were linked to the lens-specific αA-crystallin promoter and used to generate transgenic mice. Expression of either transgene resulted in a dramatic reduction in the size of the lens (microphakia), which was an unexpected result for tissue-specific inactivation of a tumor-suppressor protein. The microphakia is caused by apoptosis in the lens fiber cells, as shown by the TUNEL assay, and by degradation of the DNA into nucleosome-sized fragments. Assays for incorporation of BrdU revealed that the normally post-mitotic fiber cells were inappropriately entering the S phase of the cell cycle prior to undergoing programmed cell death. In order to assay for the roles of cyclins and cyclin-dependent kinases in both the normal and transgenic patterns of lens cell differentiation, *in situ* hybridizations have been done with cyclin A, cdk2 and cdk4 probes. In addition, the transgenic mice with lens ablation have been mated to mice that are null for p53 or that express bcl-2 in the lens. In both of these situations fiber cell death was suppressed. Our results indicate that pRb plays a crucial role in blocking the cell cycle during terminal differentiation of lens fiber cells. Inactivation of pRb causes inappropriate entry into S phase, inappropriate induction of cell cycle regulatory genes, and p53-mediated programmed cell death.

A1-428 MULTIPLE ROLES FOR ONCOGENES AND TUMOUR SUPPRESSOR GENES DURING TGF-ß1-INDUCED GROWTH INHIBITION OF TUMOUR-DERIVED HUMAN ORAL KERATINOCYTES, Vyomesh Patel, Ian Paterson, William A. Yeudall, and Stephen S. Prime, Department of Oral and Dental Science, University of Bristol Dental School, England, UK.

Transforming growth factor Beta1 (TGF-ß1) leads to the arrest of normal epithelial cells in the late G_1 phase of the cell cycle, an event linked to c-*myc* down regulation and the accumulation of the hypophosphorylated form of pRB. This study examines the effect of TGF-ß1 on certain putative oncogenes (c-myc, cyclin-D, cdk4,5) and tumour suppressor genes (RB-1, p53, p16, p21) in tumour derived human oral keratinocyte cell lines known to express a range of responses to exogenous TGF-ß1 (1-10 ng/ml; 0-48 hrs). c-*myc* expression was down regulated in the responsive cell lines (n=5) but remained unchanged in both the partially responsive (n=2) and refractory (n=1) cell lines. The ligand caused the accumulation of hypophosphorylated pRB in all cell lines. Cyclin D1, D2, cdk4 and cdk5 gene amplification was consistent with TGF-ß1-induced growth inhibition; gene amplification, however, did not result in increased expression. TGF-ß1 did not modulate gene expression of cyclin D1 and D2. The presence of $p21^{ras}$ and p53 mutation in the cell lines appeared unrelated to both TGF-ß1 induced growth inhibition and nuclear responses to the ligand.

The data indicates that whilst TGF-ß signal transduction appears to involve c-*myc*, the pathway may be independent of pRB phosphorylation and the presence of $p21^{ras}$ and p53 mutations. This suggests the existence of other signal transduction pathways possibly involving the exp ression of the cdk's and the cdk inhibitory proteins.

A1-429 MODULATION OF THE DNA-BINDING ACTIVITY OF THE HOMEOBOX PROTEINS Hoxb-7, Hoxb-8 AND ENGRAILED BY Pbx PROTEINS IS MEDIATED THROUGH DIFFERENT INTERACTION DOMAINS
Lucy T.C. Peltenburg, Marc A. van Dijk and Cornelis Murre, Department of Biology, University California San Diego, La Jolla, CA 92093-0116

The human *Pbx1* homeobox gene is located at the chromosomal breakpoint of the t(1;19) translocation that is found in approximately 25% of pre-B acute lymphoblastic leukemias. When the *Pbx1* gene is translocated in t(1;19) pre-B leukemia, the amino-terminal 88 amino acids of Pbx1 are replaced by the amino-terminal transactivation domain of the *E2A* gene, coding for the helix-loop-helix transcription factors E12 and E47. The Pbx1 homeobox protein shows strong homology to the *Drosophila* protein extradenticle.
Previously, we showed that extradenticle can interact with the products of the homeotic genes *Abd-A* and *Ubx*, as well as with the segment polarity gene product engrailed. This indicates that extradenticle functions as a co-factor in modifying the DNA-binding and regulatory properties of other homeotic gene products.
Recent studies show that Pbx1 and its family member Pbx2 can cooperatively interact with the mammalian homeobox proteins Hoxb-7, Hoxb-8 and murine engrailed-2. The affinities of the Hox and engrailed-containing complexes for several synthetic sites differ, suggesting that *in vivo* different combinations of homeobox proteins recognize distinct sites on the DNA.
Furthermore, the region of the Pbx1 protein that is needed for cooperative interaction with the homeobox proteins Hoxb-7, Hoxb-8 and mouse engrailed-2 has been mapped.We demonstrate that for cooperative binding with Pbx1, Hoxb-7 and Hoxb-8 require a region N-terminal of the homeodomain, whereas the Pbx1 homeodomain itself is sufficient for interaction with engrailed-2.
In addition, we show that the chimeric oncogene product E2A-Pbx1 has retained the ability to interact with the other homeobox proteins. Since some Hox proteins are expressed in normal pre-B cells and the Pbx1 gene is normally not expressed in those cells, these results suggest that in pre-B leukemias the expression patterns of Pbx-responsive target genes are inappropiately modulated by the transforming fusion protein.

A1-430 REARRANGEMENTS OF RET PROTO-ONCOGENE IN PAPILLARY THYROID CARCINOMAS FROM CHILDREN EXPOSED TO CHERNOBYL NUCLEAR ACCIDENT.
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The greatly increased number of papillary thyroid carcinomas reported in children who were living in Southern Belarus at the time of Chernobyl nuclear accident has risen a number of concerns regarding both a true increase in the incidence of thyroid cancer and its link to the disaster. Relevant answers to these questions could be provided by an analysis of oncogene activation in these thyroid tumors. The proto-oncogene RET has been found activated with significant frequency, by gene fusion following chromosome rearrangements, in papillary thyroid carcinoma. Moreover, gamma irradiation of in vitro cell cultures has been found capable to cause RET activation by the same mechanisms. We have analyzed by Southern-blotting, six samples of papillary thyroid carcinoma from children from the Belarus region. We found in four cases rearranged bands indicating the formation of chimeric transforming sequences. Three of them were found to belong to the oncogene RET/ptc3, the product of the fusion between ELE1 gene and the tk domain of RET. The fourth positive case was identified as RET/ptc2 (RIA gene/RET tk chimeric sequence). The DNA of all the three RET/ptc3 cases transformed NIH-3T3 cells following the standard DNA transfection assay. These results indicate that oncogenic versions of the RET proto-oncogene could play a significant role in the formation of papillary thyroid carcinomas associated with the Chernobyl nuclear accident.

This work was supported by CNR "ACRO Project" and AIRC and by JSP4 EEC Chernobyl Projects, COSU-CT93-0052.

A1-431 INSULIN STIMULATES THE DEPHOSPHORYLATION OF p125 FOCAL ADHESION KINASE (FAK): THE DISTAL C-TERMINUS OF THE INSULIN RECEPTOR β-SUBUNIT IS REQUIRED, T. S. Pillay and J. M. Olefsky, Division of Endocrinology & Metabolism and VA Medical Center, UCSD, La Jolla CA 92093-0673
The phosphorylation state of FAK in response to insulin was examined in parental and transfected Rat 1 fibroblasts expressing both wild-type (HIRc cells) and mutant human insulin receptor cDNAs lacking the C-terminal twin tyrosine phosphorylation sites (YF2) or a deletion mutant lacking the distal 43 amino acids of the β-subunit (ΔCT). Semiconfluent serum-starved cells were stimulated with 10 μM insulin for 10 minutes and then lysed and FAK was immunoprecipitated using a monoclonal antibody and analysed by antiphosphotyrosine immunoblotting. In HIRc cells insulin stimulated the tyrosine dephosphorylation of FAK. In contrast, the tyrosine phosphorylation state of FAK was unchanged in the parental Rat1 fibroblasts and the YF2 or ΔCT mutant cell lines in response to insulin. Analysis of the supernatants revealed, that FAK was only a minor component of the major Mr 120-130 phosphotyrosine band seen in HIRc cells. We conclude that: 1) In contrast to other growth factors, insulin stimulates the dephosphorylation of FAK; 2) The presence of the C-terminal tyrosines 1316 and 1322 is required for the insulin stimulated dephosphorylation of FAK suggesting a possible SH2 domain-dependent interaction; 3) Insulin may modulate integrin-mediated signaling through FAK by altering the phosphorylation state of FAK.

A1-432 MAINTENANCE OF TUMORIGENICITY FOLLOWING LOSS OF ACTIVATED N-*RAS* FROM HT1080 FIBROSARCOMA CELLS

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Activated oncogenes are observed in a variety of human tumors which suggests that they play an important role in carcinogenesis. Although "dominant" oncogenes can induce neoplastic transformation in rodent cells, normal or spontaneously immortalized human cells appear relatively resistant to transformation by activated oncogenes. Therefore, oncogenes appear to be necessary but not sufficient to induce neoplastic progression. In addition, it is unknown whether continued oncogene expression is necessary after cells have become fully malignant.

In order to determine if activated *ras* oncogene expression is necessary for maintenance of tumorigenicity, we studied the human fibrosarcoma cell line, HT1080, which contains one mutant and one wild-type copy of the N-*ras* gene. We isolated a variant of HT1080 that lacked the activated N-*ras* allele due to spontaneous chromosomal translocation and subsequent loss. This cell line demonstrated altered in vitro growth characteristics as compared to the parental HT1080 cell line. The variant grew more slowly, had a flat morphology, regained the ability to form organized actin filaments, and no longer was capable of anchorage-independent growth. Therefore, the variant appeared to have a less transformed in vitro phenotype. However, in vivo growth studies showed that the variant was still capable of forming tumors in athymic mice, although at a slower rate. In summary, although loss of the mutant N-*ras* allele probably was responsible for the in vitro growth changes observed, activated N-*ras* expression did not appear to be necessary for maintenance of the tumorigenic phenotype.

We currently are reintroducing the activated N-*ras* allele into the variant cell line in order to determine if the changes observed are due to its loss. Preliminary results suggest that there is a reversion in morphology to a more transformed phenotype.

A1-433 REGULATION OF INTRACELLULAR β-CATENIN LEVELS BY THE APC TUMOR SUPPRESSOR GENE,

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Germline mutations in the APC tumor suppressor gene result in the accrual of numerous colorectal polyps by the second or third decade of life. Mutations in the APC gene are also consistently detected in the early stages of sporadic tumor growth in the large intestine and stomach. How the loss of APC function contributes to tumor formation is not understood, but the recent finding that the APC protein associates with the cadherin-binding protein β-catenin suggests that deregulation of cell adhesion may be involved. Although the functional relationship between the β-catenin and APC protein is unknown, it is clear that certain cadherins require the association of β-catenin for their normal activity in maintaining calcium-dependent cell-cell contacts. The stability of the β-catenin-cadherin complex is in turn modulated by a postranscriptional mechanism affecting the relative stability of β-catenin itself. Since the accumulation of β-catenin correlates with the oncogenic effects of the wnt-1 oncogene, we investigated the possibility that the APC tumor suppressor may act counter to this mechanism, thereby lowering the levels of intracellular β-catenin. Introduction of wildtype APC into the SW480 colorectal cancer cell resulted in a significant decrease in the amount of total intracellular β-catenin. In particular, the excessive supply of cytoplasmic β-catenin observed in this cell was selectively depleted, as determined by imunocytochemical detection and size fractionation chromatography. By contrast, the levels of α-catenin remained unaffected. Mutational analysis revealed that the central region of APC, containing a repeated 20-amino acid sequence, was required for the reduction of β-catenin levels. The limit APC fragment harboring the in vivo activity was also capable of directly binding to β-catenin in vitro. Thus, the region of APC typically found deleted or severely truncated in tumor cells, has the capacity to bind to β-catenin in vitro and reduce its levels in vivo.

