GENE REGULATION AND SIGNALLING IN ENDOCRINE SYSTEMS

Organizers: David Moore, Jacques Drouin and Robert Lefkowitz

January 19-25, 1991

Plenary Sessions Page

Poster Sessions

Ligand-Stimulated Protein-Tyrosine Kinases (joint)

E 001 RECEPTOR PROTEIN-TYROSINE KINASES AND PHOSPHATASES Tony Hunter, Bill Boyle, Rick Lindberg, David Middlemas, Sharon Tracy, Peter van der Geer, and Jim Woodgett. The Salk Institute, P.O. Box 85800, San Diego, CA 92186

We have identified 2 novel receptor-like PTKs by screening cDNA libraries with oligonucleotide probes to consensus sequences. One of these, *eck*, isolated as a HeLa cell cDNA, is mainly expressed in tissues containing proliferating epithelial cells (skin, lung and intestine). The *eck* protein is a member of a small family of receptor PTKs, which includes *eph*, *elk* and *eek*. The *eck* protein is phosphorylated on Tyr in immunoprecipitates from epithelial cell lines, showing that the *eck* protein is a PTK. Staining of tissue sections shows that *eck* is expressed in epithelial cells in kidney and in small intestine. A second putative PTK, *trk*B, was isolated from a rat cerebellar library. The *trk*B protein is closely related to but distinct from the *trk* receptor-like PTK. *trk*B is primarily expressed in brain, as a series of RNAs ranging from ~1-13 kb. The smaller RNAs are too short to encode the intact protein. Analysis of additional *trk*B cDNAs indicates that there are mRNAs encoding 2 different truncated forms of *trk*B, which are both truncated just downstream of the TMD, and have short distinct C-termini. We are investigating the distribution of the 3 types of *trk*B mRNA and their protein products in the brain. We have identified Y706 lying in the kinase insert as a major autophosphorylation site in the murine CSF-1 receptor, and mutated Y706 and Y807 in this receptor to F or G. When expressed in Rat-2 cells the F807 and G706 mutant receptors respond to CSF-1, but significantly less well than wt receptors. G807 mutant receptors lack PTK activity and fail to respond to CSF-1. F807 receptors have 2-3 fold reduced PTK activity in vitro and cells expressing F807 receptors respond less well to CSF-1 than cells expressing wt receptors. Using PCR we have isolated a cDNA clone for a novel receptor-like PTPase, PTP-α, from an NIH 3T3 cell cDNA library. PTP-α has a rather small extracellular domain of 141 residues, including a signal peptide, which lacks Cys, is rich in Ser and Thr, and contains several potential sites for N-linked glycosylation. Like other receptor PTPases PTP-α has twin catalytic domains, and when expressed in E. coli the cytoplasmic domain has PTPase activity against a variety of peptide substrates. PTP-α is widely expressed in rat tissues as a 2.8 kb mRNA. Using antisera against recombinant PTP-α we are studying the biosynthesis and phosphorylation of PTP-α.

To determine how PKs activated at the cell surface induce nuclear events, we are examining the phosphorylation of nuclear regulatory proteins. The transcription factor cJun, which associates with cFos and binds to the TPA-response element (TRE) upstream of genes induced upon activation of PKC, is a phosphoprotein with 5 major sites of Ser and Thr phosphorylation. Three of these show decreased phosphorylation upon TPA treatment, and are clustered just upstream of the basic region and leucine zipper, which form the minimal DNA binding domain. Phosphorylation of these sites in vitro by glycogen synthase kinase 3 (GSK-3) in recombinant c-Jun protein decreases its ability to bind to a collagenase TRE. We propose that cJun function is negatively regulated by phosphorylation, and that TPA activation of transcription from TRE-dependent genes may in part involve dephosphorylation of cJun. The vJun protein has a S to F change at the position equivalent to 243 in human cJun, which is one of the cJun phosphorylation sites. A F243 mutant cJun is hypophosphorylated at all 3 GSK-3 sites when transiently expressed in rat embryo fibroblasts, and shows about 10-fold greater ability to activate transcription of a TRE/CAT reporter gene than wild type cJun. The protein kinase that phosphorylates cJun in vivo is not known, but the finding that GSK-3β is the homologue of the Drosophila segment-polarity gene *zeste-white3* suggests that GSK-3 itself may be the protein kinase. We are currently investigating whether these phosphorylations affect dimerization of cJun or the ability of the dimer to bind to the TRE. We are also trying to determine whether TPA inhibits the GSK-3 like protein kinase, or else activates a protein phosphatase specific for these phosphorylation sites.

Membrane Receptors

E 002 ANALYSIS OF DOMAINS INVOLVED IN LIGAND BINDING AND SIGNAL TRANSDUCTION OF THE PROLACTIN RECEPTOR, Paul A. Kelly, Maria Rozakis, Marc Edery, Laurence Lesueur, Suhad Ali and Jean Djiane, Laboratory of Molecular Endocrinology, Royal Victoria Hospital, 687 Pine Ave. W., Montreal, H3A 1A1 Canada, and Unité d'Endocrinologie Moléculaire, INRA, 78350 Jouy-en-Josas, France.

Prolactin (PRL), secreted by the anterior pituitary gland, is a hormone that is the best known for its reproductive actions on the mammary gland (mammogenesis and lactogenesis). However, numerous other biological functions of this hormone (salt and water balance, growth and development, additional reproductive actions, metabolic effects, behavioral effects, and immunoregulation) have been reported. It forms, with growth hormone (GH) and placental lactogen (PL), a family of polypeptide hormones. Recently, we purified and partially sequenced the PRL receptor in rat liver and cloned its cDNA, which permitted the identification of the primary structure of the mature protein. This receptor consists of 291 amino acids (aa) and contains an extracellular region of 210 aa, a single transmembrane region and a cytoplasmic domain of 57 aa. Regions of localized amino acid identity were found with the GH receptor, cloned by the Genentech Group, suggesting that the receptors for GH and PRL form a new family of single membrane-spanning receptors. Using this cDNA as a probe, we identified cDNAs that encode a long form of the PRL receptor in rabbit mammary gland (592 aa) and human hepatoma and breast cancer cells (598 aa), as well as a second long form (591 aa) of the rat prolactin receptor in the rat. The GH/PRL receptor family has recently expanded to include receptors for a number of cytokines, including IL-2(β), IL-3, IL-4, IL-6, IL-7, GM-CSF, G-CSF, and erythropoietin. The genes for the human PRL and GH receptors are found on the short arm of chromosome 5 (5p13 → p14). In the rat, the PRL receptor gene is > 100 kb, with at least 11 exons. The first 10 exons encode the long form of the the receptor, while the short form is produced by alternative splicing to include the first 9 exons and exon 11. Mutagenesis of selected amino acids of the extracellular domain have revealed that the first four cysteines in the N terminal region appear to be involved in the binding of prolactin, whereas the fifth free cysteine is outside the binding domain. Of the three potential extracellullar N-linked glycosylation sites, only the first two appear to be glycosylated. Non-glycosylated receptor is able to bind PRL. We have developed a functional assay for the receptor by cotransfecting the promoter region of a PRL-responsive gene (β-lactoglobulin) with a reporter gene (chloramphenicol acetyltransferase, CAT) along with the cDNA of the PRL receptor in CHO cells. We have determined that the long form of the receptor is functionally active in this assay, whereas the short form, which in fact is the form that predominates in the rat mammary gland, is inactive. The deletion and/or substitution of regions of the receptor molecule followed by a functional test should permit the determination of the active domains of the receptor involved in the process of signal transduction.

E 003 GLUTAMATE-OPERATED CHANNELS: CELL-SPECIFIC FUNCTIONAL SWITCHES INTRODUCED BY ALTERNATIVE SPLICING, Kari Keinänen, Bernd Sommer, Todd A. Verdoorn, William Wisden, Bert Sakmann and Peter H. Seeburg, Laboratory of Molecular Neuroendocrinology, Center for Molecular Biology, Max-Planck-Institute for Cell Physiology, 6900 Heidelberg, F.R.G and Biotechnical Laboratory, VTT, Espoo, Vuorimiehentie 5, SF-02150 Espoo, Finland. Virtually all neurons in CNS are responsive to L-glutamate (L-Glu), the major excitatory neurotransmitter in brain. L-Glu exerts its action through synaptically organized ionotropic channels which are intrinsic constituents of several pharmacologically different glutamate receptors. Molecular cloning of cDNAs encoding these receptors has revealed the existence of a family of glutamate receptors whose channels can be gated by L-Glu, AMPA, quisqualate and kainate. Furthermore the recombinantly expressed receptors contain high affinity binding sites for AMPA, but not for kainate, demonstrating that these molecules are AMPA-selective non-NMDA receptors. In a family of four abundant AMPA receptors, a small segment preceding the predicted fourth transmembrane region exists in two alternative sequence versions. These modules, designated FLIP and FLOP, are encoded on adjacent exons and impart different kinetic properties on currents evoked by L-glutamate or AMPA, but not kainate. For each receptor, the alternatively spliced mRNAs show distinct expression patterns in rat brain, particularly evident in the CA1 and CA3 fields of the hippocampal formation. These results identify a switch in the molecular and functional properties of glutamate receptors operated by alternative splicing. They reveal a novel mechanism for modifying synaptic excitatory channels that may underlie adaptive changes in a single neuron. Further functional implications of our findings will be discussed.

G Protein-Linked Receptors (joint)

E 004 REGULATION OF THE CELL CYCLE BY A NEGATIVE GROWTH FACTOR IN YEAST: *FAR1* IS AN INHIBITOR OF A G1 CYCLIN, Fred Chang and Ira Herskowitz, Department of Biochemistry and Biophysics, UCSF, San Francisco, CA 94143-0448.

We are interested in how a mating factor of *Saccharomyces cerevisiae*, α-factor, arrests the cell cycle in G1 at START. The α-factor response pathway consists of a cascade of events which involve a G-protein coupled receptor, protein kinases, a transcription factor, and "arrest" genes such as *FAR1*, which culminate in the inhibition of three G1 cyclin genes to cause cell cycle arrest. We have identified a gene *FAR1* (factor *arrest*), which is necessary for cell cycle arrest but not for other responses to α–factor, such as morphological or transcriptional induction responses. A deletion allele of *FAR1* is defective in α-factor arrest, and is not affected in mitotic division or other START controls, suggesting that *FAR1* does not have a role in more general cell cycle regulation. The nucleotide sequence of *FAR1* reveals no homologies with known proteins. *FAR1* mRNA levels are induced upon exposure to α-factor. We propose that *FAR1* is a component at the end of the α-factor response pathway which acts to arrest the cell cycle.