A1-434 SCREENING FOR p27*Kip1* GENE ALTERATIONS IN PRIMARY TUMORS,

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Alterations in cell proliferation are at the center of oncogenic processes. Orderly progression through the cell cycle is driven by cyclin dependent kinases (CDK) and cyclins, their regulatory subunits. A group of proteins that inhibit these complexes, therefore having potential tumor supressor functions, have recently been described. These include p21/WAF1/Cip1, p16/INK4/MTS1 and p27/Kip1. Until now, no alterations in p21 have been reported. However, p16 has been found to be altered by homozygous deletions or point mutations in a number of cell lines derived from melanomas and other tumor types as well as in some primary tumors. We have investigated possible p27 gene alterations in some human cancers. We examined fresh frozen pairs of normal and primary tumor tissues using Southern blots, PCR-SSCP and DNA sequence. The tumor types analyzed included: pancreatic carcinoma (ca) (n=21 pairs), bladder ca (n=15 pairs), breast ca (n= 10 pairs), renal ca (n=17 pairs), prostate ca (n=20 pairs), melanomas (n=7 pairs), sarcomas (n=20 pairs) and lung ca (n=10 pairs).
Up to now we have not found any cancer specific mutation in p27, suggesting that alterations of this gene may not be common events in human cancer.

A1-435 OVEREXPRESSION OF AN AMINO OR CARBOXYL-TERMINAL TRUNCATED MYB ONCOPROTEIN INDUCES IN VIVO B CELL LYMPHOMAS.

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The c-myb oncogene encodes a nuclear DNA-binding transcriptional transactivator that is terminally-truncated in a variety of naturally-occurring hematopoietic tumor cell types. To directly assess the role of these terminal deletions in the activation of the oncogenic ability of myb, we have produced retroviruses expressing amino- or carboxyl-terminal truncated versions of myb and injected these into chicken embryos.

A consistent minority of birds infected with any of these viruses were found to harbor widely metastatic B cell lymphomas (see table below). Most of these tumors contained clonal retroviral integrations within the 5' end of the endogenous cellular c-myb oncogene. These "insertional" lymphomas therefore each expressed a 5' truncated c-myb mRNA and an amino-terminal truncated protein (without its first 78 amino acids).

Unexpectedly, 5 of the 12 lymphomas in the animals infected with a C-terminal truncated myb (T-myb) did not contain c-myb proviral insertions. These "non-insertional" lymphomas harbored an intact T-myb provirus and expressed the expected T-myb protein. The overexpression of a C-terminal truncated myb can therefore directly induce in vivo B cell lymphomas. The loss of an intact N or C terminus is therefore sufficient for the direct activation of myb's lymphomagenic activity.

The N-terminal sequence deleted from the insertional lymphomas includes a casein kinase II phosphorylation site and an adjacent acidic region. The C-terminal sequence deleted from the non-insertional lymphomas includes the transcriptional inhibition domain. The deletion of these regions in oncogenically-active forms of myb suggests that these sequences may function to attenuate the activity or expression of other growth-regulatory factors. We are now fine-mapping these crucial control regions and identifying the factors and/or genes with which they interact.

Construct (amino acids 0–700; CK II P, DNA Binding Domain, Transcription Activation Domain, LZ, Transcription Inhibition Domain (2))	# Birds Infected	Total Lymphomas (%)	Insertional Tumors (%)
C-myb (1–699)	50	4 (8%)	0-2 (0-50%)
ΔN-myb (79–699)	60	11 (18%)	10 (91%)
VCC-myb (130–699)	22	5 (23%)	5 (100%)
T-myb (1–485)	52	12 (23%)	7 (58%)
RCAMV-0 (No myb insert)	27	4 (15%)	4 (100%)

A1-436 REPRESSION OF THE TYROSINASE GENE PROMOTER BY TUMOR SUPPRESSOR P53 IN HUMAN MELANOMA CELLS, Sikha Rauth and Julia Kichina, Department of Surgical Oncology and Department of Genetics, University of Illinois at Chicago, Chicago, IL 60612

The nuclear phosphoprotein p53, implicated in the control of cell proliferation, can modulate expression of several genes. Here, we investigated whether p53 regulates activity of promoter of tyrosinase, the essential gene in melanin synthesis. Experiments in which tyrosinase promoter-CAT construct was transiently cotransfected along with expression vectors for wild and mutant p53 into pigmented human melanoma cells, either lacking p53 expression (UISO-MEL-6) or expressing low levels of normal p53 mRNA (UISO-MEL-7), revealed that both wild type and mutant p53 could repress gene expression from tyrosinase promoter in a dose-dependent manner. These results suggest that p53 protein is involved in tyrosinase gene regulation, and hence melanogenesis, in melanoma cells.

A1-437 DIFFERENTIAL EFFECTS OF CYCLINS D1 AND E ON pRb PHOSPHORYLATION, Dalia Resnitzky[1] and Steven I. Reed[2], [1]Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel. [2]Department of Mol. Biol. Scripps Research Institute, N. Torrey Pines Rd. La Jolla Ca, 92037.

Ectopic expression of cyclins D1 and E was previously shown to accelerate the G1/S phase transition, indicating that both classes of G1 cyclin control an event(s) that is rate-limiting for entry into S phase. In order to explore the molecular basis for G1 cyclins action, we used rat fibroblast cell lines capable of expressing cyclins D1 and E prematurely and measured the effect of cyclin expression on phosphorylation of the retinoblastoma susceptibility gene product (pRb). We found that while expression of either cyclin advances the G1/S phase transition, expression of cyclin D1 leads to immediate appearance of hyperphosphorylated pRb, while expression of cyclin E does not. Ectopic expression of both cyclins E and D1 in the same cell caused an additive effect on acceleration of the G1/S phase transition. Based on these results, we suggest that cyclins D1 and E function parallel to each other, rather than redundantly or in a sequential order, in regulation of the G1/S phase transition.

A1-438 TWO DIFFERENT ETS RELATED TRANSCRIPTION FACTORS - PU.1 AND GABP - COMPETE FOR BINDING SITES IN THE PROMOTER OF THE ß2 LEUKOCYTE INTEGRIN, CD18. Alan Rosmarin, David Caprio, David Kirsch, Nick Rockwell, Carl Simkevich; Department of Medicine; Brown University/The Miriam Hospital, Providence, RI 02906.

CD18 (ß2 leukocyte integrin), the common ß chain of the cell adhesion molecules LFA-1, Mo1, and p150/95, plays a crucial role in cell-cell and cell-matrix interactions of leukocytes. CD18 expression is limited to leukocytes, and it is transcriptionally regulated during myeloid (monocytic and granulocytic) differentiation. We have cloned the CD18 gene in order to characterize the *cis* elements and *trans* acting factors which regulate gene expression during myeloid differentiation. Following transient transfection, the CD18 promoter is active in myeloid and B and T lymphoid cell lines, but not in non-hematopoietic cells. Tissue specific and myeloid inducible activity is encoded within 79 nt of the CD18 transcriptional start site. Within this minimal promoter, we have identified binding sites for the myeloid and B lymphoid restricted *ets*-related transcription factor, PU.1. Mutagenesis of these sites significantly decreases CD18 promoter activity. PU.1 from myeloid and B lymphoid nuclear extracts, binds to these critical DNA elements. We also identify a very low mobility species that binds to this region of the CD18 promoter. This species consists of the ubiquitous transcription factors GABPα and GABPß binding in a high molecular weight complex. GABPα is an *ets*-related transcription factor which forms a heteromeric complex with the *notch*-related transcription factor, GABPß. Purified GABPα binds to the CD18 promoter as a monomer or homodimer; purified GABPß alone does not bind to CD18. Together these factors exhibit cooperative, high affinity binding. We demonstrate that the ubiquitous, low mobility binding complex represents a tetramer of GABPα and GABPß. GABP complexes bind to the CD18 promoter through the same sites as PU.1. Thus, a tissue specific *ets* factor, PU.1, and a complex containing a ubiquitously expressed *ets* factor, GABPα, compete for sites which are critical for the tissue specific and myeloid inducible expression of CD18.

A1-439 COOPERATION OF WILD-TYPE p53 AND c-myc IN APOPTOSIS OF A RAT HEPATOCELLULAR CARCINOMA CELL LINE(FAA-HTC1), Yoshinori Saito and Katsuhiro Ogawa, Department of Pathology, Asahikawa Medical College, Asahikawa, Hokkaido, Japan

FAA-HTC1 cells, a rat hepatocellular carcinoma cell line, expressed no detectable p53 with unregulated c-myc, and became apoptosis by treatment with chemotherapeutic compounds. We expressed wild-type p53 in this cell line by introducing a dexamethasone-inducible wild-type rat p53 expression vector. On the other hand, c-myc could be suppressed by treatment with the c-myc antisense oligonucleotides. Apoptosis was induced by expression of p53 in the p53-transformants, which was significantly inhibited by suppression of c-myc. Treatment with a low dose of cycloheximide markedly enhanced apoptosis, suggesting that the apoptotic pathway potentiated by c-myc and/or p53 may be further facilitated by a loss of putative survival protein(s). On the other hand, although either p53 expression or c-myc suppression only weakly affected cell growth, simultaneous p53 expression and c-myc suppression significantly inhibited cell proliferation. These results indicate that p53 can act either as an apoptosis-inducer or growth-suppressor within the same cell depending on the status of c-myc.

A1-440 INVESTIGATIONS OF INTERACTIONS BETWEEN MEMBERS OF THE Bcl-2 PROTEIN FAMILY USING YEAST TWO-HYBRID SYSTEM.

Takaaki Sato, Hiroko Kobayashi, Motoi Hanada, Sharon Bodrug, Shinji Irie, Hong-Gang Wang, John C. Reed. La Jolla Cancer Research Foundation, Oncogene & Tumor Suppressor Gene Program. La Jolla, CA 92037

Interactions of the Bcl-2 protein with itself and other members of the Bcl-2 family, including Bcl-X-L, Bcl-X-S, Mcl-1 and Bax were explored using a yeast two-hybrid system. Fusion proteins were created by linking Bcl-2 family proteins to a LexA DNA binding protein or a B42 trans-activation domain. Protein-protein interactions were examined by expression of these fusion proteins in *S. cerevisiae* carrying a lacZ gene under control of a LexA operator. Using this approach, evidence for Bcl-2 protein homo-dimerization was obtained. Bcl-2 also interacted with Bcl-X-L and Mcl-1, as well as the dominant inhibitors Bax and Bcl-X-S. Bcl-X-L displayed the same pattern of the combinatorial interactions with Bcl-2 family proteins as Bcl-2. Our preliminary attempts to map the region within Bcl-2 that are necessary for homodimerization suggest a head-to-tail model for this protein-protein interaction (Sato, T. et al., *Proc. Natl. Acad. Sci. USA*, in press). Furthermore, the demonstration that a LexA/Bax protein has a lethal phenotype in yeast suggests that Bax is a cell death effector whose activity is neutralized by Bcl-2 and Bcl-X-L, and raises possibilities for exploiting yeast for identification of novel genes that participate in or regulate the Bax death pathway. The candidate genes isolated by yeast two-hybrid will be discussed.

A1-441 THE C-ABL NUCLEAR TYROSINE KINASE BINDS TO THE P53 TUMOR SUPPRESSOR PROTEIN AND SUPPRESSES GROWTH IN A P53 DEPENDENT FASHION.

Charles L. Sawyers, Andrei Goga, Xuan Liu, Tina Hambuch, Kristen Senechal, Tyler Jacks, Arnold Berk, Owen N. Witte. Departments of Medicine, Microbiology and Molecular Genetics, Molecular Biology Institute and Howard Hughes Medical Institute, University of California Los Angeles, CA and Center for Cancer Research, Massachusetts Institute of Technology, Boston, MA.