Genetic evidence demonstrates that *FAR1* acts to inhibit specifically one of the three G1 cyclins,*CLN2* : a *far1 cln2* double mutant arrests in response to α-factor. Because the other G1 cyclins, *CLN1* and *CLN3*, can functionally substitute for *CLN2*, other effectors must inhibit these products. A drop in *CLN2* mRNA levels upon exposure to α-factor is dependent on *FAR1*. We propose that *FAR1* acts to repress *CLN2* mRNA levels, either on the level of transcription or mRNA stability.

E 005 POSITIVE AND NEGATIVE REGULATION OF THE MATING SIGNAL TRANSDUCTION PATHWAY IN YEAST. H. G. Dohlman, J. Thorner (Dept. of Molecular and Cell Biology, Univ. California, Berkeley, CA 94720) Klim King, Marc G. Caron, and Robert J. Lefkowitz (Depts. of Biochemistry, Cell Biology, and Medicine, Duke University, Durham, NC 27710)

Eukaryotic microorganisms, such as the yeast S.cerevisiae, have proven useful for the study of G protein-mediated signal transduction processes analogous to those in animal cells. Evolutionary conservation of function has in some instances allowed substitution by mammalian genes for the corresponding genes in yeast. To facilitate functional and mechanistic studies of receptor/G protein interaction, high level expression of the human β_2-adrenergic receptor (βAR) was achieved; βAR expressed in yeast displayed characteristic ligand binding affinities, specificity, and stereoselectivity. In yeast cells lacking the endogenous G protein α subunit (GPA1 gene product), partial activation of the yeast pheromone response pathway by adrenergic agonists was observed in cells coexpressing βAR and a mammalian G protein α subunit. Hence, the mammalian receptor and $G_s\alpha$ subunit can couple to each other and to downstream effectors when expressed in yeast. This in vivo reconstitution system will be useful for examining many aspects of the ligand binding and G protein activating functions of the βAR and other cell surface receptors. Similarly, the role of negative regulatory components identified genetically in yeast is being examined in mammalian cells. For example, the yeast SST2 gene product plays a critical role in recovery from pheromone-induced responses. The mechanism of SST2 action is poorly understood, but homologous gene products may play a role in desensitization of G protein-mediated signaling in mammals as well.

E 006 MOLECULAR ANALYSES OF β-ADRENERGIC REGULATION, W.P. Hausdorff, J.A. Pitcher, J. Ostrowski, M.G. Caron, and R.J. Lefkowitz, HHMI, Depts. of Medicine and Cell Biology, Duke Univ. Medical Center, Durham, NC 27710.

Continuous exposure of cells expressing β_2-adrenergic receptors (β_2AR) to epinephrine results in a rapid attenuation of the adenylyl cyclase response. Mutation of the carboxyl terminal tail of β_2AR has previously been shown to impair a variety of regulatory processes affecting β_2AR, including agonist-induced phosphorylation of the receptor, the rapid (and reversible) loss of hydrophilic ligand binding proposed to reflect sequestration of the receptor away from the plasma membrane, and a long-term loss of all ligand binding to β_2AR (down-regulation). We describe a series of substitution mutations in the proximal portion of this domain of the receptor with selective effects on the rapid regulatory processes. Although phosphorylation of β_2AR is required for rapid desensitization, these findings indicate that neither β_2AR phosphorylation nor sequestration is a prerequisite for long term down regulation of β_2AR. In addition, these results identify a 10 amino acid stretch of the carboxyl terminal domain of β_2AR that is involved in rapid regulation of the receptor.

Activation and Function of Nuclear Receptors

E 007 MULTIFACTORIAL TRANSCRIPTIONAL CONTROL OF THE MMTV PROMOTER.

Ulf Brüggemeier, Domingo Barettino, Mathias Truss, Martha Kalff, Emily P. Slater and Miguel Beato, IMT, E.-Mannkoppf-Str. 2, 355 Marburg, F.R.G.

Gene regulation by steroid hormones is mediated by the hormone receptors that interact with hormone responsive elements (HRE) near the regulated promoters. In the mouse mamma tumor virus (MMTV) promoter there is a complex HRE in the long terminal repeat region of the provirus that is precisely organized in a phased nucleosome. Following hormone treatment *in vivo*, the chromatin over the HRE becomes more accessible to digestion by DNaseI, a phenomenon that correlates with binding of transcription factors, including nuclear factor I (NFI), to the MMTV promoter. However, NFI acts as a basal transcription factor on the MMTV promoter *in vitro* and does not cooperate with the hormone receptors in terms of binding to free DNA. There is an apparent discrepancy between the behavior of the MMTV promoter *in vivo*, where it is silent in the absence of hormone, and *in vitro*, where it is enhanced by progesterone receptor but constitutively active. This paradox can be solved by considering the occlusive effect of chromatin structure on promoter activity. The precise positioning of the DNA double helix on the surface of the histone octamer precludes binding of NFI to its cognate sequence, while still allowing recognition of the HRE by the hormone receptors. We postulate that receptor binding to the nucleosomally organized MMTV promoter disrupts the chromatin structure enabling NFI binding and subsequent formation of a stable transcription complex. However, promoters lacking the NFI binding site are still responsive to hormone stimulation *in vivo* and *in vitro*. This effect is mediated by a tandem of degenerated octamer motifs located immediately upstream of the TATA box that jointly bind OTF-1 (Oct-1, NFIII) with similar affinity as the octamer consensus. In transfection experiments, mutation of these octamer motifs interferes with the hormonal response of the MMTV promoter. *In vitro*, these mutations do not influence basal transcription, but completely abolish the stimulatory effect of purified progesterone receptor. Progesterone receptor bound to the HRE facilitates binding of OTF-1 to the two octamer motifs. Thus, OTF-1 is a natural mediator of progesterone induction of the MMTV promoter and acts through cooperation with the hormone receptor for binding to DNA. We conclude that the hormonal regulation of the MMTV promoter can occur via two different mechanisms, one involving removal of nucleosome repression followed by NFI binding, and the other mediated by direct interaction between the hormone receptors and OTF-1. Whether these two mechanisms are mutually exclusive or can take place simultaneously on the same DNA molecule remains to be established.

E 008 THE ROLE OF LIGAND BINDING IN OESTROGEN RECEPTOR FUNCTION,

Malcolm G. Parker, Paul S. Danielian, Catherine E. Emmas, Stephen E. Fawell, Jacqueline A. Lees and Roger White, Molecular Endocrinology Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, U.K.

The oestrogen receptor is a member of a large family of transcription factors whose activity depends on the binding of a hormonal ligand. A region near the C-terminus of the receptor which is responsible for oestrogen binding overlaps sequences involved in receptor dimerisation (1) and is adjacent to sequences called TAF-2 involved in transcriptional activation (2). Agonists such as oestradiol appear to 'transform' or 'activate' receptor by favouring dimerisation thereby increasing DNA binding affinity and, secondly, induce the activity of TAF-2. The partial agonist/antagonist, tamoxifen, promotes DNA binding but fails to induce the activity of TAF-2, its agonist activity arising from the N-terminal transcriptional activation function TAF-1 (2,3). The pure antioestrogen, ICI 164,384, on the other hand, disrupts receptor dimerisation and inhibits DNA binding so that neither TAF-1 or TAF-2 would be active on target genes (4). The ability of these three ligands to produce such marked differences in the function of the receptor is likely to form the basis for the mechanism of action of other oestrogens and antioestrogens in vivo. Moreover, since the region involved in ligand binding, dimerisation and transcriptional activation appears to be conserved in the ligand binding domain of all nuclear hormone receptors, it is possible that other hormone agonists and antagonists function by similar mechanisms.

1. Fawell, S.E., Lees, J.A., White, R. & Parker, M.G. (1990). Cell 60:953-962
2. Lees, J.A., Fawell, S.E. & Parker, M.G. (1989). Nucleic Acids Res. 17:5477-5488
3. Berry, M., Metzger, D. & Chambon, P. (1990). EMBO J. 9:2811-2818
4. Fawell, S.E., White, R., Hoare, S., Sydenham, M., Page, M. & Parker, M.G. (1990) Proc. Natl. Acad. Sci. USA 87:6883-6887

E 009 GLUCOCORTICOID RECEPTOR BINDING TO hsp90 AND RECEPTOR FUNCTION IN VITRO, William B. Pratt, Department of Pharmacology, The University of Michigan Medical School, Ann Arbor, MI 48109

The steroid/thyroid receptor family can be divided into two subclasses distinguished by their ability to form or not to form a stable complex with hsp90. Receptors that do not bind hsp90, such as retinoic acid and thyroid hormone receptors, proceed directly after synthesis to very tight association with the nucleus. In contrast, receptors that bind hsp90 (e.g. glucocorticoid, mineralocorticoid, sex hormone and dioxin receptors) are either retained in the cytoplasm (e.g. glucocorticoid) or in the nucleus (e.g. estrogen, progesterone) in a "docking" complex composed of several proteins. The docking complex is recovered in the cytosolic fraction after cell rupture, and exposure of cells to hormone results in transfer of the receptor from the docking mode to tight association with nuclear components.

Binding of hormone to the glucocorticoid receptor in mouse L cell cytosol promotes the dissociation of hsp90 from the receptor and transformation of the receptor from a non-DNA-binding to a DNA-binding state. If hsp90 is dissociated from the steroid-free receptor, the unliganded receptor will bind to DNA and the transformation process can be reversed by incubating the DNA-bound receptor with rabbit reticulocyte lysate. In this case, the lysate directs the rapid, temperature-dependent removal of the mouse receptor from DNA, regenerating a soluble receptor that is now bound to rabbit hsp90 and has no DNA binding activity. The glucocorticoid receptor requires hsp90 to be in a high affinity steroid binding conformation and the rabbit reticulocyte lysate also converts the receptor from a non-steroid-binding form to a steroid-binding form. It is a reasonable speculation that the reticulocyte lysate performs a protein unfolding function with subsequent or simultaneous trapping of the unfolded state by hsp90. Interestingly, the steroid-bound receptor is not a substrate for reticulocyte-directed hsp90 reassociation. This leads one to speculate that dissociation of the steroid is accompanied by a conformational "relaxation" of the receptor such that unfolding and hsp90 trapping of the unfolded state can occur. Steroid receptors (including the glucocorticoid receptor) are known to associate with hsp70, which has a well defined unfoldase function. If reassociation of hsp90 indeed requires unfolding of the receptor, then hsp70 may be a reasonable candidate for the receptor unfoldase activity.

It is established that hsp90 interacts with the hormone binding domain of the glucocorticoid receptor. From examination of hsp90 association with mutant glucocorticoid receptors deleted for various regions of the hormone binding domain, we find that amino acids 632-659 (mouse) are necessary for formation of a stable hsp90 complex. Peptides spanning portions of this region inhibit reticulocyte lysate-directed reconstitution of a receptor-hsp90 complex. The 632-659 region contains a short proline-containing hydrophobic segment and an adjacent dipole-plus-cysteine motif that is conserved in all of the receptors that bind hsp90 but is not present in the receptors that do not bind hsp90. (Supported by NIH grants DK31573 and CA28010.)

E 010 REGULATION OF GENE TRANSCRIPTION AND mRNA DEGRADATION BY CLONED ESTROGEN RECEPTORS, David J. Shapiro, David A. Nielsen. Ann Nardulli, Tsu-Chung-Chang, and Gary Jenkins, Department of Biochemistry, University of Illinois, Urbana, IL 61801

The massive induction of the hepatic mRNA encoding the egg yolk precursor protein, vitellogenin, is achieved through an estrogen-mediated increase of more than 10,000 fold in the absolute rate of vitellogenin gene transcription, and by a >20 fold increase in the cytoplasmic stability of vitellogenin mRNA. Although circulating estradiol levels in male Xenopus laevis are sufficient to load a substantial fraction of the estrogen receptor with hormone, the vitellogenin genes are transcriptionally silent in the absence of exogenous estrogen. Several types of data suggest that binding of unliganded estrogen receptor to the estrogen response elements (EREs) in the 5'-flanking region of the vitellogenin genes plays an important role in transcriptional silencing of the genes. Unliganded estrogen receptor binds to the ERE with an affinity close to that exhibited by estradiol-ER complex. Unliganded ER suppresses basal transcription of vitellogenin promoters in transfected cells. Mutant forms of the cloned Xenopus estrogen receptor, which do not bind estradiol, are dominant negative mutants which compete for binding with hormone-receptor complex in co-transfection assays. In our initial experiments, cell-free extracts supplemented with estrogen receptor exhibit both estrogen dependent activation of transcription from an ERE-containing promoter and suppression of transcription by a truncated mutant ER expressed in E. coli and purified to homogeneity.

To analyze the molecular basis of the estrogen stabilization of vitellogenin mRNA we demonstrated that a mini-vitellogenin mRNA lacking more than 5,000 nucleotides of coding sequence exhibited estradiol-ER dependent stabilization in our homologous Xenopus liver cell line. Analysis of additional mutants led to the identification of two segments of the mini-vitellogenin mRNA essential for estrogen-mediated stabilization. Our studies also suggest the presence of a sequence in the 3'-untranslated region of many, but not all, mRNAs which helps to determine the location of the 3' end of the mRNA. These studies also identified a possible intermediate in the AUUU-dependent degradation of many oncogene and growth factor mRNAs.

Cell-Specific Gene Expression

E 011 TRANSCRIPTIONAL REGULATION OF THE RAT INSULIN 1 GENE, Johan Ericsson, Torbjörn Norberg, Helena Ohlsson Stefan Thor and Thomas Edlund, Department of Microbiology, University of Umeå, S-901 87 Umeå, Sweden

Expression of the rat insulin 1 gene is restricted to pancreatic β cells via transcriptional control mechanisms operating through well defined cis-acting DNA elements in the 5' flanking DNA of the gene. Of particular importance are two 9 bp elements (IEB 1 and IEB2) located at -108 and -233. In vitro analyses have shown that a β cell specific complex (insulin enhancer factor 1 - IEF 1) is capable of recognizing these sequences. Transcription activities of point mutations in IEB 1 closely parallel the in vitro binding properties of IEF 1, strongly implicating IEF 1 as a β cell specific transcriptional activator of the rat insulin 1 gene. IEB 1 and IEB 2 contain the CAxxTG consensus sequence implicated in binding of helix-loop-helix (HLH) proteins. IEF 1 interacts with antibodies to and heterodimerizes with an HLH protein, thus indicating that HLH proteins play a role in determining cell specific gene expression in lineages other than the muscle cell lineage.
A third region (-200 to -230) of the insulin enhancer is also needed for full expression of the insulin gene. Using the expression screening technique we have isolated a cDNA encoding a protein, Isl-1, capable of binding to this DNA region. Isl-1 contains a homeodomain with greatest similarity to those of the C. elegans proteins encoded by mec-3 and lin-11. In addition, Isl-1, like the mec-3 and lin-11 gene products, contains a novel Cys-His domain, the LIM domain, which is reminiscent of known metal binding domains. In pancreas Isl-1 is only expressed in the endocrine islet cells. The expression is not restricted to β cells but is also observed in glucagon and somatostatin producing cells. In adults Isl-1 is also expressed in a subset of hormone producing cells in the anterior pituitary and in cell lines derived from neuroendocrine cells of the lung. In addition, Isl-1, is expressed in the central nervous system (brain, spinal cord and ganglia) of the developing rat. This pattern of expression indicates a function for Isl-1 in the development and/or maintenance of neuroendocrine cells.

E 012 TRANSCRIPTIONAL STRATEGIES IN DEVELOPMENT OF SPECIFIC CELL PHENOTYPES IN THE MAMMALIAN NEUROENDOCRINE SYSTEM. M.G. Rosenfeld, R. Chen, E.B. Crenshaw, R. Guerrero, X. He, H.A. Ingraham, S. Li, C.R. Lin, M. Mathis and J. Voss. Howard Hughes Medical Institute, School of Medicine, University of California, San Diego 9500 Gilman Drive, La Jolla, California, USA 92093-0648. The anterior pituitary gland is derived from a common primordium originating in Rathke's pouch; following proliferation, the five classical specific cell types appear in a stereotypical order during ontogeny, defined on the basis of the trophic hormone that they elaborate. The structurally-related prolactin and growth hormone genes are expressed in discrete cell types - lactotrophs and somatotrophs, respectively - with their expression virtually limited to the pituitary gland. The lactotrophs and somatotrophs are the last cell types to appear in the developing rat anterior pituitary.

Cis-active elements in the prolactin and growth hormone genes have been identified and used to isolate and clone the pituitary-specific transcription factor, that we refer to as Pit-1. The coding sequence of Pit-1 predicted a 291 amino acid 32,900 kDa protein and was found to be a member of a novel family of transcription factors that contain a highly conserved domain, referred to as the POU-domain. Expression of Pit-1 in non-pituitary cell lines activates expression of both prolactin and growth hormone fusion genes, even at levels of Pit-1 expression <10-fold those present in pituitary (G/C) cells. We find that expression of the cloned Pit-1 structural gene in bacteria generates a 32,900 kDa protein that specifically binds to the tissue-specific elements in the distal and proximal regions of the prolactin gene as well as the growth hormone promoter, and activates *in vitro* transcription of both prolactin and growth hormone genes at equivalent concentrations.

The ontogeny of Pit-1 expression is consistent with a critical role for this factor in initial activation of prolactin and growth hormone gene expression, since the appearance of Pit-1 transcripts and protein (e15) precede the initial appearance of prolactin and growth hormone transcripts (e16). Pit-1 protein is selectively expressed in three cell types in the mature pituitary-somatotrophs, lactotrophs, and thyrotrophs. Because characterization of mutations of the Pit-1 gene would clarify the role of POU-domain genes in mammalian development, three types of pituitary-dependent dwarfism were examined in mice. The mutation of the dwarf locus, (dw, Snell; dw^J, Jackson) interrupts normal development of the anterior pituitary, resulting in the loss of expression of growth hormone, prolactin and thyroid stimulating hormone, and loss of somatotroph, lactotroph and thyrotroph cell types. We have proven that mutations in the gene encoding Pit-1 occur in both characterized alleles of the dwarf locus. These data, together, indicate that Pit-1 is necessary for the specification of phenotype of three cell types within the anterior pituitary, and directly link a transcription factor to commitment and progression events in mammalian organogenesis. We have subsequently cloned eight additional mammalian and two *Drosophila* members of the POU-domain gene family, and demonstrated that all of the known POU-domain genes are expressed during neural development, and exhibit precisely restricted temporal and spatial patterns of gene expression. In this regard, the POU-domain family of transcription factors resemble the developmental patterns of the hierarchy of regulatory genes that are sequentially activated during *Drosophila* development.

E 014 CLONING OF INSULIN GENE TRANSCRIPTION FACTORS, Michael J. Peyton, Sheau-Yann Shieh, Helena H. Ritchie, Sophia Y. Tsai, and Ming-Jer Tsai, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030

The insulin gene is a cell specific gene whose expression is limited to the beta cells of the Islets of Langerhans in the pancreas. In order to understand the mechanism of this cell specific gene regulation, we have undertaken an analysis of the Rat Insulin II promoter. Linker-scanning mutations have defined seven important Rat Insulin Promoter Elements (RIPE 1-7).

One of these elements, RIPE1 (-60 to -40) is bound by a ubiquitous factor, COUP-TF. We purified this protein and raised antibodies which were used to screen a lambda gt11 library. Sequence analysis of the clone we obtained indicates that it is a member of the steroid receptor superfamily. It contains a highly conserved DNA binding domain composed of two zinc fingers. In addition, two other regions (II and III) in the putative ligand binding domain are also conserved. Using the cDNA as a probe, we have isolated two related but distinct genes. The two zinc fingers of the DNA binding domain of each gene are encoded by a single exon. Therefore, COUP-TF is quite unique among the members of the steroid receptor superfamily.

Another element, RIPE3 [-125 to -86], is capable of conferring tissue specific expression on a heterologous minimal promoter. Further analysis demonstrated that RIPE3 is composed of two sub-elements, RIPE3a and RIPE3b. RIPE3a and RIPE3b appear to cooperate as neither sub-element alone stimulates promoter activity to the same extent as the entire element. Using RIPE3 as a probe to screen an expression library, we were able to isolate a clone, RIPE3a-BP, whose protein product binds specifically to the RIPE3a site. Sequence analysis of this clone determined it to be a member of the helix-loop-helix family of transcription factors. Northern blotting detected a predominant message of 7 kb which is seen in a variety of tissues. Currently, we are analyzing the role of this factor in insulin gene transcription.