Overexpression of the nuclear tyrosine kinase c-Abl suppresses growth and causes G1 arrest in fibroblasts. To determine if c-Abl acts through growth suppression pathways mediated by p53 or RB, we measured c-Abl growth suppression in primary embryo fibroblasts from mice deficient in p53 or RB. c-Abl suppressed growth in RB -/- cells but not in p53 -/- cells. A temperature sensitive dominant negative p53 mutant impaired c-Abl growth arrest in a temperature dependent fashion in fibroblasts with an intact p53 gene. We also find that c-Abl forms a physical complex with p53 through an interaction between a C-terminal domain in c-Abl and a C-terminal domain in p53. Deletion of the p53 binding site within c-Abl leads to loss of c-Abl growth suppression and gain of transforming activity. These results demonstrate that c-Abl binds p53 and arrests growth in a p53-dependent fashion, perhaps by activating a p53 growth suppression pathway.

A1-442 EXPRESSION OF ril, A NOVEL LIM GENE, IS DOWN-REGULATED IN *ras*-TRANSFORMED CELLS AND RESTORED IN PHENOTYPIC REVERTANTS,

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Expression cloning can identify alterations at the mRNA level that are specific for the conversion from the normal to the malignant phenotype. Several candidate tumor suppressor genes (*H-rev*) have been identified by differential expression cloning on the assumption that they are expressed in phenotypically normal rat cells and repressed in closely related *H-ras*-transformed cells. Of particular interest are those genes whose expression is restored upon phenotypic reversion of transformed cells. Some of these genes may even be effective in restoring the normal phenotype in cells transformed by oncogenes.The genes coding for lysyl oxidase (*H-rev142*) and for a novel 18K-protein (*H-rev107*) were recovered as cDNAs by subtraction hybridization (1,2). The *ril* gene (*H-rev18*), a novel member of the heterogeneous class of genes encoding proteins with a LIM/double zinc finger domain, is down-regulated in *H-ras*-transformed fibroblasts, but expressed in normal fibroblasts, phenotypic *ras*-revertants and cells refractory toward *ras*-mediated transformation. The predicted protein product of *ril* consists of 330 amino acids, has an isoelectric point of 8.22 and a single LIM domain, but no homeodomain (HD). LIM-only proteins may serve as adaptor proteins which regulate the activity of LIM/HD transcription factors. The *ril* gene is highly conserved in evolution. Northern analysis and *in situ* hybridization showed *ril* expression in somites of developing mice, meiotic spermatocytes and a wide variety of tissues of adult mice. This pattern of expression suggests a role in the control of growth and differentiation, analogously to other LIM domain genes.

1) Canc. Res. 53, 4670-4675, 1993. 2) Oncogene 9, 479-490, 1994.

A1-443 p53 MUTATION AND MDM2/SAS-AMPLIFICATION IN HUMAN OSTEOSARCOMA,

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The tumor suppressor gene p53 is inactivated in most human malignancies either by missense mutations or by binding to oncogenic proteins. 12 human osteosarcomas (OS) (11 high-grade, 1 low-grade) were examined for p53, MDM2, and SAS gene aberrations (9 primary, 1 local relapse, 2 metastatic). Point mutations within the hot spot region of p53 (exons 5-8) were detected in one fourth of OS (3 of 12) screened by temperature gradient gel electrophoresis (TGGE).
Southern analysis revealed one gross alteration in a further tumor which exhibited a wild type (wt) p53 in TGGE.
MDM2 amplification was found in 2 primary OS one of which developed lung metastases with a co-amplification of the MDM2 and SAS gene. In this subset of OS, no further SAS amplification was observed. Supporting former results tumors with p53 alterations were devoid of MDM2 amplification and vice versa.
On RNA level MDM2 amplification coincided with a high expression of the MDM2 transcript. Monoclonal antibodies (PAb 1801, 240) specific for p53 revealed an intranuclear p53 gene product not only in tumors with detectable p53 gene aberration but also in those with MDM2 amplification combined with wt p53. This may reflect an upregulation of wt p53 in response to the MDM2 mediated inhibition of its transcription activating function or an accumulation of wt p53 by altered protein kinetics.

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A1-444 REGULATION OF THE E2F2 AND E2F3 GENE PROMOTERS DURING THE CELL CYCLE. Rosalie C. Sears and Joseph R. Nevins, Department of Genetics, Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC 27710

Regulation of the cell cycle is tightly controlled through a series of positive and negative growth signals. The E2F transcription factor plays a key role in regulating the progression of cells through the G1-phase of the cell cycle into DNA synthesis. The E2F transcription factor activity is known to involve the products of multiple E2F family member genes as well as a dimerization partner termed DP-1. We recently characterized the promoter region of the E2F1 gene to understand how this important protein is itself regulated during the cell cycle (Johnson et al., 1994, Genes & Dev. 8:1514-1525). It was observed that E2F1 expression is tightly controlled through autoregulation so that it is rapidly switched on just prior to the initiation of S-phase, where E2F is known to activate a number of genes expressing proteins used during DNA synthesis. Like E2F1, the E2F2 mRNA appears to accumulate in late G1 following stimulation of quiescent cells. We are now cloning and characterizing the promoters for the E2F2 and E2F3 genes in an attempt to understand how these additional E2F family members are regulated during the cell cycle. An initial examination of the E2F2 promoter shows that it is similar to the E2F1 promoter in that it contains a series of E2F binding sites. The presence of E2F sites in the E2F2 promoter suggests that it is also autoregulated, which could account for the increased expression of E2F2 message at the G1/S-phase boundary. Further characterization of the regulation of the E2F2 promoter, as well as the E2F3 promoter, is in progress. This approach will hopefully elucidate some of the differences between these other E2F proteins and provide insight into the roles of E2F2 and E2F3 in controlling cell cycle progression.

A1-445 p53 BINDS SINGLE STRANDED DNA ENDS THROUGH C-TERMINAL DOMAIN AND INTERNAL DNA SEGMENTS VIA THE MIDDLE DOMAIN, Galina Selivanova*, Georgy Bakalkin#, Tatjana Yakovleva#, Elena Kiseleva^, Laszlo Szekely*, Lars Terenius#, and Klas G. Wiman*., *Microbiology and Tumor Biology Center, and Departments of #Drug Dependence Research and ^Cell and Molecular Biology, Karolinska Institute, S-17177, Stockholm, Sweden.

We have previously shown that wild type p53 can bind single stranded (ss) DNA ends and catalyze renaturation of short ss complementary molecles. Using p53 deletion mutants we have mapped the DNA end binding site and renaturation activity to the C-terminal domain of p53 protein (residues 320-393). Besides the end of the ss DNA molecule, p53 can also bind internal segments of long DNA molecules via the binding site (internal DNA site) distinct from the binding site for DNA ends (DNA end site). The internal p53 binding site was mapped to the central region of the molecule (residues 99-307). Importantly, that in the presence of excess of ssDNA ends we observed stimulation of p53 binding to ssDNA via its int binding site. Thus, interaction of C-terminal domain with ss DNA ends activates p53 central core DNA binding site. Although both int and specific DNA binding sites are mapped to the same domain, it is not clear yet, whether they are distinct or not. The observation that p53 binds to ssDNA ends have led us to suggest that p53 may serve as an intracellular sensor of DNA strand breaks in vivo (Bakalkin et al, 1994). It is conceivable, that interaction of C-terminal domain with ssDNA ends of damaged DNA can cause p53 protein conformational shift analogous to that achieved in vitro by different modifications of the C-terminus, leading to the activation of specific DNA binding.

Bakalkin, G., Yakovleva, T., Selivanova G., Magnusson, K.P., Szekely, L., Kiseleva, E., Klein, G., Terenius, L., and Wiman, K.G. (1994). Proc. Natl. Acad. Sci. USA 91, 413-417.

A1-446 FUNCTIONAL MODULATION OF THE WT1 PROTEIN BY ITS PHOSPHORYLATION, Kentaro Semba[1,2] and Tony Hunter[1], [1]The Salk Institute, La Jolla, CA 92037, [2]The Institute of Medical Science, University of Tokyo, Tokyo 108, JAPAN

The Wilms' tumor predisposition gene WT1 encodes an EGR1-like DNA binding protein that has four contiguous zinc finger motifs. The WT1 protein binds to a set of DNA binding sequences including 5'-GCGGGGGCG-3' and functions as a transcriptional repressor. However nothing is known about modulation of its function by extracellular signal. Here we show 1) the WT1 protein is phosphorylated *in vivo* , 2) forskolin enhances phosphorylation at three sites in the WT1 protein *in vivo,* 3) two of them are Ser-365 and Ser-393 in the zinc finger motifs, 4) *in vitro* phosphorylation of the WT1 zinc finger domain by PKA abolishes its DNA binding activity, 5) this reaction is reversible because the phosphorylated WT1 protein restores its DNA binding activity after treatment with BAP (bacterial alkaline phosphatase), 6) double mutant at Ser-365 and Ser-393 retains its DNA binding activity even after incubation with PKA. These findings suggest that activation of PKA by forskolin *in vivo* suppresses the function of the WT1 protein through phosphorylation in its DNA binding domain. Recently we found that TPA also induces phosphorylation of the WT1 protein at an unknown site. We are currently determining the third phosphorylation site by PKA and a new phosphorylation site induced by TPA.

A1-447 TGFβ EFFECTS ON CELL CYCLE PROGRESSION OF NORMAL AND IMMORTAL HUMAN MAMMARY EPITHELIAL CELLS IN CULTURE, M. Stampfer[1], J. Garbe[1], C. Pan[1], D. Alexander[1], J. Hosoda[1], P. Yaswen[1], S. Reed[2], and J. Slingerland[3], [1]Lawrence Berkeley Laboratory, Berkeley, CA, 94720, [2]The Scripps Research Institute, La Jolla CA; [3]The University of Toronto, Toronto, Ontario, Canada M5S 1A8.

To specifically examine the pathway of TGFβ induced growth inhibition the effects of TGFβ on cell cycle regulation and specialized functions have been examined and compared in normal finite life span human mammary epithelial cell (HMEC) strains and two immortally transformed cell lines derived from normal HMEC following in vitro exposure to benzo(a)pyrene. Normal HMEC are all growth inhibited by TGFβ. Both immortal cell lines can give rise to populations which are resistant to growth arrest in the presence of TGFβ, as well as populations which are largely growth inhibited. All of these HMEC can respond to TGFβ with increased synthesis and secretion of extracellular matrix associated proteins. HMEC were Go growth arrested by blockage of EGF receptor signal transduction, and then allowed to synchronously enter the cell cycle by re-exposure to EGF with and without TGFβ. DNA synthesis began 10-12 hrs following EGF exposure. TGFβ added at 10 hrs or later did not inhibit DNA synthesis. TGFβ did not affect the initial burst of early response gene expression seen upon exit from Go. There was no correlation between levels of myc and extent of TGFβ induced growth inhibition. While protein levels of cyclins D1, D2, and E were minimally suppressed and Cdk2 and Cdk4 levels were unaffected by TGFβ in all lines, cyclin E- and A-associated kinase activities were suppressed in TGFβ sensitive lines. In sensitive cells, TGFβ inhibited the phosphorylation of the retinoblastoma protein that normally precedes the G1-to-S phase transition and inhibited the association of cyclin D1 with Cdk4. We and others have previously shown that while cyclin E/Cdk2 complex formation and Cdk2 phosphorylation are not inhibited by TGFβ, kinase activation is inhibited by the action of a cyclin/cdk inhibitor, the recently cloned $p27^{kip-1}$. To better understand the role of p27 during the cell cycle, its expression, its presence in cyclin E/Cdk2 and cyclin D1/Cdk4 complexes and its inhibitory activity are being examined in TGFβ sensitive and resistant cell lines during G1 and S phase.