Nuclear Receptors and Transcription

E 013 CELL SPECIFICITY OF PRO-OPIOMELANOCORTIN GENE REGULATION, Jacques Drouin, Mona Nemer, Yu Lin Sun, Tom Schmidt† and Marc Therrien, Laboratory of Molecular Genetics, Institut de recherches cliniques de Montréal, Montréal (Québec) CANADA, H2W 1R7 and †Department of Physiology, University of Iowa, Iowa City, IA 52242

Expression of the pro-opiomelanocortin (POMC) gene is restricted to two cell types of the pituitary gland: the corticotrophs of the anterior lobe and the melanotrophs of the intermediate lobe. A short 543 bp 5' flanking fragment of the rat POMC gene confers cell-specific expression in transgenic mice, in transfection experiments and in an *in vitro* transcription system. Using *in vitro* DNA binding with nuclear extracts, together with deletion and replacement mutagenesis, we have defined nine different regulatory elements which contribute to POMC promoter activity in transfection experiments using the POMC-expressing AtT-20 cell line as host. Whereas some of these elements appear to bind well-characterized transcription factors, at least five elements appear to represent novel DNA binding activities. However, most of these DNA binding proteins are not restricted in their tissue or cell distribution: it has thus far, not been possible to identify a single putative transcription factor which by itself could provide the molecular basis for cell-specific expression of POMC. Rather, it might be the combinatorial use of these factors which imparts specificity of transcription.

Glucocorticoids, which are synthesized in response to POMC-derived ACTH, exert a specific negative feedback on ACTH secretion and POMC transcription in the anterior pituitary. We have previously identified in the proximal POMC promoter an *in vitro* binding site for the glucocorticoid receptor (GR). This binding site is somewhat similar in DNA sequence to GREs; however, when it was mutated to a consensus GRE sequence, the POMC promoter was converted from glucocorticoid-repressed to glucocorticoid-inducible. Since oligonucleotides containing this binding site do not confer either inducibility or repression to a heterologous promoter, we tested whether GR interacts differently with this site and with consensus GREs. Using purified rat liver GR in a gel retardation assay, we have characterized a novel GR:DNA interaction with the POMC binding site. Whereas typical GREs bind GR dimers, the POMC binding site appeared to bind three GR subunits which interact with both sides of the DNA helix. The interaction between GR bound to this site and other factors elements in the POMC promoter is under investigation in order to define the molecular basis of glucocorticoid-dependent repression.

E 015 NEW WRINKLES IN RETINOID PHYSIOLOGY, R.M. Evans, Gene Expression, Laboratory, The Salk Institue, Howard Hughes Medical Institute, PO Box 85800, San Diego, CA

We have previously identified receptors for steroids, retinoids and thyroid hormones. The homologies of these receptors and the cDNAs which encode them define the existence of a superfamily of related regulatory proteins. These molecules all contain homologous structures that include domains required for DNA binding, ligand binding and transcriptional activation.

The retinoids comprise a group of compounds including retinoic acid, retinol (vitamin A), and a series of natural and synthetic derivatives that exert profound effects on vertebrate development and cellular differentiation in a wide variety of systems. At least three different genes encoding retinoic acid receptor subtypes (α, β, and γ) have now been identified. These retinoic acid receptors (RARs) by virtue of their homology, form a subfamily within the superfamily of steroid/thyroid hormone receptors. This multiplicity of receptor subtypes increases the difficulty of understanding the contribution of any particular gene product. To begin to address this issue we have created dominant negative retinoic acid receptors which function to block the activity of the endogenous counterparts. In transfection experiments, these dominant negative derivatives block retinoic acid induced differentiation of cultured teratocarcinoma cell lines. In addition to characterizing the functional role of the receptors we are attempting to identify genes whose activities controlled by retinoic acid and in particular the binding sites for the RARs in those genes. This has led to the characterization of a response element in the beta retinoic acid receptor gene that serves as a potent enhancer for the RARs. This response element is unusual in that it contains two tandem (as opposed to inverted) repeats. *In situ* hybridization patterns indicate that the RAR subtypes are expressed in distinct patterns throughout development and in the mature organism, indicating that they may mediate different functions. One important question is whether all the actions of retinoic acid are mediated through these proteins or whether additional retinoic acid substrates and regulatory networks exist.

Recently, we have described the cloning and characterization of a new class of receptor-like protein which responds to retinoic acid. This protein is substantially different in primary structure and ligand specificity from the products of the previously described RAR genes. We refer to this gene product as the retinoic X receptor (RXR) to distinguish it from the RAR family. We have also isolated a second related product (RXRβ)whose activity is also induced by retinoic acid. Northern blot analysis indicates that both RXRα and β are expressed during in the developing embryo and unlike the RARs, they continue to be expressed at high levels in visceral organs of the adult animal including liver, kidney, lung and muscle. The discovery of the RXR subfamily further confounds the analysis of the role of retinoids in development and differentiation and underscores the need of a genetic approach to begin to address their function. To pursue this issue we have recently isolated a RXR homolog from the fruitfly *Drosophila* and have demonstrated that this corresponds to the segmentation gene *ultraspiracle* (usp). One important outcome from this study is the demonstration that a distantly related organism utilizes a common regulatory pathway to orchestrate its development and provides the first genetic evidence for the essentiality of a retinoid receptor in animal development.

E 016 MECHANISMS OF STEROID RECEPTOR GENE TRANSCRIPTION, O'Malley, B.W., Bagchi, M., Tsai, S.Y. and Tsai, M.-J., Dept. of Cell Biology, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030.

Highly purified chicken progesterone receptor (cPR) is capable of stimulating RNA synthesis from a GRE/PRE linked promoter when added to a HeLa cell nuclear extract. Stimulation of cell-free transcription by the progesterone receptor requires the presence of progesterone response elements (PREs) in the template and can be specifically inhibited by the addition of competitor oligonucleotides containing PREs, indicating that the receptor itself is responsible for activation. Using this *in vitro* system we have analyzed the role of steroid hormone receptor in the initiation of target gene transcription. Our results indicate that progesterone receptor recruits and/or stabilizes general transcription factors at a target gene promoter (TATA element). This template committed complex of transactivators is now capable of directing efficient initiation of transcription by RNA polymerase II. In separate test experiments, we have found that estrogen receptor and glucocorticoid receptor act in an analogous fashion to regulate transcription *in vitro*.

We have used this *in vitro* transcription system to study also the role of hormone in the regulation of receptor function. It is well documented that a specific ligand, the steroid hormone, is important for steroid receptor function. We and others have previously demonstrated that binding of progesterone to its cognate PRE requires progesterone using crude T47D nuclear extract prepared in the absence of hormone. Such a salt extract from T47D cells is inactive in enhancing target gene expression. Upon addition of hormone, the progesterone receptor present in the extract was capable of binding to PREs and enhancing transcription of PRE containing target genes *in vitro*. Therefore, hormone is required to "transform" an inactive receptor to an active form which binds and transactivates the target gene. We have analyzed the role of hormone-induced dissociation of attendant heat shock proteins from receptor. We find that via gentle purification through a series of three columns, the vast majority of hsp90, p59, and hsp70 can be removed from receptor. At this point, the receptor is still dependent upon the addition of authentic progestins for efficient transactivation of a target gene. Our results reveal that activation of receptor is dependent upon at least two hormone-induced and distinct reactions: (1) dissociation of heat shock proteins and (2) allosteric activation and/or covalent modification of receptor.

Regulation of Transcription by Phosphorylation (joint)

E 017 REGULATION AND FUNCTION OF YEAST TRANSCRIPTIONAL ACTIVATOR ADR1, Clyde L. Denis, Robert C. Vallari, William J. Cook, Deborah C. Audino, Daniel L. Chase, Susan C. Fontaine, Stephen P. Mosley, and Lynne T. Bemis, Department of Biochemistry, University of New Hampshire, Durham, NH 03824

ADR1, a multiply phosphorylated protein consisting of 1323 amino acids, is required for the transcription of the glucose-repressible alcohol dehydrogenase (ADH2) from Saccharomyces cerevisiae. Several functional domains in the ADR1 protein have been identified. Two zinc-fingers in its N-terminal region are required by ADR1 to bind to the UAS1 region of ADH2. At least two other widely separated regions appear to be important for its activation of transcription. The activity of ADR1 is regulated by glucose through several different mechanisms. Glucose reduces ADR1 mRNA levels and inhibits its translation. The region involved in the translational regulation of ADR1 has been localized to the center of its coding region. In addition, ADR1 appears to be post-translationally regulated. Phosphorylation of ser-230 by cAMP-dependent protein kinase (cAPK) has been shown to inhibit ADR1 function (1). cAMP levels in yeast are known to increase in response to glucose-induced activation of the adenyl cyclase effectors CDC25 and RAS. Mutations in ADR1 that occur between residues 227 and 239 increase ADR1 activity by sixty-fold under glucose repressed conditions. Many but not all of these mutations reduce or eliminate cAPK phosphorylation of ADR1 or synthetic peptide substrates modeled on the ser-230 phosphorylation region. These results suggest that the ADR1 mutations enhance ADR1 activity by structural alterations of the 227-239 region that include but are not limited to effects on the phosphorylation state of ADR1. These alterations either allosterically activate ADR1 or affect its contact with another protein. In addition to cAPK control of ADR1, two other protein kinases, CCR1 (SNF1) and SCH9, have been found to be required for ADH2 expression. CCR1 and SCH9, however, act on factors that function independently of ADR1 to control ADH2. The mechanism by which glucose coordinates the control of these multiple protein kinases remains unclear.

(1) Cherry, J.R., Johnson, T.R., Dollard, C., Shuster, J.R., and Denis, C.L. (1989) Cyclic AMP-Dependent Protein Kinase Phosphorylates and Inactivates the Yeast Transcriptional Activator ADR1. Cell 56, 409-419.

E 018 *TRANS*-ACTIVATION BY VIRAL ONCOGENE PRODUCTS. Joseph R. Nevins, Duke University Medical Center, P.O. Box 3054, Durham, NC 27710

The analysis of transcriptional control in animal cells has been facilitated through the use of simple viral systems that encode transcriptional regulatory proteins. Perhaps the best studied of the viral *trans*-activators is the E1A gene of adenovirus. Recent studies of E1A-mediated *trans*-activation have demonstrated that multiple cellular transcription factors are likely targeted by E1A to effect an activation of transcription. At least two of these factors, E2F and E4F, are regulated via changes in DNA binding activity, dependent on a phosphorylation event. E2F DNA binding activity is further regulated through an interaction with a product of the early viral E4 gene allowing E2F to bind cooperatively and form a stable complex on the promoter. E2F can also be found in association with cellular proteins in extracts of a variety of uninfected cells. The interaction of these cellular proteins with E2F prevents E4 binding. E1A proteins are capable of dissociating E2F from these cellular complexes, allowing the E4 protein to interact. The ability of E1A to dissociate E2F from these interactions is dependent on sequences in conserved domain 1 and 2 of E1A, a region of E1A sequence that is required for oncogenic activity and that is homologous to other oncogenes such as SV40 T antigen and human papillomavirus E7. Interestingly, T ag and E7 also appear to *trans*-activate by targeting the E2F factor. Indeed, E7 is also capable of disrupting the E2F complexes, suggesting that a variety of viral *trans*-acting proteins may utilize common mechanisms for transcription factor control.

Finally, we also find that E2F activity is regulated during a cell proliferative response, consistent with the presence of E2F binding sites in a number of cellular genes regulated by cell proliferation. Moreover, the interaction of cellular proteins with E2F varies during the proliferative response indicating a regulatory role for these interactions. Since these complexes are disrupted by viral oncogene products, this activity may contribute to the alteration of cell growth control by these oncogenes.

Nuclear Receptors and Development

E 019 **DNA-PROTEIN AND PROTEIN-PROTEIN INTERACTIONS OF THYROID HORMONE RECEPTORS** Jae W. Lee, Gregory A. Brent*, John W. Harney*, P. Reed Larsen* and David D. Moore, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114, and *Howard Hughes Medical Institute, Brigham and Women's Hospital, Boston, MA 02115

We have carried out an extensive functional analysis of the thyroid hormone response element (T3RE) from the rat growth hormone promoter. This complex element consists of three copies of a hexameric consensus sequence, of which the first two are arranged as direct repeats and the third is inverted. Point mutations that decrease the similarity of any of the three repeats to the consensus decrease T3 induction, while increasing the match of the second or third increases response. Remarkably, a minimal T3RE can be constructed from two copies of the consensus sequence arranged either as direct repeats separated by 4 base pairs or as directly adjacent inverted repeats. Both the direct and inverted elements are bound by dimers of T3 receptors. The possibility that a number of potential heterodimers involving T3, retinoic acid and perhaps other receptors may also interact with the rGH sequence and other T3REs significantly complicates this situation. To understand the complex interactions of these receptors, we have developed a genetic test for dimerization, based on the bacteriophage λ repressor. By itself, the amino terminal DNA binding domain of the repressor cannot dimerize efficiently, and bacteria expressing such a truncated protein are not resistant to λ infection. However, repressor function can be rescued by addition of a heterologous dimerization domain. We have found that expression of a chimeric protein consisting of the amino terminal repressor domain plus the ligand binding domain from the β1-type T3 receptor confers full resistance to λ infection. We are using λ genetics to analyse the details of the process of T3 receptor dimerization and to identify other proteins that can interact with the receptor.

E 020 STEROID-TRIGGERED REGULATORY HIERARCHIES IN *DROSOPHILA* DEVELOPMENT: REGULATION AND FUNCTION OF THE *E75* ECDYSONE-INDUCIBLE GENE, William A. Segraves, Howard Hughes Medical Institute, Gene Expression Lab, The Salk Institute, La Jolla, CA 92037.

The insect molting hormone ecdysone controls genetic regulatory hierarchies manifested by the induction of polytene chromosome puffs in the larval salivary gland. It has been proposed that the products of the transiently active early ecdysone-inducible genes regulate late ecdysone responses including the repression of early ecdysone-inducible genes and the induction of late ecdysone-inducible genes[1].

The early ecdysone-inducible *E75* gene responsible for the 75B puff is expressed in a variety of tissues and developmental stages, encoding three different proteins which are members of the steroid receptor superfamily. Genetic analysis of *E75* demonstrates that these proteins have functionally distinct roles in the regulation of larval molting and metamorphosis.

In functional studies, the major larval E75 protein, E75 A, is capable of hormone-independent repression of its own promoter. This protein may thus be one of the early ecdysone-inducible products responsible for the secondary repression of early genes. Regions of the E75 A protein required for repression have been identified, and the relationship between autoregulatory response elements and ecdysone response elements in the E75 A promoter has been studied.

The similarity between the E75 proteins and members of the steroid receptor superfamily within all highly conserved regions of the hormone binding domain suggests that the E75 proteins are receptors. Nonetheless, the putative E75 hormone binding domain cannot be activated by known steroid, thyroid, retinoid or insect juvenile hormones, suggesting that the E75 ligand is a novel hormone which might act through E75 to modulate ecdysone response. The finding in Drosophila and other invertebrates of a growing number of receptor-like molecules for which no ligand has been identified suggests that there may be many undiscovered invertebrate hormones.

1. Ashburner, M. (1990) Cell 61, 1-3

Developmental Regulation of Gene Expression

E 021 DEVELOPMENTAL AND HORMONAL REGULATION OF MAMMARY GLAND GENE EXPRESSION

Jeffrey M. Rosen, Trevor Dale, Sean Gavigan and Thomas Bühler. Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030.

Regulatory sequences that are important for the hormonal, developmental and cell-specific control of milk protein gene expression are being defined by DNA-mediated gene transfer into both cells in culture and transgenic mice and by the analysis of trans-acting factors interacting with these sequences. Mammary gland development is unique in that it occurs primarily postnatally, and it is, therefore, accessible to direct study and analysis. We are studying the expression of genes that may regulate initial ductal proliferation in the 5 to 10 week old virgin mouse, as well as alveolar development during pregnancy. In addition, we are studying the regulation of milk protein genes, which act as markers of terminal differentiation in the mammary gland. Milk protein gene expression is regulated by a complex interaction of hormonal, cell-substratum and cell-cell interactions. The regulation of casein gene expression is a particularly useful model in which to study the action of prolactin. The prolactin and growth hormone receptors have been shown to be part of a larger hematopoietin receptor superfamily. Induction of casein gene expression requires the synergistic interaction of prolactin, insulin and glucocorticoids. The effect of glucocorticoids on casein gene expression appears to be mediated by an indirect mechanism, which involves effects at both the transcriptional and post-transcriptional levels. While proximal promoter sequences in the β-casein gene are sufficient to elicit developmental, cell substratum and hormonal regulation, other more distal elements appear to be required for the efficient expression of this gene in transgenic mice. In contrast, the entire rat whey acidic protein(WAP) gene is expressed at levels comparable to the endogenous gene in transgenic mice. Sequences within the 3' untranslated region of the WAP gene appear to be important to maintain a high level of expression. Experiments are underway to define whether these sequences are acting as classical enhancers or are important for post-transcriptional regulation of mRNA stability.

Supported by USDA grant 88-37266-3951 and NIH grant CA-16303.

E 022 EXTINCTION OF GENE ACTIVITY BY INTERFERENCE WITH THE cAMP SIGNAL TRANSDUCTION PATHWAY, G. Schütz, M. Boshart, F. Weih, Institute of Cell and Tumor Biology, German Cancer Research Center, Im Neuenheimer Feld 280, D-6900 Heidelberg, FRG. The tyrosine aminotransferase (TAT) gene is specifically expressed in liver cells and is inducible by glucocorticoids as well as via the cAMP pathway. In fibroblasts and other non-liver cells the TAT gene is subject to negative regulation by the trans-dominant tissue specific extinguisher locus (*Tse-1*). We have identified a liver specific enhancer 3.6 kb upstream of the transcription start site of the TAT gene. This enhancer is responsive to induction by cAMP and is the target for negative regulation by *Tse-1*. In a hepatoma microcell hybrid line which contains only a small segment of human fibroblast chromosome 17 carrying *Tse-1*, induction by cAMP is able to overcome extinction, thus revealing a functional antagonism between *Tse-1* and the signal transduction pathway. A detailed mutational analysis of the enhancer reveals a bipartite structure: Two distinct sequence motifs are absolutely essential for function of this enhancer: a cAMP response element (CRE), which is the target for repression by *Tse-1*, and a hepatocyte-specific element. The specificity of the enhancer is generated by the combination of these two essential elements, which are fully interdependent. Using genomic footprinting we have shown that the CRE sequence is occupied by protein in uninduced cells and that this footprint is transiently increased upon cAMP induction. This observation suggested that both, basal and cAMP inducible activity is dependent on the same protein recognizing the CRE site. Interference with kinase A dependent phosphorylation by overexpression of a regulatory subunit of the protein kinase A did indeed abrogate basal TAT gene transcription and CRE binding activity, thus generating a phenotype identical to the extinguished state. Therefore, we analyzed the role of components of the protein kinase A signaling pathway in extinction. We will present evidence that *Tse-1* is indeed a component of this pathway directly interfering with the cAMP signal transduction pathway.

E 023 POSITION-SPECIFIC ACTIVATION OF HOMEOBOX GENES IN LIMB DEVELOPMENT, Cliff Tabin, Hans-Georg Simon, and Bruce Morgan, Department of Genetics, Harvard Medical School, Boston, MA 02115.

Homeobox genes are believed to pay important roles in vertebrate development. This belief is predicated upon their spatial and temporal patterns of expression during development as well as by analogy to homologous genes in invertebrates whose function is better understood. We have focused on a few members of this gene family that are expressed in localized patterns in the limb to begin functional dissection of their roles in development.

We have studied several homeobox genes which are expressed differentially in the developing forelimb and the developing hindlimb. Techniques have been developed that allow these genes to be introduced specifically into the cells of the limb bud, creating a "transgenic limb." This will allow the functions of these genes to be directly investigated.

Another homeobox gene, Hox7.1, is specifically expressed by the subapical limb mesenchymal cells. The expression of Hox7.1 is induced by the overlying specialized ectoderm, the Apical Ectodermal Ridge (AER). *In collaboration with David Sasson of Boston University Medical School and Ken* Muneoka of Tulane University, we have set up an in vitro limb system. Limb mesenchymal cells can be plated in a dish. When an AER is placed on top, the cells contacting the AER turn on Hox7.1, multiply under the ectoderm and bud out of the dish. Those in vitro limbs undergo proper morphogenic steps ultimately making in vitro limbs including digits. This provides an in vitro assay to screen for factors which activate Hox7.1 and stimulate outgrowth in the absence of an AER.

Regulation of Transcription by Second Messengers (joint)

E 024 MULTIPLE REGULATION OF THE NF-κB TRANSCRIPTION FACTOR BY PROTEIN SUBUNITS, Patrick A. Baeuerle, Egenhart Link, Lienhard Schmitz, Ralf Schreck, Manuela B. Urban and Ulrike Zabel, Laboratory of Molecular Biology and Biochemistry, Gene Center, Ludwig-Maximilian-University, D-8033 Martinsried, West Germany.