A1-448 EXAMINATION OF A NOVEL DIFFERENTIALLY EXPRESSED GENE PRODUCT IDENTIFIED IN HUMAN GLIOMA CELL SUPPRESSED HYBRIDS CONTAINING AN INSERTED CHROMOSOME 10. Peter A. Steck, Mark A. Pershouse, Samar Jasser, W. K. Alfred Yung, and Azra Hadi Ligon, Department of Neuro-Oncology, University of Texas M. D. Anderson Cancer Center, Houston, TX 77030.

The loss of large segments of chromosome 10 is the most common genetic alteration in human glioblastomas. To examine the potential significance of the loss of chromosome 10 genes, subtractive hybridization was performed between a human glioblastoma cell's mRNA and cDNA from a microcell-hybrid containing an inserted chromosome 10. The hybrid cells exhibited a suppression of their *in vitro* and *in vivo* tumorigenic phenotype. Sixty-one clones were isolated and found to include mitochondrial genes, various known genes, and novel gene sequences. Three sequences, one from each of the above classes of genes, were shown to be differentially expressed in two glioma model systems representing the parental glioma cells and their suppressed hybrids containing a chromosome 10. One clone (17) appeared to encode for novel gene product. Screening of a fetal human brain expression library to identify full length clones yielded at least two independent gene products. One clone sfb 8-1 was shown to be differentially expressed as a 2.6 kb mRNA. Preliminary characterization suggests sfb8-1 maps to chromosome 10. Clone sfb 5.6 was also isolated and identifies 5 mRNAs of 0.8, 1.6, 2.4, 5, and 8 kb and were not differentially expressed. Searches of GenBank have shown no significant homology of either gene product to other known genes. Both series of clones were found to be expressed predominantly in the brain (glial cells), with lower quantities in the lung and heart. The homology between two series of clones is related to a 3' repeat element. The biochemical and biological significance of these novel gene products in the oncogenesis and phenotype of brain tumors is currently under investigation.

A1-449 ACTIVATION OF MYC TRIGGERS HYPER-PHOSPHORYLATION OF THE RETINO-BLASTOMA PROTEIN

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Activation of the c-*myc* proto-oncogene as a conditional MYCER chimera in confluent RAT1A fibroblasts triggers re-entry into the cell cycle. We now show that this process is accompanied by rapid hyperphosphorylation of the retinoblastoma protein, pRb. Surprisingly, we observe no change in the amounts of cyclin D1, cdk4, cyclin E and cdk2 proteins during the time interval in which pRb hyper-phosphorylation occurs. Also, cyclin D1 and cdk4 appear to be assembled even before activation of MYC. However, cyclin D1-associated kinase activity is low before activation of MYCER but increases after the addition of hormone. The mechanism of this activation is currently being investigated.

A1-450 **THE hBRG1 AND hBRM TRANSCRIPTIONAL ACTIVATORS BIND TO AND COOPERATE WITH MEMBERS OF THE RB-FAMILY OF PROTEINS TO INDUCE GROWTH ARREST.** Bruce E. Strober, Joshua L. Dunaief, Sushovan Guha, and Stephen P. Goff, Department of Biochemistry and Molecular Biophysics and the Howard Hughes Medical Institute, Columbia University College of Physicians and Surgeons, New York, NY 10032

hBRG1 and hBRM are highly similar proteins that represent the human homologs of both the yeast *SNF2/SWI2* and *Drosophila brahma* transcriptional activators. Previously we showed that pRB binds to hBRG1 both *in vitro* and *in vivo*. Further, this interaction is necessary for hBRG1's ability to act as a tumor suppressor. Transfection of hBRG1 into the *hBRG-*, *hBRM- RB+* human carcinoma cell line SW13 induces the formation of flat, growth-arrested cells, and both an RB non-binding mutant of hBRG1 and the sequestration of RB (and its family members) by the adenovirus E1A protein abolishes flat cell formation. Two lines of evidence indicate that hBRG1 also can functionally bind to other members of the RB family. First, an antibody (C36) that recognizes pRB and its family members p107, p130, and p80/90 is able to co-immunoprecipitate hBRG1 in the *RB*-minus cell lines WERI-1 and Y79. Second, the cotransfection of an RB non-binding mutant of E1A (E1A;928) only partially restores the flat cell activity of hBRG1. This suggests that hBRG1's growth suppressing activity in part relies on its ability to bind to the other Rb-family members that remain sequestered by E1A;928. In addition, we have found that the hBRM protein exhibits growth arresting activity in the cell line SW13, and also is able to bind pRb *in vitro*. We demonstrate here that both hBRM and hBRG1 cooperate with the various members of the RB-family to induce growth arrest in SW13 cells.

A1-451 STUDYING C-SRC REGULATION USING FISSION YEAST: STRUCTURAL REQUIREMENTS AND IDENTIFICATION OF NOVEL REGULATORS,

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Expression of c-Src in *S.pombe* is lethal. Coexpression of the Csk tyrosine kinase counteracts the lethal phenotype by phosphorylating Tyr527 of Src, thereby repressing its catalytic activity. We have used this system to study which residues in Src and in Csk are crucial for regulation. The SH2 and the SH3 domains of Src are both required for regulation to occur and for the protein to stably acquire an inactive conformation associated with low kinase activity. In contrast, the myristylation signal and the unique domain are not necessary. Detailed mutagenesis of the Src SH3 domain has shown that the same binding surface is involved in interaction with heterologous proteins and in the intramolecular regulation of Src. Hybrid Src molecules bearing the SH3 domain of Fyn but not of Lck or spectrin are regulated by c-terminal phosphorylation. We have also tested the role of the SH3 and SH2 domains of Csk using this system, and found that both domains are dispensable for efficient phosphorylation and regulation of Src. Src molecules bearing a phenylalanine to tyrosine substitution at the autophoshorylation site (Tyr416) are unable to kill *S.pombe* and phosphorylate endogenous proteins poorly.

We have obtained several clones from human cDNA libraries able to antagonize the lethal effect of Src in fission yeast. The clones are currently being analysed.

A1-452 TWO MECHANISMS OF TRANSCRIPTIONAL ACTIVATION BY HTLV-1 TAX THROUGH NF-κB BINDING SITE.

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Human T-cell leukemia virus type 1 (HTLV-1) is an etiologic agent of adult T-cell leukemia (ATL) and tropical spastic paraparesis (HAM/TSP). The viral trans-activator Tax protein has been thought to contribute to the pathogenesis of these diseases through activation of the viral and cellular gene expression. The responsive elements for Tax trans-activation are the HTLV-1 21-bp enhancer, the NF-κB binding site and the CArG box. We previously reported that Tax binds to some of the enhancer binding proteins (e.g. CREB, CREM, NF-κB p50 and SRF) and associates with these enhancer DNA sequences. Thus, We proposed that *indirect association of Tax to enhancer DNA is a common mechanism of* Tax trans-activation. Supportig this conclusion, we found that Tax also binds to other NF-κB family proteins, p65 and c-Rel, through the Rel homology domain. Tax binding to these NF-κB proteins in nucleus in fact stimulated the transcription directed by the NF-κB binding site.

In addition to the association of Tax to DNA, Tax also binds to the ankyrin motifs of IκB proteins, inhibitors of NF-κB proteins. Tax binding to the IκB proteins inhibits the formation of NF-κB/IκB complexes in the cytoplasm, resulting in nuclear translocation of active NF-κB. We will focus on the interaction of Tax with IκB-α protein, and discuss the molecular mechanism of trans-activation by Tax.

A1-453 PLEOTROPIC CHANGES IN TRANSCRIPTIONAL CONTROL INDUCED BY RAS AND ONCOGENIC TRANSCRIPTION FACTORS,

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The human teratocarcinoma cell line PA-1 serves as a good model for differentiation in vitro. Oncogenic transformation by N-*ras* in these cells results in inhibition of retinoic acid induced differentiation. The transcriptional activity of AP-2 (measured by a 3X AP-2 binding site *tk*-CAT plasmid) is inhibited in PA-1 cells *transformed by the N-ras oncogene due to dramatic overexpression of AP-2 mRNA.* Stable transfection of AP-2 expression vectors results in inhibition of differentiation and inhibition of AP-2 transcriptional activity in a fashion similar to N-*ras* transformation. AP-2 overexpression also results inhibition of other transcription factors which is mediated by its activation domain. In addition overexpression of the activation domain from AP-2 or VP-16 is able to inhibit endogenous AP-2 activity, transform the cells and inhibit differentiation. Since the activation domains alone can transform these cells and VP-16 has no natural targets for mediating changes in gene expression, our data indicate that oncogenic transcription factors may not function by changing expression of their normal targets genes but rather may function through *pleotropic effects on cofactors shared by multiple transcription factors.* This represents a new mechanism, "transcriptional-interference", by which oncogenes and transcription factors can have biological effects on cells.

Homeodomain proteins are transcriptional factors that play a regulatory role in mediating pattern formation in developing embryos. The induction of homeobox (HOX) gene expression by the developmental morphogen, retinoic acid represents a relevant differentiation marker in these cells. The induction of homeobox mRNAs, by retinoic acid, is delayed in the PA-1 cells transformed by an activated N-*ras* oncogene. Five kb of upstream DNA from the mRNA start site of human HOX A4 (previously called HOX 1.4) gene is required for full retinoic acid induction. Transfection of PA-1 cell variants in the presence or absence of retinoic acid treatment revealed regions containing inducible promoter activity; *Ras* and retinoic acid sensitive promoter activity was localized to 0.45 kb of sequence, between -2950 and -3335 upstream of the start site. This region of the promoter contains an AP-2 binding site, one RARE and another highly conserved binding site. These studies reveal that like AP-2 activity, the transcriptional activity of the HOX A4 promoter is inhibited in these cells as compared to the activity in cells with normal AP-2 mRNA expression. Thus "transcriptional interference" can mediate oncogenic transformation and inhibition of differentiation and differentiation-specific gene expression.

A1-454 THE SUPPRESSIVE ACTION OF *BCL*-2 ONCOGENE MAY CONTRIBUTE TO TISSUE DAMAGE IN AUTOIMMUNE RHEUMATIC DISEASES,

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Autoimmune rheumatic diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and Sjögren's Syndrome (SS) are characterized by lymphocyte activation, immune dysregulation, and predisposition to lymphoid malignancy. MRL/lpr mice, a genetic model for these diseases, are unable to undergo apoptosis or programmed cell death (PCD) due to a retrotransposon insertion into the *Fas* gene which diminishes the production of *Fas* protein required for PCD. This abnormality probably explains the accumulation of double-negative ($CD4^-CD8^-$) $B220^+$ T cells which infiltrate the synovium, salivary glands, and other tissues in these animals.

The exact mechanism linking defective PCD to autoimmune disease in patients is unknown. We hypothesize that this mechanism involves the suppressive action of the *bcl-2* oncogene which normally functions to limit clonal expansion of activated lymphocytes by regulating PCD. We find, using *in situ* hybridization and immunostaining techniques, high *Fas* expression in T cells infiltrating SS salivary glands. The suppressor oncogene *bcl-2* is also abundantly present. Interestingly, expression of *bcl-2* is detected also in the hyperplastic RA synovium. The *bcl-2* oncogene may inhibit PCD allowing persistence of cells in tissues despite their strong *Fas* positivity. Furthermore, these cells unable to undergo PCD could initiate a *Fas*-dependent death pathway resulting in autoimmune tissue damage, inflammation, and organ failure. If this hypothesis is correct, it would lend further credibility to the autogene hypothesis (Mountz & Talal, *Immunol Today*, 14: 532-536, 1993) which suggests that genes such as *Fas* and *bcl-2* contribute to the pathogenesis of autoimmune diseases in a manner analogous to how oncogenes contribute to cancer.

A1-455 DIFFERENTIAL EXPRESSION AND CELL CYCLE REGULATION OF THE CDK4 INHIBITOR $p16^{Ink4}$,

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$p16^{Ink4}$ (Inhibitor of Cyclin-dependent kinase 4) is a cell cycle regulator that specifically binds to and inhibits Cdk4. Recently, the human *mts1* (multiple tumor suppressor 1) gene was found to be identical to *ink4* which is deleted or mutated in various primary tumors and in a large number of transformed cell lines. We have surveyed by immunoblotting the protein levels of $p16^{Ink4}$ in normal and transformed human cells. We determined that $p16^{Ink4}$ was differentially expressed in normal diploid cells derived from different tissues, in contrast to another cell cycle inhibitor, $p21^{Waf1}$, which is ubiquitously expressed. In some tumor cell lines $p16^{Ink4}$ protein was not detected, presumably because of a homozygous deletion of its gene. By contrast, it was found to overexpress in other cell lines when compared to levels in their normal counterparts. Interestingly, high levels of $p16^{Ink4}$ protein correlated with functional inactivation of the retinoblastoma gene product. We also found that $p16^{Ink4}$ protein expression varies during the cell cycle peaking during S phase. These results show a functional relationship between $p16^{Ink4}$ and the retinoblastoma gene product and indicate that $p16^{Ink4}$ is required for Cdk4 inhibition only at the G1/S transition at the time when Cdk4 kinase activity is no longer necessary.

A1-456 IDENTIFICATION OF A NOVEL HUMAN PAPILLOMAVIRUS TYPES 16 AND 18 INTEGRATION LOCUS IN CHROMOSOME 12 IN GENITAL TUMORS. TAWHEED A., PETIT C*. and ORTH G. Unité des Papillomavirus, *Unité de Génétique Moléculaire Humaine, Institut Pasteur, 25 Rue du Dr. Roux, 75015 Paris, FRANCE.

We report here, the identification of a first human papillomavirus (HPV) integration locus, localised in the chromosomal region 12q14-q15, in two tumeur cells derived from a precancerous genital lesion associated with HPV16, and a cervical cancer associated with HPV18. We showed that the cellular sequences adjacent to 3' end of the viral integrated sequences are located in a 0.5 Mb DNA fragment flanked by two CpG islands. The physical Map of this region revealed that the distance between the two integration sites is 25 kb and the HPV 16 integration site is located 60 kb downstream from the CpG island. Two putative open reading frames (ORF) were identified upstream from the integration sites. The first contains the CpG islands and is transcribed in skeletal muscles. The second ORF encodes a protein containing seven sequence motifs rich in proline similar to those which bind to the SH3 domain of proteins implicated in the tyrosine kinase signal transduction pathway. The two ORFs do not show any homology with sequences in the data banks. The results demonstrate the existence of novel target genes, located near the HPV integration locus, in the region q14-q15 of chromosome 12 known to harbour the breakpoints of chromosomal translocations in lipoma, uterine leiomyoma and adenoma of the salivary gland .

A1-457 CLONING OF A NOVEL GENE, ELL, THAT FUSES TO MLL IN A t(11;19)(q23;p13.1) IN ACUTE MYELOID LEUKEMIA. M.J. Thirman, D.A. Levitan, H. Kobayashi, M.C. Simon, and J.D. Rowley. Departments of Medicine, Section of Hematology/Oncology, Molecular Genetics and Cell Biology, and the Howard Hughes Medical Institute, University of Chicago, Chicago, IL.

The t(11;19)(q23;p13.1) is a recurring cytogenetic abnormality in AML. We have previously shown that this translocation as well as other recurring 11q23 aberrations involve the MLL gene on chromosome 11, band q23. This translocation is distinct from another type of (11;19) translocation with a 19p13.3 breakpoint that results in the fusion of MLL to the ENL gene. By PCR screening of a cDNA library prepared from a patient's leukemia cells with this translocation, we obtained a fusion transcript containing exon 7 of MLL and sequences of an unknown gene. The sequences of this novel gene were amplified and were used as a probe to screen a fetal brain cDNA library. On northern blot analysis, this cDNA fragment detects a 4.4 kb transcript that is abundant in peripheral blood leukocytes, skeletal muscle, placenta, and testis, and expressed at lower levels in multiple tissues. In addition, a 2.8 kb transcript is present in peripheral blood, testis, and placenta. On zoo blots, this gene is evolutionarily conserved in 10 mammalian species as well as in chicken, frog, and fish. We have named this gene, ELL, for eleven-nineteen lysine-rich leukemia gene. ELL contains a predicted open reading frame of 621 amino acids; 576 amino acids of 3' ELL fuse to 5' MLL sequences as a result of the translocation. A highly basic, lysine-rich motif of ELL is homologous to similar regions of several proteins including the DNA binding domain of poly(ADP-ribose) polymerase. ELL is not homologous to other MLL partner genes. The characterization of the normal functions of this previously unidentified gene as well as its altered function when fused to MLL will be critical to further our understanding of the mechanisms of leukemogenesis.

A1-458 p53 GENE LOSS AND ALTERATION DURING PROSTATE CANCER PROGRESSION AND METASTASIS IN A NOVEL MOUSE MODEL: RELEVANCE TO HUMAN PROSTATE CANCER. TL Timme,. JA Eastham, SH Park, C Ren, D Kadmon, LA Donehower, A Bradley and TC Thompson, Baylor College of Medicine, Houston, TX 77030.

We modified the mouse prostate reconstitution (MPR) model system by using p53 "knockout" urogenital sinus tissue as a target for transduction of initiating oncogenic alterations. The *ras* and *myc* oncogenes were transduced using recombinant Zipras/myc 9 retrovirus into wild type p53 (+/+), heterozygous p53 (+/-), or nullizygous p53 (-/-). Normal prostatic morphology was seen in control, BAGα-infected MPRs with all p53 allelotypes. Epithelial hyperplasia was present in 21 of 21 *ras+myc*-infected +/+ MPRs and a small focal cancer with no evidence of metastasis was observed in one of these. Within 3 weeks, 14 of 14 +/- MPRs and 5 of 5 -/- MPRs had malignant *ras+myc*-induced adenocarcinoma at the primary grafting site. The -/- primary site tumors were larger and more aggressive than the +/- tumors. Metastatic deposits were found in 95% of the mice and the pattern of metastasis was remarkably similar to that in human prostate cancer with gross metastatic deposits in the lung, lymph nodes, bone and liver of many animals. Multiple cell lines derived from MPR primary site tumors and metastatic deposits were analyzed by Southern blotting. Loss of p53 function occurred in all the +/- MPRs by gene loss or interstitial deletion of the wild-type p53 allele, or by abrogation of mRNA expression. Clonal analysis based on retroviral integration patterns in primary site tumor DNA and in cell lines derived from metastatic sites suggested that the acquisition of the metastatic phenotype requires at least one other genetic event in addition to *ras + myc* initiation and loss of p53. In general, -/- primary site tumors were more polyclonal than +/- primary site tumors. The clonal pattern of metastatic cell lines from +/-MPRs was detected in the primary site tumor, whereas the metastatic cell lines from 2 of 3 -/- MPRs contained unique clonal patterns not detected in the primary site tumor. Our model system provides a unique *in vivo* experimental system to initiate carcinogenesis with defined genetic alterations in a background of normal prostate cells and then track clonal progression through the metastatic phenotype. Hopefully this revised MPR model will help to further elucidate the pathways involved in metastatic prostate cancer.

A1-459 HETEROGENEOUS RIBONUCLEOPROTEIN PARTICLE PROTEIN K IS A DNA BINDING TRANSACTIVATOR.
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We have previously reported that heterogeneous ribonucleoprotein K (hnRNP K) binds to the pyrimidine-rich strand of the CT-element found in the human *c-myc* gene and activates CT-reporter driven gene expression, *in vivo*. We now characterize the DNA and protein requirements for the interaction of hnRNP K with the CT-element. First, hnRNP K is shown to bind preferentially single-stranded DNA (ssDNA) over native DNA or RNA. Using specific oligo- ribonucleotide or deoxyribonucletide probes with specific or non-specific, RNA or DNA competitors, EMSA revealed hnRNP K to be a DNA binding protein. Specific binding was not simply a reflection for binding to pyrimidine-rich sequences as the number and arrangement of individual CT-elements governed binding with hnRNP K; at least two CT repeats separated by at least three nucleotides are required for binding indicating the existence of particular stereochemical constraints regulating CT-hnRNP K complex formation. Deletion analysis showed that hnRNP K possesses several non-overlapping, DNA binding domains, each capable of specific binding with the CT-element and preferring DNA over RNA. Each DNA sequence recognition domain is composed of at least one K homology(KH) motif, while a larger portion of hnRNP K may be required for RNA binding. Additional experiments indicate that the amino terminal 35 residues of hnRNP K are necessary for transactivating the CT-element. These results indicate that hnRNP K is a DNA binding protein and transcriptional activator.

A1-460 OVEREXPRESSION OF THE SPI 1 ONCOGENE AND OF A DOMINANT NEGATIVE FORM OF P53 CONFERS DISTINCT PHENOTYPES TO ERYTHROID PROGENITORS

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The erythroleukemia induced in the mouse by the SFFV component of the Friend leukemia virus is first characterized by the polyclonal expansion of non leukemic erythroid progenitors as the result of the constitutive activation of the EpoR by the SFFV env gene product, followed by the emergence of leukemic clones. Two molecular events are associated with this leukemic phase: the overexpression of Spi1/Pu1, a member of the Ets family of transcriptional regulators, as the result of an SFFV proviral insertion, and the inactivation of the p53 tumor suppressor gene. To study the effects of these genetic alterations on the differentiation program of erythroid progenitors and their ability to cooperate with oncogenic protein tyrosine kinases, we first analysed the consequences of the expression of Spi1/Pu1 and of a dominant negative form of p53 in ts-v-sea transformed avian primary erythroblasts. Expression of p53(V135A) was found to be without effect on the ability of ts-v-sea erythroblasts to undergo Epo-dependent terminal differentiation following inactivation of v-sea function. In contrast, ts-v-sea-transformed erythroid clones expressing Spi1/Pu1 failed to differentiate in erythrocytes but continued to proliferate in conditions non permissive for ts-sea function. In another series of experiments, we examined the effect of Spi1/Pu1 and p53(V135A) on self-renewal of normal avian erythroid progenitors in the presence of SCF, TGFα and estradiol. Whereas Spi1/Pu1 appeared insufficient to transform these cells, expression of p53(V135A) was found to suppress their strict requirement for TGFα and SCF, allowing their proliferation in the presence of estradiol only.

These results indicate that the genetic defects characteristic of Friend leukemic cells each interfere in a distinct manner with the normal control of erythroid progenitors proliferation and differentiation.

A1-461 OVEREXPRESSION OF TYPE I COLLAGEN SUPPRESSES THE TUMORIGENICITY OF KI RAS TRANSFORMED MOUSE FIBROBLASTS, Helen Travers and John D. Norton, CRC Department of Gene Regulation, Paterson Institute For Cancer Research, Christie Hospital NHS Trust, Manchester M20, UK.

Transformed fibroblasts exhibit reduced adhesion to substrata, a characteristic which is attributable to reduced expression/ increased degradation of extracellular matrix (ECM) proteins eg type I collagen. Stable overexpression of cDNAs of focal adhesion/ microfilament associated proteins such as vimentin, vinculin, and α-actinin has been shown to suppress the tumorigenicity of a variety of malignant cell types. To directly assess the role of reduced expression of type I collagen in cellular transformation, a Ki ras transformed NIH3T3 cell line was transfected with an α2 type I collagen expression construct. A number of stable transfectants analysed showed a flatter, more anchorage dependent morphology relative to control cell lines. These clones also displayed reduced tumorigenicity in nude mice, a reduced ability to clone in soft agar, and a slower growth rate together with a lower saturation density. Moreover, suppression of the transformed phenotype by the addition of exogenous type I collagen protein is correlated with the downregulation of ras oncogene responsive NVL-3 VL30 (Virus Like 30s) gene expression. These results suggest that in addition to suppressing tumorigenicity by promoting cellular adhesion ECM proteins, such as collagen, may also act to subvert oncoprotein signalling pathways associated with cellular transformation.