In many cell types, the NF-κB transcription activator is involved in the rapid induction of gene expression upon stimulation of cells with agents such as viruses, parasites and cytokines. Most of its known target genes encode cytokines, immunoreceptors and acute phase proteins. The NF-κB system comprises three protein subunits: p50, the DNA binding subunit, p65, a non DNA-binding accessory protein of p50, and IκB, the inhibitory subunit. The inactive cytoplasmic form of NF-κB is composed of p50, p65 and IκB. Upon stimulation of cells, IκB is released and allows association of two p50-p65 dimers to a heterotetrameric complex. The heterotetramer is the active form of NF-κB found in nuclei. While in the inactive cytoplasmic form p65 serves to bind IκB, it has a modulatory role for the DNA binding of p50 in the nucleus. If p65 is bound to p50, κB motifs of low symmetry are preferred binding sites, the DNA bending induced by p50 is increased and the position of the bending center is shifted. In addition p65 might help to dimerize p50, to provide a transcription activation domain and to serve as receptor for IκB. The latter would allow inactivation of NF-κB and a subsequent transport of the factor back to the cytoplasm. Novel functional aspects of the NF-κB system and a more detailed characterization of the protein subunits will be presented.

E 025 POST-TRANSCRIPTIONAL AND TRANSCRIPTIONAL CONTROL OF AP-1 (JUN/FOS) ACTIVITY, Michael Karin, Tod Smeal, Bernard Binetruy, Hsin-Fang Yang-Yen, Jean-Claude Chambard, Tiliang Deng and Adriana Radler-Pohl, Department of Pharmacology, School of Medicine, M-036, University of California
San Diego, La Jolla, CA 92093.

AP-1 is a transcriptional activator composed of homo-and heterodimeric Jun and Jun/Fos complexes. It is involved in the activation of various target genes, such as: collagenase, stromelysin, IL2 and TGFβ1, by tumor promoters, growth factors and cytokines. In addition AP-1 activity is elevated in response to expression of transforming oncogenes including *H-ras*, *v-src*, and *v-raf* and is required for cell proliferation. AP-1 activity is subject to complex regulation both transcriptionally and post-transcriptionally. Transcriptional control determines which of the *jun* and *fos* genes is expressed at any given time in any given cell type. Therefore, transcriptional control determines the amount and composition of the AP-1 complex. Transcription of the *jun* and *fos* genes is subject to both positive and negative autoregulation and is highly inducible in response to various stimuli including those associated with cell proliferation.

AP-1 activity is also regulated at the post-transcriptional level. Both cJun and cFos are phosphoproteins that are subject to regulated phosphorylation. In the case of cJun, phosphorylation of sites near the DNA-binding domain inhibits its DNA-binding activity while dephosphorylation reverses this inhibition. It is also possible that phosphorylation of cJun on other sites increases its ability to activate transcription without affecting its DNA binding activity.

Another mechanism that modulates AP-1 activity is transcriptional interference by members of the nuclear receptor family. For example, the glucocorticoid receptor was recently shown to interact with cJun and cFos to form a protein complex that is no longer capable of binding to either the AP-1 recognition site or hormone response elements.

Late Abstracts

ANALYSIS OF RETOINIC ACID RECEPTOR PROMOTERS IN TRANSGENIC ANIMALS,
P. Chambon, CNRS/LGME and INSERM/U.184, Faculté de Médecine, 67085 STRASBOURG Cédex, France.

Retinoic acid (RA) has a wide range of effects on cellular growth and differentiation and is thought to be a morphogen which specifies positional cues during embryogenesis. The genes for 3 classes of retinoic acid receptors (RARs) have been characterized in mouse and man, RAR-α, RAR-β and RAR-γ, as well as isoforms which arise through alternative splicing and differ in their 5'-untranslated sequences and A regions. The finding that the 3 RAR genes are expressed in a unique and developmentally specific fashion during embryogenesis, supports the notion that RARs play an important role in development.

To learn more about the regulatory elements which direct the expression of RARs, we have begun to characterize the promoter regions of RAR-α, RAR-β and RAR-γ genes. For all three genes we have isolated mouse genomic 5'-flanking sequences which direct expression of the CAT reporter gene in transient transfection experiments. To determine if these promoter sequences contain the information necessary to specify RAR gene specific patterns of expression during development, transgenic animals have been generated which express the β-galactosidase reporter gene under the control of RAR gene flanking sequences. By comparing the patterns of β-galactosidase expression observed in the transgenic animals, to the results of in situ hybridization experiments, it has been possible to define genomic sequences important for directing the unique and specific patterns of RAR transcription during murine embryogenesis.

TRANSCRIPTIONAL CONTROL OF cAMP-INDUCIBLE GENES,
Marc R. Montminy, Gustavo A. Gonzalez, and R. Scott Struthers, The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA 92037

How are extracellular signals transduced into physiological responses? Many of these events occur through altered patterns of gene expression mediated by second messenger pathways and their associated protein kinases. The cloning of the transcription factor CREB (cAMP Regulatory Element Binding protein) and subsequent functional characterization of the clone has enabled our laboratory to elucidate mechanisms by which protein kinases govern gene transcription. The CREB protein has a cluster of phosphorylation sites, including a site for cAMP dependent protein kinase, situated between two putative glutamine rich activator domains. Furthermore, protein kinase C, CAM kinase II, glycogen synthesase kinase III and casein kinase II also phosphorylate CREB in this region, suggesting that multiple signal transduction pathways may act within the centrally located "kinase inducible domain" (KID) to regulate CREB activity. Although phosphorylation of SER 133 by protein kinase A is required for transactivation, it does not appear to be sufficient. Deletion mapping has identified regions distinct from the protein kinase A phosphorylation site that are indispensable for the transactivation activity of CREB. These motifs include the two glutamine rich regions flanking the KID. Our results suggest that CREB can activate a number of different promoters through a glutamine type activator. It is likely, therefore, that phosphorylation regulates the transcriptional activity of CREB by causing structural changes in the KID region which render the Q regions transcriptionally competent to interact with proteins of the polymerase II complex.

DEVELOPMENTAL REGULATION OF CARDIAC ANF GENE EXPRESSION, Mona Nemer, Jacques Drouin, Stefania Argentin, Ali Ardati and Stéphane Tremblay, Laboratory of Molecular Genetics, Institut de recherches cliniques de Montréal, Montréal (Québec) CANADA, H2W 1R7

Tissue-specific gene expression plays a key role in growth and differentiation of cardiac muscle cells, as reflected by the expression of specific genes during the different stages of heart development. In addition, within the heart itself, atria and ventricles express specific subset of these genes suggesting that despite their common embryological origin, atrial and ventricular myocytes follow distinct paths of differentiation.

In the past few years, we have used the atrial natriuretic factor (ANF) gene as a model to study signal transduction in the endocrine heart. ANF, a peptide hormone with potent natriuretic, diuretic and vasodilatory - hence hypotensive - properties, is the major secretory product of the heart. The ANF gene is expressed primarily in atrial and ventricular myocytes where it is under differential hormonal and developmental control. In particular, we have found that ventricular expression of the ANF gene correlates positively with growth stimulation and inversely with cardiac differentiation: ANF mRNA and peptides decrease precipitously during the first week of postnatal development in parallel with the decrease in mitotic activity of ventricular cells. However, in the adult ventricle, ANF gene expression is dramatically induced in all conditions of cardiac growth stimulation or cardiac hypertrophy. Thus, the ANF gene appears to be a good marker to study tissue-specific expression during various stage of heart development. In order to define the regulatory elements of the ANF gene, we have fused ANF promoter fragments up to -3.7 kb to the human growth hormone gene and transfected these constructs into primary cardiocyte cultures derived from newborn atria and ventricles. The ANF promoter was functional only in cardiac cells and sequences up to -1.6 kb were required for full activity. Using a combination of mutagenesis and *in vitro* binding of nuclear extracts to ANF upstream fragments, we have identified several promoter elements which contribute to cardiac-specific expression and to modulation during cardiac growth and differentiation. One of these elements, which is present in several copies in functionally relevant promoter regions, binds cardiac-specific nuclear protein(s). The distribution of this protein correlates perfectly with the relative expression of the ANF gene in various cardiac tissues and is higher in newborn versus adult ventricles. These findings suggest that this cardiac protein is itself target for developmental regulation and thus, it may be involved both in tissue-specific and developmental control of ANF and possibly other cardiac genes.

SIGNAL TRANSDUCTION BY RECEPTORS WITH TYROSINE KINASE ACTIVITY, Joseph Schlessinger, Department of Pharmacology, New York University Medical Center, New York, NY 10016 The membrane receptor of epidermal growth factor (EGF-receptor) is composed of a large extracellular ligand binding domain, a singletransmembrane region and a cytoplasmic domain, a single transmembrane region and a cytoplasmic domain containing protein tyrosine kinase activity. We have formulated an allosteric oligomerization model for activation of the catalytic properties of neighboring cytoplasmic domains. In Vitro site directed mutagenesis was used to generate various EGF-receptor mutants. Using this approach, it was shown that the kinase activity of EGF receptor is essential for signal transduction and for normal receptor trafficking, while autophosphorylation is not crucial for receptor signaling. It was also shown that EGF is able to stimulate tyrosine phosphorylation of phospholipase C-γ and EGF also induced activation of c-raf, suggesting that this enzyme may be involved in cascade of kinases initiated by tyrosine phosphorylation. The precise composition of the transmembrane domain is not essential for receptor activity, further supporting the oligomerization model for receptor activation. Binding experiments of EGF to various chicken/human EGF receptor chimera has allowed the identification of domain III of the extra-cellular domain of EGF-receptor as a major ligand binding domain. Some interactions are also provided by domain I which, together with domain III appear to constitute the binding region for EGF.

SIGNAL TRANSDUCTION BY PDGF RECEPTORS INVOLVES DIMERIZATION AND ASSOCIATION WITH SIGNALING MOLECULES, L.T. Williams, J.A. Escobedo, V.A. Fried, H. Ueno, and W.M. Kavanaugh Howard Hughes Medical Institute and Cardiovascular Research Institute, University of California San Francisco, CA 94143 and Department of Cell Biology and Anatomy, New York University, Valhalla, New York 10595

To study the role of receptor dimerization in signal transduction we have used mutants of the PDGF β receptor (PDGFR) that bind PDGF but lack intrinsic kinase activity. When co-expressed with wild type receptors, these mutants formed heterodimers with wild type PDGFR in a PDGF-dependent manner. A heterodimer consisting of wild type PDGFR and a PDGFR with a truncated tyrosine kinase domain was defective in autophosphorylation, whereas the ligand-induced homodimer of wild type receptor was autophosphorylated. When co-expressed with wild type receptors in Xenopus oocytes, the mutant PDGFR abolished signal transduction by wild type PDGFR but did not affect signal transduction by wild type FGF receptors (FGFR). A similar mutant of the FGFR blocked signal transduction by wild type FGFR but not by PDGFR. These findings show that receptor dimerization is required for PDGF receptor signal transduction, suggest that autophosphorylation is intermolecular reaction between the components of a receptor dimer and establish a unique approach to inhibit receptor function in vivo.