A1-462 TIMING AND CHARACTERIZATION OF P53 ALTERATIONS IN UV-B INDUCED SKIN TUMORS OF HAIRLESS MICE.

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UV radiation causes severe DNA damage and is a known inducer of skin cancer in humans as well as in animal modelsystems. To identify (the timing of) onco-genetic events in UV carcinogenesis, we examined UV-B induced skin tumors of albino hairless mice (HRA) for mutations in genes of the **ras** family and the **p53** tumor suppressor gene. Mice were irradiated daily during 75 min with UV-B, starting at 10 weeks of age. The daily surface exposure of the animals was 150 mJ/cm^2. All mice developed multiple dorsal skin tumors, predominantly of the SCC type. After 12 weeks the first tumors were collected from 6 mice (group A, 13 tumors). After 18 weeks another 6 mice (group B, 37 tumors > 1mm) and after 25 weeks the remaining 12 mice (group C, 32 tumors > 3mm) were sacrified. Only one point mutation in the **K-ras** gene was detected and no point mutations were observed in the codons 12, 13, or 61 of the **H-ras** and **N-ras** gene. Using the CM5 polyclonal antibody raised against mouse **p53**, we detected high steady state levels of **p53** in all three groups. Staining was observed in more than 90% of the tumors, primarily in the basal cells (especially in group A). So overexpression of **p53** is already present in early precursor lesions of SCC's, such as actinic keratosis. To investigate if the immunoreactivity is correlated with mutant alleles of the **p53** gene, we amplified and sequenced the exons 4-8 of the **p53** gene and also used the C-19 antibody against **waf-1** encoded p21 on serial coupes stained for overexpression of **p53**. In about 50 % of the tumors point mutations in the conserved domains III, IV and V were detected. The majority was observed in exon 8 with codon 267 as a hotspot. Most point mutations are at dipyrimidine sites and the presence of these mutations on the non-transcribed strand correlates well with the preferential repair of the trancribed strand, as measured after a single UV-B exposure in vivo.

A1-463 MOLECULAR HETEROGENEITY OF THE ESTROGEN RECEPTOR IN HUMAN BREAST CANCER. Laura J. Van 't Veer, Mariska A.J. van Dijk, Arno N. Floore. Department of Pathology, Netherlands Cancer Institute, Amsterdam, The Netherlands.

The estrogen receptor (hER) is an important regulator of growth and differentiation in the mammary gland, and is also implicated in the development of breast cancer. Elevated levels of the estrogen receptor are found in 60 percent of mammary carcinomas and its presence correlates well with disease-free and overall survival when patients are treated with antiestrogens. However, forty percent of the patients with an ER positive tumor do not respond to the treatment. Recent findings suggest that variant estrogen receptors might be present in these tumors that are non-functional and non-responsive to antiestrogens.

Estrogen receptors are nuclear receptors, that upon hormone binding act as transcriptional activators of target genes by binding to a specific DNA sequence, the estrogen responsive element (ERE), in the vicinity of these genes. We are using a yeast growth assay to determine the transcriptional activity of the estrogen receptors present in mammary carcinoma. In this assay, PCR products of the ORF of hER cDNA (2004 bp) are directly cloned into a yeast expression vector in the yeast *Saccharomyces cerevisiae in vivo*. Subsequently, the transcriptional activity of the cloned PCR products is tested, using a hER activated reporter gene, whose product is required for growth of the yeast. Each yeast colony represents a single hER molecule and is tested individually for hER activity. This allows us to discriminate between constitutively active, inactive and estrogen inducible hER variants. This assay is also suitable to appreciate heterogeneity of estrogen receptors within one specimen. So far, we have indeed been able to identify different hER variants that are present in the mammary carcinoma cell lines T47D and MCF7 on the basis of their different functional activity. The nature of these variants is subsequently determined by DNA-sequencing. The prevalence of variant receptors in mammary carcinoma is currently being analysed.

A1-464 THE RAS ADAPTOR PROTEINS, GRB2 and Shc, INTERACT WITH PI 3-KINASE IN BCR/abl TRANSFORMED CHRONIC LEUKEMIA CELLS.
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BCR/abl fusion protein is expressed by the BCR/abl chimeric gene which is the hallmark of human chronic myelogenous leukemia (CML). Activation of the Ras pathway and phosphatidylinositol 3-kinase (PI 3-kinase) is implicated in BCR/abl induced transformation. Two adaptor proteins, Shc and GRB2, link activated growth factor receptors to the Ras signalling pathway. GRB2 protein SH2 domain binds directly to p-Y177 on BCR/abl. Our results demonstrate that PI 3-kinase binds to Shc in *BCR/abl transformed chronic leukemia cells. In* contrast, GRB2 associates with PI 3-kinase protein in parental and BCR/abl transformed hematopoietic cells which suggest interaction via SH3 domains. GRB2 immunoprecipitates from BCR/abl transformed cells have an 18-fold increase in PI 3-Kinase activity as compared to parental cells. We also found that GRB2 translocates from the cytosol to membrane fractions in BCR/abl transformed BaF3 cells. These results show BCR/abl dependent interaction of PI 3-Kinase with proteins of the Ras pathway in transformed hematopoietic cells.

A1-465 CD39, A PUTATIVE TUMOR SUPPRESSOR GENE MAPPING TO THE X CHROMOSOME. Joseph R. Voland, John Hirai, and Homero Sepulveda, Department of Biology and Cancer Center University of California San Diego La Jolla, California 92093

We have previously reported the cDNA sequence of a transmembrane protein, 721P, expressed on the cell surface of syncytiotrophoblast, endothelium, and activated lymphocytes and have shown that it contains a cytoplasmic domain homologous to the *myc* family of oncogenes. This similarity includes a leucine zipper, and basic region approximately 60% homologous to the leucine zipper and basic region of *myc*. (Voland *et al., Proc Nat Acad Sci* 89:10425, 1992).

We now present evidence that this protein is CD39, originally described as a B cell activation antigen. We have transfected this gene, under the control of a CMV promoter/enhancer, into the choriocarcinoma cell lines BeWo, JEG and JaR, as well as the fibrosarcoma line HT1080. The transfected cells show a reduced proliferative capacity, a decreased mitotic index, and fail to produce tumors in nude mice. Mutational analysis of the cDNA shows that removal of the putative basic region results not only in a loss of the "tumor suppressor" effect, but also in an increase in proliferation of the cells.

These results suggest that the 721P protein may play an important role in the differentiation of several cell types, and that the regions of the molecule homologous to *myc* are important in the function of this molecule.

We have mapped the gene coding for CD39 to the X chromosome using an Oncor hybrid blot. Detailed mapping studies to pinpoint the localization of the gene on this chromosome are underway. Several diseases map to the X chromosome, including X-linked lymphoproliferative disease, and once the location of the CD39 gene has been determined, we will be able to better determine the role of CD39 in the development of disease.

A1-466 STRUCTURE AND FUNCTION ANALYSIS OF THE ALTERNATIVELY SPLICED WT1 PROTEIN, Zhaoyi Wang, Qing Qing Qiu and Thomas F. Deuel, Division of Hematology, Jewish Hospital at Washington University Medical Center, St. Louis, MO 63110

The Wilms' tumor susceptibility gene, wt1, encodes a transcription factor of the zinc finger protein family. Mutations in the wt1 gene product have been detected in both sporadic and familial Wilms' tumors, suggesting that alterations in WT1 may disrupt its normal function as a transcriptional regulator. The transcripts of wt1 are alternatively spliced; the major transcript of wt1 encodes a WT1 protein [WT1(+KTS) +17AA] that contains three amino acids (+KTS) between the third and fourth zinc fingers and a serine-rich, 17 amino acid (+17AA) domain N-terminal to the zinc finger region. We now show that the WT1(+KTS) forms functionally bind to a unique G+C-rich sequence within the PDGF A-chain promoter. We also show that WT1 (+KTS)+17AA functions as a strong transcription repressor and that +17AA alone fused to the zinc-finger domain of WT1 or to the heterologous DNA binding domain of GAL4 functions independently as a repressor. Deletion of four serine residues within +17AA abolishes the repressor activity of +17AA. These results indicate that wt1 products with +17 AA contain an additional dominant repressor domain and that the presence or absence of +KTS determines alternative DNA binding specificity.
We have also defined the functional domains of WT1 and demonstrated that amino acid residues 85-124 and 181-250 of WT1 are required for transcriptional repression and activation, respectively. In order to seek proteins that interact with the repressor domain of WT1, we co-transfected increasing concentrations of the repressor domain of WT1 fused to the GAL4 DNA binding domain with fixed concentrations of the wild type (wt) WT1 and observed a progressive loss of the wt WT1 repressor activity and an increase of wt WT1 dependent activation activity. The activation domain of WT1 was without effect in this assay. These results establish independent domains of WT1 that activate and repress transcription and suggest that the repressor domain of WT1 binds to an interactive nuclear protein that is essential for the repressor activity of WT1.

A1-467 THE MECHANISM OF ACTIVE TRANSCRIPTIONAL REPRESSION BY THE RETINOBLASTOMA PROTEIN, Steven J. Weintraub, Kevin N.B. Chow, Robin X. Luo, Steven Zhang, Song He, and Douglas C. Dean, Departments of Medicine and Cell Biology, Washington University School of Medicine, St. Louis, MO 63110.

Deletion or mutation of the retinoblastoma protein (Rb) is associated with the loss of cellular growth control. Rb is thought to regulate cellular proliferation by inhibiting expression of cell cycle genes. One target of Rb in the cell is the E2F family of transcription factors (referred to collectively as E2F). E2F sites have been found in the promoters of a number of cell cycle genes, and the interaction of Rb with E2F has been shown to block *trans*-activation by E2F. Thus, it was initially postulated that Rb controlled the activity of these genes by inactivating E2F. We, however, found that when wild-type Rb was expressed in Rb(-) cells, E2F sites switched from enhancers to silencers. These results suggested that Rb was doing more than simply inactivating E2F—the Rb-E2F complex appeared to be an active transcriptional repressor.

Here, we demonstrate that Rb is an active transcriptional repressor which is solely responsible for the repressor activity of the Rb-E2F complex, and we investigate the mechanism by which Rb represses transcription. We present evidence that Rb is selectively recruited to promoters through a high affinity interaction with E2F, and that, once it is tethered to the promoter by E2F, Rb can interact simultaneously with E2F and surrounding transcription factors. This interaction with Rb appears to quench the activity of these transcription factors by blocking their interaction with the basal transcription complex. The ability of Rb to bind concurrently with E2F and other transcription factors allows the Rb-E2F complex to function as an active transcriptional repressor.

Previously it has been demonstrated that binding of Rb inactivates E2F. Here, we demonstrate a novel activity for Rb—it can bind and inactivate surrounding transcription factors while it is bound to the promoter by E2F. We propose that resulting repressor activity of the Rb-E2F complex is crucial for inhibiting expression of cell cycle genes that contain enhancers in addition to E2F sites.

A1-468 CONFINING THE BREAKPOINT REGION FOR THE PAPILLARY RENAL CELL CARCINOMA ASSOCIATED TRANSLOCATION t(X;1)(p11;q21), Marian A. J. Weterman, Monique Wilbrink, Richard J. Sinke, Bauke de Jong*, and Ad Geurts van Kessel, Department of Human Genetics, University Hospital Nijmegen, Nijmegen, the Netherlands and * Dept. of Medical Genetics, University of Groningen, Groningen, the Netherlands

The chromosomal translocation t(X;1)(p11;q21) has been described as the sole anomaly in a case of papillary renal cell carcinoma (1). Other cases confirmed the presence of this translocation, sometimes next to numerical aberrations (2), which makes it a good candidate for a primary change within this type of tumor. Previously, we mapped the breakpoint regions between the loci DXS226 and DXS146/DXS255 (3) on the short arm of chromosome X and in the 1q21 region distal to the FcgammaR1 locus on chromosome 1.

In the present study, we applied a series of additional markers to narrow down the area of interest on both translocation chromosomes using FISH and a panel of somatic cell hybrids containing der(X), der(1), or chromosomes X or 1 only. The current data indicate that the breakpoint maps between DXS255 and TFE3 on chromosome X and centromerically to APOA2 on chromosome 1, thereby considerably reducing the breakpoint area.

1). de Jong *et al.*, Cancer Genet. Cytogenet., **21**:165-169, 1986
2). Meloni *et al.*, Cancer Genet Cytogenet., **65**:1-6, 1993
3). Sinke *et al.*, Hum. Genet., **92**:305-308, 1993

A1-469 THE EBV-ENCODED LMP1 PROTEIN BLOCKS p53-INDUCED APOPTOSIS BUT NOT G1 ARREST BY A MECHANISM DOWNSTREAM OF WAF1, Klas G. Wiman, Yisong Wang, George Klein, and Ismail Okan, Microbiology and Tumor Biology Center, The Karolinska Institute, S-171 77 Stockholm, Sweden

We have previously demonstrated that exogenous wild type p53 induces apoptosis in the Burkitt lymphoma line BL41 that carries endogenous mutant p53, using a temperature sensitive Val-135 mutant p53 construct expressed as mutant p53 at 37°C and wild type p53 at 32°C. We also found that wild type p53-induced apoptosis is prevented by bcl-2 in a mouse T lymphoma line. The Epstein-Barr virus (EBV)-encoded latent membrane protein LMP1 has been shown to protect Burkitt lymphoma cells from apoptosis induced by low serum. In order to test whether LMP1 is able to block wild type p53-induced apoptosis, we introduced LMP1 in the BL41 cells expressing the ts p53 construct using a retroviral LMP1 expression vector. Cell cycle analysis demonstrated that the cells expressing LMP1 were arrested in G1 upon temperature shift to 32°C. However, they did not enter apoptosis, as shown by the absence of characterisitc morphological changes, DNA fragmentation, and positive Tunel staining. Trypan blue staining showed that the cells remained viable at 32°C, although the cell number did not increase. WAF1 mRNA was induced at 32°C in both the ts p53-transfected and ts p53-transfected/ LMP1-infected BL41 cells. Thus, LMP1 expression prevents p53-induced apoptosis by a mechanism downstream of WAF1. The LMP1-infected cells expressed elevated levels of bcl-2 protein. Therefore, our data suggest that LMP1 protects cells from p53-induced apoptosis by upregulating bcl-2 expression.

A1-470 A GENETIC ANALYSIS OF CYCLIN E: HUMAN CYCLIN E MUTANTS AND INTERACTING PROTEINS, Kwang-Ai Won and Steven I. Reed, Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037

Human cyclin E was originally isolated by complementing yeast G1 cyclins, and accumulating evidence points to cyclin E as a rate-limiting G1 cyclin in mammals. Deregulated expression and activity of the proteins that control entry into S phase could lead to uncontrolled cell growth which would result in the development of tumors. Indeed, G1 cyclins and kinases have been shown to be overexpressed by gene translocation and amplification in some cancers. To understand the molecular interactions of cyclin E with other components of the cell division cycle control machinery, we employed genetic approaches to isolate functional cyclin E mutants and cyclin E-interacting factors.

The cyclin E gene was subjected to random mutagenesis by a modified PCR procedure, and the products were inserted into a yeast expression vector containing an attenuated *GAL1* promoter. The library was transformed into a yeast strain in which the kinase CDC28 is replaced by the human kinase CDK2, and transformants were screened for lethality resulting from high cyclin E-CDK2 activity in presence of galactose. We have obtained hyperactive and hyperstable mutant forms from this screen, and are now mapping the residues of cyclin E responsible for the hyperfunctional phenotypes.

Based on the observation that overexpression of wild type cyclin E driven by the intact *GAL1* promoter results in a very high kinase activity that is lethal to yeast cells, we started another genetic screen to isolate KES (CDK2-cyclin E suppressor) which rescue the lethality of cyclin E in the CDK2 strain. The cloning of KES cDNAs and their functional relationship with cyclin E-CDK2 kinase will be discussed.

A1-471 Targeted degradation of a forcal adhesion protein during prolonged mitotic arrest of CHO cells and fibroblasts. Ryuji Yamaguchi, Hitoshi Kimura, and Hisataka Sabe Institute for Virus Research, Kyoto University, Sakyoku, Kyoto 606, Japan.

Adhesive cells *in vitro* contact the extracellular matrix (ECM) through specialized structures called focal adhesion plaques (FAPs). During M-phase of the cell-cycle, cells release their hold on the extracellular matrix and disassemble most FAPs by an as yet unknown mechanism. Tyrosine residues of several FAP components are dephophorylated when cell's adherence to the ECM is lost. The eventual aim of our study is to understand the mechanism governing assembly and disassembly of FAPs during the cell cycle. Towards this end, we arrested cells in M-phase using pharmacological agents (nocodazole and demecolcine) and examined steady-state levels as well as phodsphorylation states of FAP proteins.

CHO cells, mouse NIH 3T3 and rat 3Y1 fibroblasts were treated with nocodazole or demecolcine for various time periods, and M-phase cells were collected by mechanical shake-off. We harvested protein from M-phase arrested cells, from cells that were still adherent to the same tissue culture plates, and from cells kept in suspension in soft agar in the presence and absence of drugs.

We found that the tyrosin phosophorylation of several FAP proteins are greatly reduced. Moreover, we found that one of the focal adhesion proteins was specifically degraded in M-phase cells treated with nocodazole or demecolcine for prolonged periods of time. This protein was not degraded in identically treated adherent or soft agar suspended-cells. Nor did we see degradation of any other FAP proteins examined. Currently, we are investigating possible mechanisms for the targeted degradation of the protein.

A1-472 ALTERATIONS OF A ZINC FINGER GENE, *BCL-6*, IN B CELL LYMPHOMA,

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As a consequence of aberrations affecting chromosome 3q27, a substantial fraction (30-40%) of diffuse large cell lymphoma (DLCL), characterized by extranodal origin and favorable prognosis (4), are associated with rearrangements of the BCL-6 gene (1-3). The *BCL-6* gene encodes a 89 kD nuclear phosphoprotein featuring six C-terminal C_2H_2-type zinc-fingers and a Zinc finger N-terminal (ZIN/POZ) domain, a protein-protein interaction domain common to a subset of zinc-finger proteins including several Drosophila developmental regulators (2). In both human and murine B-cell lines, *BCL-6* is preferentially expressed in mature B-cells where its transcription is regulated by two promoters in the first exon region. In the majority of cases carrying *BCL-6* rearrangements, the *BCL-6* promoter region is truncated. In three cases studied, (3;14)(q27;q32) translocations were shown to juxtapose sequences from the immunoglobulin (Ig) loci to exon 2-10 of *BCL-6*, leading to the expression of *BCL-6* under the control of Ig promoters. To determine whether *BCL-6* may be altered by mechanisms other than chromosomal rearrangements, the presence of mutations in either coding or regulatory *BCL-6* sequences was investigated by PCR/SSCP analysis. No mutations were detected in the entire *BCL-6* coding region (exons 3-10) in 22 DLCL samples tested. In contrast, alterations within a 4 kb region spanning the *BCL-6* first exon were detected in 14 of 18 DLCL cases (78%) carrying unrearranged *BCL-6* genes, but not in 190 other biopsies including most types of solid tumors. These alterations were only found in tumor- but not in normal DNA from the same patients suggesting their somatic origin. Nucleotide sequence analysis indicated that the detected alterations include point mutations and/or small deletions. Thus, considering both rearrangements and mutations, >80% of DLCL carry alterations in the 5' non coding region of *BCL-6* . The frequency, clustering, and disease-association of these alterations suggest that they may contribute to lymphomagenesis, presumably by altering *BCL-6* gene expression.

1. Ye, BH et al., *Cancer Res.* 53:2732, 1993.
2. Ye, BH et al., *Science* 262:747, 1993.
3. Lo Coco, F et al., *Blood* 83:1757, 1994.
4. Offit, K et al., *New Engl. J. Med.* 331, 74, 1994

A1-473 **Partial Rescue of RB1-/- Mice by an RB1 Minigene**

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Homozygous RB1 knock out mice (RB1-/-) die in utero by embryonic day E15.5 with defective neurogenesis and hematopoiesis. The early death of the RB1-/- embryos precluded studies on the role of RB1 in the development of tissues which are formed post E15.5. We therefore set up to disrupt RB1 in specific tissues using the CRE/lox recombination system. Our strategy involves the generation of transgenic mice in which the null mutation in RB1 will be complemented by a mouse RB1 minigene flanked by two loxP sites (Rblox;RB1-/-). The minigene Rblox will then be disrupted in the tissue of choice by expressing the CRE gene under the control of a tissue specific promoter.

We produced Rblox transgenic animals, with an RB1 minigene consisting of 1.3 of promoter DNA sequence the first exon and intron of RB1 (Zacksenhaus et al., 1993 Oncogene 8:2343), and the remaining RB1 cDNA. Breeding of several independent Rblox;RB1+/- with RB1+/- has, so far, yielded no viable offspring with the desired genotype. By inbreeding Rblox(founder #4);RB1+/-, we have obtained Rblox-1(#4);RB1-/- newborn mice; these neonates died at the day of birth (P1). We are testing these P1 pups for pRb expression, copy number of the transgene and abnormalities in the nervous system, hematopoiesis and other tissues. The early death of the Rblox(#4);RB1-/- pups is indicative of only partial complementation and should provide valuable information about the role of pRb in the development of specific tissues post E15.5.

A1-474 THE MLL AT HOOK DOMAIN BINDS TO CRUCIFORM DNA AND TO SCAFFOLD ATTACHMENT REGIONS,

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We have recently cloned the *MLL* gene which is disrupted in recurring chromosomal translocations involving chromosome band 11q23 in AML and ALL. MLL is a putative transcription factor with homology to several other proteins including the zinc fingers and other domains of the Drosophila trithorax protein and the AT-hook DNA binding motif of high mobility group proteins (HMG). In translocations involving *MLL*, the protein produced from the der(11) chromosome which contains the AT-hook domain is thought to be responsible for leukemogenesis. We assessed the DNA binding capability of the MLL AT-hook domain using bacterially expressed and purified AT-hook protein. In a gel mobility shift assay, the MLL AT-hook domain could bind cruciform DNA, recognizing structure rather than sequence of the target DNA. This binding could be specifically competed with Hoechst 33258 dye and with distamycin. In a nitrocellulose protein-DNA binding assay, the MLL AT-hook domain could bind to AT-rich SARs from the interferon alpha-2 gene, but not to non-SAR DNA from the same gene. We are investigating MLL AT-hook domain binding on SAR fragments from a variety of genes to determine whether binding is universal or limited to a subset of SARs. It is unclear what role the AT-hook binding to DNA may play *in vivo*, but it is likely that DNA binding could affect downstream gene regulation. It seems likely that the two DNA-binding domains of MLL, AT-hooks and zinc fingers, normally act together to determine downstream target genes. The AT-hook domain retained on the leukemogenic der(11) would potentially recognize a different DNA target than the one normally recognized by the intact MLL protein, thus contributing to leukemogenesis.

A1-475 PYGMY GENE, A MUTANT ALLELE OF THE TRANSCRIPTIONAL ACCESSORY FACTOR , HMGI-C

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Body size control is one of the most fundamental questions in developmental biology. In the mouse, a number of developmental mutants have been identified by their dwarf phenotypes, but only one cannot be explained by disruption of the growth hormone pathway. This is the pygmy mutant and a molecular analysis revealed a common deletion area of 56 kb in 3 mutant alleles. We searched 100kb of genomic sequence surrounding the common deletion and putative exons were obtained by using the exon trapping system. When the gene data base was searched, this gene was found to have 100% homology with a known gene, HMGI-C.