Autophosphorylated PDGFR associates with several cellular molecules and phosphorylates some of them on tyrosines. We have recently focused on an 85 kDa protein (P85) that associates with ligand-activated PDGFR and is one of the major tyrosine-phosphorylated proteins in PDGF-stimulated cells. We purified P85 using tyrosine-phosphorylated PDGFR as an affinity reagent and cloned the cDNA that encodes P85. P85 contains two SH2 domains but no identifiable kinase motifs. Thus P85, like GTPase activating protein, may associate with PDGF receptor through its SH2 domains. We showed that P85 binds to a 16 amino acid segment of PDGFR the that contains phosphotyrosine at position 719. This segment of PDGFR also binds phosphatidylinositol 3 kinase (PI3 kinase), an enzyme that has been implicated in the regulation of growth of both normal and transformed cells. P85 expressed in cos cells competes with PI3 kinase and 110 kDa protein for binding to the receptor. Thus P85 is either a subunit of PI3 kinase or an antagonist that competes with PI3 kinase for binding to the receptor. Thus signaling seems to involve ligand-induced receptor dimer formation, intermolecular autophosphorylation of receptors, and the binding of SH2 domain-containing proteins to autophosphorylation sites on the receptor. The SH2 domains appear to recognize phosphotyrosine in a specific sequence context.

Gene Regulation I

E 100 ESTROGEN AND THYROID HORMONE REGULATE OXYTOCIN GENE PROMOTOR ACTIVITY

Roger A.H. Adan and J. Peter H. Burbach, Department of Pharmacology, Rudolf Magnus Institute, University of Utrecht, Vondellaan 6, 3521 GD Utrecht, The Netherlands

Oxytocin (OT) is a neuropeptide involved in the control of reproductive functions and with a number of central actions. It is synthesized in various groups of hypothalamic neurons in which the expression of the OT gene is critically regulated during development, pregnancy and lactation. The present studies are focussed on endocrine factors involved in the regulation of OT gene transcription and their mode of action. Plasmids having a 5'-flanking region of the rat OT gene (-363/+16) or the human OT gene (-382/+41) cloned in front of the firefly luciferase gene were co-transfected with expression vectors for the human estrogen receptor or the rat α thyroid hormone (T_3)receptor in P19 EC cells, or in estrogen receptor expressing MCF-7 cells. Estrogen strongly stimulated rat and human OT promotor activity. In the rat gene two regions each associated with approximately 15-fold stimulation of promotor activity were located between nucleotides -172 and -149 and between -148 and +16. The former region contains the imperfect palindrome GGTGACCTTGACC which differs in one nucleotide from the estrogen response element (ERE) consensus. The latter has two half ERE motives TGACC. Constructs having the 5'-flanking region of the bovine OT gene did not respond to estrogen (experiment together with N. Walther and R. Ivell, Hamburg, FRG), indicating that the corresponding ERE-like motive CATAACCTTGACC nor the half-palindromes could function as an ERE. T_3 also stimulated rat and human OT promoter activities, but less strongly than estrogen. Responsiveness accounting for about 5-fold T_3 stimulation was located between nucleotides -172 to -148 containing the imperfect ERE. There was no cooperativity between the activated T_3 and estrogen receptors. However, there was interaction between the effects of the two receptors. The presence of unoccupied estrogen receptor suppressed T_3 stimulation by about 50%. The unoccupied thyroid hormone receptor reduced the estrogen stimulation slightly. The data show that T_3 and estrogen are regulators of OT gene expression and interact at the ERE of the OT gene.

E 101 THE 5′ FLANKING REGION OF HUMAN CATHEPSIN D GENE MEDIATES ITS ESTROGEN TRANSCRIPTIONAL REGULATION.

Patrick Augereau, Vincent Cavaillès and Henri Rochefort, INSERM U. 148, (Hormones et Cancer), 60 rue de Navacelles, 34090 Montpellier, France.

Cathepsin D is a mitogenic lysosomal aspartyl protease induced by estrogens in mammary cancer cells (1). We previously showed that it is regulated at the mRNA level, and run-on experiments suggested that this regulation operates mainly at the initiation of transcription (2,3). We have cloned the cathepsin D gene and its 5′ flanking sequences from MCF7 cells. Using transient transfection experiments in various cell lines, we demonstrate that the 5′ region is able to confer estrogen responsiveness to an heterologous promoter. Sequence analysis has revealed a potential ERE in the proximal promoter region. We are now studying interactions of the ER as well as other transcriptional factors with the cathepsin D gene promoter sequences.

1. H. Rochefort et al (1987). J. Cell. Biochem. **35**,17-29.
2. P. Augereau et al (1988). Mol. Endocr. **2**, 186-192.
3. V. Cavaillès et al (1988). Nucleic Acids Research **16**, 1903-1919.

E 102 ACTIVIN-A MODULATES POMC mRNA LEVELS AND GENE EXPRESSION IN A CLONAL AtT20 CELL LINE, *Louise M. Bilezikjian, Amy L. Blount and Wylie Vale,* Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA 92037

Activins, members of the TGFß family of growth and differentiation factors, were initially isolated based on their ability to stimulate FSH secretion from rat anterior pituitary cultures. The proteins are dimers consisting of any combination of $ß_A$ and $ß_B$ chains and Activin-A is a homodimer of two $β_A$ subunits. The present studies were performed using corticotrope-derived AtT20 tumor cells. Activin-A (0.7 nM) inhibited basal ACTH secretion in a time- and dose-dependent manner with a maximal effect at 72h at 0.07 nM. Activin-A also decreased POMC mRNA accumulation in a dose- and time-dependent manner; a 50% and a 30% reduction of mRNA levels were evident within 18h and 72h of treatment with 0.7 nM Activin-A, respectively. In order to determine if this effect was transcriptionally mediated, fusion genes were constructed using 5'-flanking sequences of POMC inserted upstream of the coding region of the CAT gene. In transient expression assays using AtT20 cells, a 60h treatment with Activin-A inhibited CAT activity by approximately 70%. Deletion analysis indicated that this inhibition was mediated by sequences present within a fragment of the POMC gene spanning from -84 to +250 relative to the initiation site. These results suggest that Activin-A may play an important regulatory role in corticotropic cell function.

E 103 β-ADRENERGIC INDUCTION OF THE MITOCHONDRIAL UNCOUPLING PROTEIN GENE, Bert B. Boyer and Leslie P. Kozak, The Jackson Laboratory, Bar Harbor, ME 04609

Non-shivering thermogenesis is initiated by the cold-induced β-adrenergic stimulation of brown fat, and leads to induction of the mitochondrial uncoupling protein gene (*Ucp*). The induction in gene expression takes place within minutes of exposing animals to the cold. Accordingly, this provides us with a unique model system for analyzing mechanisms by which adrenergic hormones activate gene expression. To identify DNA regulatory sequences involved in the induction of *Ucp*, we surveyed 50 kb of chromatin surrounding the *Ucp* gene for its hypersensitivity to DNase I. We have mapped eight DNase I hypersensitive sites (DHS) within 18Kb of the first exon of *Ucp* chromatin DNA from brown fat. These DHS were compared to other tissues and cells in culture. To evaluate these DHS as regulatory regions, transgenic mice were produced which showed tissue-specific and cold-inducible expression of a *Ucp* minigene containing four proximal DHS. Four additional transgenic lines were generated with a second transgene containing a 1.8 kb deletion in the 5′ flanking DNA and expression is undetectable in all tissues analyzed. Based on these observations, we propose that a *cis*-regulatory element between -3 and -1.2 kb is required for *Ucp* expression *in vivo*. We are evaluating whether the fat cell-specific DHS located within this region is required for expression.

E 104 GLUCOCORTICOID RECEPTOR-DEPENDENT REMODELLING OF MMTV CHROMATIN IS MODULATED BY HISTONE ACETYLATION/DEACETYLATION, Emery H. Bresnick, Sam John, Charles Rories and Gordon L. Hager, Laboratory of Experimental Carcinogenesis, NCI, Bethesda, MD 20892

Activation of MMTV transcription by the hormone-bound glucocorticoid receptor (GR) results in disruption of a single nucleosome (nuc-B) in an array of phased nucleosomes on the MMTV promoter. We have hypothesized that GR binding to glucocorticoid regulatory elements that are positioned on nuc-B is a primary signal to disrupt nuc-B during transcription initiation. Limited treatment of cells with sodium butyrate, a histone deacetylase inhibitor, prevents GR-dependent promoter activation and nuc-B disruption. To determine if the inhibitory effect of butyrate is accompanied by changes in the structure or stability of MMTV chromatin, we employed cell lines that stably express MMTV LTR-reporter chimeras in BPV-based episomes. Although butyrate prevents hormone-induced endonuclease cutting in the nuc-B region, MMTV chromatin containing hyperacetylated histones does not differ from normal chromatin in sensitivity to nucleases. In addition, hyperacetylation does not affect nucleosome phasing. A synthetic DNA fragment spanning the nuc-B region was reconstituted into monosomes with normal or hyperacetylated core particles. Analysis of monosome structure and stability by nondenaturing PAGE, restriction enzyme access, Exo III and DNase I footprinting reveals no effect of hyperacetylation. These results provide evidence that hyperacetylation does not induce a major change in MMTV chromatin structure, such as unfolding of nucleosomes. We are currently testing the hypothesis that hyperacetylation of conserved lysines on the amino terminal tails of core histones may modulate GR binding to nuc-B. PCR-mediated amplification of nuclease digested chromatin is being used to examine detailed structural features of inactive and active MMTV chromatin.