With such a large deletion present in the available mutants, definitive proof that HMGI-C is responsible for the pygmy phenotype is currently being confirmed by gene targeting. So far, 14 male chimearic mice have been generated. There is compelling evidence to suggest the HMGI family of proteins play a significant role in transcription either, as accessory or architectural factors. For example, there is evidence that HMGI/(Y) interacts with NF-kB and ATF-2 transcription factors in the virus induction of the human IFN-B gene. Since HMGI-C is expressed during embryogenesis, we have constructed a fusion protein library using mRNA from 12.5 dpc mouse embryo. At present, we are screening the library to detect proteins that may interact with HMGI-C in the two hybrid system.

Late Abstracts

MOLECULAR CLONING OF THE GENE (*AF10*) INVOLVED IN THE t(10;11)(P12;Q23) TRANSLOCATION IN ACUTE LEUKAEMIA REVEALS A NEW CLASS OF CONSERVED ZINC FINGER/LEUCINE ZIPPER PROTEINS.

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The t(10;11)(p12;q23) translocation is a recurrent event in acute myeloid leukaemias (AML). The fusion of novel sequences into mRNA from the HRX gene was observed in three leukaemias with either simple or complex versions of the t(10;11) translocation. The translocated gene on chromosome 10 (AF10) has been molecularly cloned and its sequence analysed. The predicted product of AF10 is a 109 kDa protein of 1027 amino acids. Northern analysis indicates that the AF10 gene is expressed in a variety of human tissues as a 5.5kb mRNA, with particularly high expression in testis. An N-terminal zinc finger domain has been identified in AF10 which has 93% identity to the zinc finger domain of AF17, recently identified in the t(11;17) translocation. AF10 also contains a region with the characteristics of a leucine zipper, related to the same feature in AF17, located such that it would be included in the HRX/AF10 fusion products expressed from the der(11) chromosome in the three leukaemias analysed. Database searches have revealed that AF10 is related to a human gene of unknown function (BR140). We have identified a C Elegans gene (CEZF) which has a zinc finger region with 62% identity and 78% similarity to the corresponding feature in AF10. Additionally, a region with the potential to encode a leucine zipper region has been located in CEF54F2 with homology to that of AF10. Thus these four genes AF10, AF17, BR140 and CEZF share certain conserved features which suggest they may represent a new class of transcription factors.

AFFINITY MODULATION OF INTEGRIN $\alpha5\beta1$ BY V-SRC.

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The integrins form a family of heterodimeric cell surface receptors for ligands present in the extracellular matrix or expressed on cell surfaces. They constitute a major class of cell adhesion molecules. Chicken embryo fibroblasts transformed by v-src can be rounded up and detached by monoclonal antibodies which interfere with binding of ligands to b1 integrin. This indicates that the $\beta1$ family of integrins provide the major source of substrate adhesion. Analysis of the adhesion properties of the transformed cells show that they adhere primarily to fibronectin with a weaker adhesion to vitronectin and laminin matrix substrates. This adhesion appears to be primarily mediated by $\alpha5\beta1$ integrin. In contrast to earlier data, we find little reduction in the level of $\alpha5\beta1$ and no evidence that $\alpha3\beta1$ participates in adhesion. The affinity of $\alpha5\beta1$ for fibronectin was measured using a modified cell adhesion assay and controlling the affinity of $\alpha5\beta1$ by exchanging of the divalent cation or reaction with a monoclonal antibody to the a5 subunit which has the ability to activate or stabilize the higher affinity form of the receptor. By comparing the adhesion without external activation of the receptor (i.e. relying on intracellular complexes to activate the receptor) with adhesion in the presence of Mn^{++} or U1α antibody which activate the receptor by altering the conformation of the extracellular domain (i.e. extracellular activation), we measured the relative activation state of $\alpha5\beta1$ in the cell. In normal CEF there was very little difference in the levels of adhesion measured in the presence of Mg^{++} and Mn^{++} indicating that $\alpha5\beta1$ could be efficiently activated in these cells; whereas in the v-src transformed cells there was a large increase in adhesion in the presence of Mn^{++} indicating that $\alpha5\beta1$ is mostly in the low affinity state although it is present on the cell surface and capable of participating in cell adhesion.

DIMERIZATION BETWEEN NF-IL6 AND FOS/JUN MODULATES GP130 SIGNALING,

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Gp130 is a receptor component shared by IL-6, oncostatin M, leukemia inhibitory factor, ciliary neurotrophic factor and IL-11. Ligand binding induces rapid tyrosine phosphorylation of gp130 and STATs (signal transducers and activators of transcription) by Jak-Tyk tyrosine kinases. Our recent evidence indicates that although tyrosine phosphorylation is required for activation and nuclear translocation of STATs, association between STATs is ligand-dependent and specified by post-translational modification of STATS other than tyrosine phosphorylation.

The Jak-STAT activity, however, declines within minutes after activation. Propagation of stable signals mediated by gp130 involves a delayed (or concurrent) pathway that leads to activation of NF-IL6 (C/EBPβ) and Fos/Jun. NF-IL6 functions as a dimeric transcription activator or inhibitor, depending on the ratio of activator to inhibitor isoforms translated from in-frame AUGs of the same mRNA species. We have showed that gp130 signaling regulates the ratio of NF-IL6 isoforms to favor the activator form, and is modulated by the cross talk between NF-IL6 and AP-1. NF-IL6 dimerizes with Fos/Jun through the leucine zipper region in the absence of DNA, resulting in acquisition of new DNA binding specificities by the heterodimers and modulation of transactivation by NF-IL6 and Fos/Jun. Most importantly, dimerization between NF-IL6 and Jun occurs in the cells in a lineage-dependent manner, but only when both NF-IL6 and Jun are activated by IL-6. These results provide an example for regulated transcription factor interaction, and suggest a physiologic role for NF-IL6/AP-1 dimerization in the determination of promoter specificity in gp130 signaling. The significance of NF-IL6/AP-1 interaction in linking the Jak-STAT and the delayed (concurrent) pathways will be discussed.

REGULATION OF RAPAMYCIN SENSITIVE PHOSPHORYLATION SITES OF $P70^{S6K}$,

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$p70^{s6k}/p85^{s6k}$ is responsible for the multiple phosphorylation of ribosomal protein S6. The kinase is activated through multiple phosphorylation on serine/threonine residues, and lies on a novel $p21^{ras}$-/$p42^{mapk}$-independent signalling pathway. The four major phosphorylation sites associated with $p70^{s6k}/p85^{s6k}$ activation characterized to date are clustered in 14 amino acids within a putative autoregulatory domain and each is followed immediately by a proline residue. The immunosuppressant rapamycin was recently shown to block the phosphorylation and activation of the $p70^{s6k}/p85^{s6k}$ and inhibit cell growth. In transient transfection studies rapamycin-induced inactivation of $p70^{s6k}/p85^{s6k}$ has been shown to result from the dephosphorylation of a novel set of sites distinct from those above. Recent studies further show that these sites are also rapidly phosphorylated in response to mitogen stimulation of quiescent cells. Rapamycin treatment of serum-stimulated Swiss 3T3 cells also leads to the dephosphorylation of these same sites. However, phosphorylation apparently proceeds in the proline directed sites of the autoinhibitory domain, suggesting there are at least two independent pathways regulating $p70^{s6k}/p85^{s6k}$ activation. In addition the effect of rapamycin on these sites is reversed by FK506, demonstrating that the dephosphorylation of these sites is mediated through the rapamycin-FKBP12 immunophilin complex. Identification of these sites will be a necessary step in establishing the mode of $p70^{s6k}/p85^{s6k}$ activation.

STIMULATION OF THE PDGF-β RECEPTOR SIGNALING PATHWAY ACTIVATES PKC-δ,

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The murine myeloid progenitor cell line, 32D, was recently shown to undergo monocytic differentiation when protein kinase C (PKC)-δ was overexpressed and activated by 12-O-tetradecanoylphorbol-13-acetate (TPA). Tyrosine phosphorylation of PKC-δ occurred when PKC-δ-transfected 32D cells were stimulated by TPA. In order to elucidate the role played by PKC-δ in response to activation of a receptor tyrosine kinase, we transfected platelet-derived growth factor-β receptor (PDGF-βR) alone (32D/PDGF-βR) or together with PKC-δ (32D/PDGF-βR/PKC-δ) into 32D cells. NIH-3T3 cells which endogenously express both PDGF-αR and PDGF-βR were also transfected with PKC-δ (NIH-3T3/PKC-δ). Like TPA treatment, PDGF-BB stimulation caused striking phosphorylation of PKC-δ *in vivo* and translocation of some PKC-δ from the cytosol to the membrane fraction in both cell systems. Some of the phosphorylation induced by PDGF-BB treatment was found to be on tyrosine residue(s). Tyrosine-phophorylated PKC-δ was observed only in the membrane fraction after PDGF-BB or TPA stimulation. The enzymatic activity of PKC-δ in the membrane fraction also increased after TPA or PDGF stimulation, providing a positive correlation between PKC-δ tyrosine phosphorylation and its activation. Overnight treatment of 32D/PDGF-βR/PKC-δ cells with PDGF-BB induced monocytic differentiation as judged by an increase in expression of cell surface macrophage differentiation markers. PDGF-BB had much weaker effects on 32D/PDGF-βR cell differentiation, suggesting that increased PKC-δ expression enhanced monocytic differentiation. These results indicate that PKC-δ is a downstream molecule in the PDGFR signaling pathway which may play a pivotal role in PDGF-βR-mediated cell differentiation.

BCL-2 ACTIVATION IN DIFFERENTIATED NEUROBLASTOMA IS ASSOCIATED WITH RESISTANCE TO CHEMOTHERAPY-AND RADIATION-INDUCED APOPTOSIS.

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Overexpression of the Bcl-2 oncogene inhibits apoptosis induced by DNA damaging agents thus providing a mechanism for tumor resistance to chemotherapy and radiation. Metastatic neuroblastoma (NB) is one of the most lethal solid tumors of childhood with a 20% long-term survival rate despite intensive therapy. Previous studies have shown that 80% of NB tumors express Bcl-2 following chemotherapy, suggesting that Bcl-2 activation in NB may contribute to drug resistance and poor clinical response. In an effort to improve survival for metastatic NB, novel treatment strategies which incorporate differentiating agents such as retinoic acid (RA) are being developed. Therefore we wished to determine the relationship of Bcl-2 expression to chemotherapy and radiation induced apoptosis in differentiated NB cells. Human NB cells (SH-SY5Y) were incubated with either RA, tetradecanoylphorbol-13-acetate (TPA), or nerve growth factor (NGF) to induce differentiation. Bcl-2 expression was measured in both differentiated as well as undifferentiated SH-SY5Y, and the cells were then utilized in short term cytotoxicity assays to compare their relative levels of sensitivity to vincristine, etoposide, cis-platinum, and radiation. Apoptosis induced by these DNA damaging agents was assessed by DNA fragmentation assays. Up-regulation of Bcl-2 was detected in RA and TPA-differentiated SH-SY5Y. Paralleling this induction of Bcl-2, RA and TPA-differentiated NB cells were significantly more resistant to DNA damage induced by several chemotherapeutic drugs and radiation as compared to undifferentiated controls. In contrast, NGF-mediated differentiation of SH-SY5Y did not result in Bcl-2 induction nor inhibition of chemotherapy/radiation-induced apoptosis. Collectively these results suggest that Bcl-2 expression in differentiated NB correlates with tumor cell resistance to DNA damaging agents. Moreover, NGF may be more clinically useful as a NB differentiating agent since it does not induce Bcl-2.