E 105 EXPRESSION OF BOVINE B-CASEIN/CAT GENE FUSIONS IN C1D9 MOUSE MAMMARY EPITHELIAL CELLS: REGULATION BY HORMONES AND EXTRACELLULAR MATRIX. G.F. Casperson[1], C. Schmidhauser[2], C.A. Myers[1], and M. Bissell[2]
[1]Monsanto Company, Department of Biological Sciences, Chesterfield MO, and [2]Lawrence Berkeley Laboratory, Division of Cell and Molecular Biology, Berkeley CA

In order to analyze the cis-acting elements of the bovine B-casein (bBcas) promoter, we have developed a system in which stably transfected COMMA-1D mouse mammary epithelial cells express bBcas/CAT gene fusions. The COMMA-1D cells used in these studies were an epithelial-enriched subpopulation of this heterogenous cell line designated C1D 9. In C1D 9 cells cultured on EHS matrix, 37% of the cells stained for B-casein by immunocytochemistry (as compared to 8% for the parental C1D cells) and the level of endogenous mouse B-casein mRNA was only slightly lower than that in primary mouse mammary epithelial cells cultured under the same conditions. The bovine B-casein gene was cloned from a bovine genomic DNA library and the 5′ flanking DNA was used to construct a series of CAT fusions. Fragments of the bBcas 5′ flanking (promoter) DNA with varying 5′ termini were fused to the bacterial CAT gene at nucleotide +42 of the first, noncoding bBcas exon. The smallest bBcas promoter fragment (5′ terminus at -700; designated -700,+42) drove significant CAT activity in C1D 9 cells. CAT expression was modulated by hydrocortisone, insulin and extracellular matrix and was extremely dependent upon prolactin. Successively longer fragments of bBcas 5′ flanking DNA (5′ termini at -1700, -2800, and -3800), each fused to CAT at +42, gave rise to progressively higher levels of CAT expression but with qualitatively similar modulation by hormones and ECM. Almost no CAT activity was expressed from these same constructs when transfected into non-mammary epithelial cells. Availability of a cell culture-based system in which transfected major milk protein genes exhibit their full range of transcriptional regulation will be a valuable tool in determining the mechanisms of hormonal and developmental regulation of these genes.

E 106 FACTOR-ASSISTED DNA BINDING AS A POSSIBLE GENERAL MECHANISM FOR STEROID RECEPTORS, Alice H. Cavanaugh and S. Stoney Simons, Jr., LMCB/ NIDDK/NIH, Bethesda, Maryland 20892

We have previously demonstrated that activated glucocorticoid receptor-steroid complexes from rat hepatoma tissue culture (HTC) cells exist as at least two populations, one of which requires an additional factor for binding to DNA. This factor is of low molecular weight (700-3,000 daltons) and is found predominantly in extracts of crude HTC cell nuclei. This behavior is not unique to HTC cells and has now been observed for HeLa cells. Thus activated HeLa cell glucocorticoid receptor-steroid complexes are heterogeneous with efficient DNA binding of one subpopulation requiring a small M_r factor from HeLa nuclei. A similar factor was also found in extracts of rat kidney and liver nuclei. The factor isolated from these sources was identical by all criteria examined to that found in the HTC cell nuclear extracts, indicating neither tissue nor species specificity. Finally, activated human progesterone receptor complexes were also found to be heterogeneous with a small M_r nuclear factor being required for the DNA binding of one subpopulation of complexes. These results with different tissues and receptors suggest that the involvement of a small M_r factor in the DNA binding of activated complexes may be a general mechanism for all steroid receptors.

E 107 TRANSCRIPTIONAL REGULATION OF BOVINE ADRENODOXIN GENE EXPRESSION. Jeou-Yuan Chen, Evan R. Simpson, and Michael R. Waterman, Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX 75235.

The bovine adrenodoxin gene gives rise to two species of mRNA which differ only at their 5'-ends. The synthesis of these two types of mRNA in bovine adrenocortical cells is regulated transcriptionally by ACTH via the action of cAMP. Examination of the 5'-end of the adrenodoxin gene revealed that each mRNA contains sequences from a different exon as its leader sequence. To understand the mechanisms involved in the regulation of adrenodoxin gene expression, we have studied the promoter and regulatory sequences of this gene by linking different portions of adrenodoxin gene to different reporter genes followed by transient expression assays of the chimeric genes. Our results clearly demonstrated that the bovine adrenodoxin gene contains two functional promoters; one, ADXP1 is located in the 5'-flanking region and a second, stronger promoter, ADXP2 maps within intron 1. Furthermore, the transcription initiated from each promoter is stimulated by cAMP. The regulatory sequence associated with each promoter which is responsible for cAMP-dependent regulation has been located by deletional analyses. Neither one of these adrenodoxin cAMP-responsive elements shares sequence homology to the consensus cAMP responsive element (5'-TGACGTCA-3') or to each other. To understand the biological role of each promoter and each cAMP-responsive element, we are currently investigating the differential usage of these two promoters and their interactions to each cAMP-responsive element.

E 108 ACTIVATION OF THE COLLAGEN IV PROMOTER-ENHANCER IN F9 TERATOCARCINOMA CELLS BY 3-DEAZAADENOSINE ANALOGS -- INDIRECT INHIBITORS OF METHYLATION, Peter K. Chiang, Peter D. Burbelo*, Sheryl A. Brugh, Richard K. Gordon and Y. Yamada*, Walter Reed Army Institute of Research, Washington, DC 20307-5100, and *NIDR, NIH, Bethesda, MD 20892

3-Deazaadenosine analogs can function as inhibitors and also as alternative substrates of S-adenosylhomocysteine (AdoHcy) hydrolase. In cells treated with the analogs, AdoHcy invariably accumulates, and sometimes novel congeners of AdoHcy are generated. F9 teratocarcinoma cells stably transfected with collagen IV promoter-enhancer-CAT constructs were treated with 3-deazaadenosine, 3-deaza-(±)aristeromycin and 3-deazaneplanocin. The 3-deaza analogs exerted a differential effect on the CAT gene activity. In cells transfected with a promoter-enhancer-CAT construct (p48), 10 μM 3-deaza-(±)aristeromycin or deazaneplanocin activated the transcriptional activity without affecting differentiation, while higher concentrations of 3-deazaadenosine were required for activation. However, the same 3-deaza analogs were without effect on the CAT activity in F9 cells transfected with the β-actin promoter-CAT construct. The 3-deaza analogs most likely affect DNA methylation because their results are consistent with the previous observation that the integrated collagen IV promoter-enhancer construct was activated with 5-azacytidine.

E 109 REQUIREMENT OF A SECOND TISSUE-SPECIFIC 5' REGULATORY ELEMENT FOR THE HORMONE DEPENDENT TRANSCRIPTIONAL RESPONSE OF THE α-AMYLASE 2 GENE IN EXOCRINE PANCREAS CELLS, Moira Cockell, Otto Hagenbüchle and Peter Wellauer, Department of cellular differentiation, I.S.R.E.C. Lausanne, Switzerland, CH1066

The expression of different exocrine pancreas-specific genes depends on a bipartite regulatory sequence which binds a pancreas-specific transcription factor PTF1 (Cockell et al. MCB vol 9, 1989). Expression of the α-amylase 2 gene is stimulated in exocrine cells that are exposed to glucocorticoid hormone. This stimulation of transcription by hormone occurs by an indirect mechanism, since it requires ongoing protein synthesis. No alteration in binding of proteins to the 5' DNA of α-amylase 2 genes is detected in nuclear extracts made from cells exposed to hormone. A second cis-acting regulatory element has been identified which is necessary in order to detect hormone dependent transcription of transfected α-amylase 2 genes. Binding activity recognising this element is detected in nuclear extracts from an exocrine pancreatic cell-line and from both pancreas and liver tissues. This binding activity is not detected in a number of other cell-lines of non-pancreatic origin nor in kidney, spleen, salivary gland and brain tissue. Proteins binding to this regulatory site have been purified from an exocrine pancreatic cell-line by DNA affinity chromatography. Polypeptides of apparent M.W. 70 and 80 kd co-elute with the binding activity after two successive rounds of affinity chromatography.

E 110 A cAMP RESPONSE ELEMENT IN THE β_2-ADRENERGIC RECEPTOR GENE CONFERS TRANSCRIPTIONAL AUTOREGULATION BY cAMP. S.Collins*, J.Altschmied*, M.G.Caron,P.Mellon*and R.Lefkowitz*, HHMI, Duke Univ. Med. Ctr.,Durham,NC 27710, Salk Institute La Jolla, CA 92136.

The β_2-adrenergic receptor (β_2AR) gene is transcriptionally upregulated in response to agonist or forskolin stimulated cAMP levels. Previous studies show this auto-regulation resides in the 5'-flanking region of the β_2AR gene (PNAS, 86:4853,1989). A 34 bp sequence from the β_2AR promoter (-70 to -37), containing the sequence GTACGTCA, confers responsiveness to cAMP in either orientation 5' to a thymidine kinase promoter-CAT reporter gene when transfected into rat C6 glioma or human JEG-3 chorio-carcinoma cells. Specific mutations in this sequence completely abolished stimulation. Overexpression of the catalytic unit of protein kinase A fully substituted for the induction of CAT activity by forskolin.

A 43 kD transcription factor (CREB; cAMP response element binding protein) confers cAMP responsiveness through binding to specific sequences. Purified CREB bound to the β_2AR cAMP response element (CRE) in gel-mobility shift assays with an affinity identical to that for the CRE from the human glycoprotein hormone α-subunit gene, and failed to bind to mutated elements.

These results demonstrate an autoregulatory mechanism by which a receptor (β_2AR) stimulatory for adenylyl cyclase exerts positive feedback regulation on its own expression.

E 111 CLONING AND EXPRESSION OF AN ADIPOCYTE SPECIFIC GENE: IDENTIFICATION OF A NOVEL GENE REGULATORY SEQUENCE ELEMENT, Ulrich Danesch and Gordon M. Ringold, Institute of Cancer and Developmental Biology, Syntex Research, Palo Alto, CA 94304

Our laboratory has cloned several cDNAs whose mRNAs are induced during differentiation of TAI adipocytes. One of these cDNA clones, referred to as FSP27, encodes a 1.8 kb mRNA which is detectable only in differentiated adipocytes. This tissue specific expression can be turned off by dedifferentiating TAI adipocytes with TNF or TPA together with a Ca-ionophore. In the mouse, FSP27 expression is restricted to fat tissue and can not be detected in any other organ. The sequence of the FSP27 cDNA, coding for a protein of relative molecular weight of 27 kd, shows no homology to any existing protein as revealed by a computer aided search of current protein data banks. In addition to the cDNA, we cloned the FSP27 gene from TAI cells. A fusion gene containing only 176 bp of 5' upstream flanking sequences of the FSP27 gene and a CAT reporter plasmid is expressed and regulated in a tissue specific manner during adipocyte differentiation of the adipogenic cell lines TAI and 3T3-LI. No expression can be found in JZ hepatoma or L fibroblast cells. Deleting sequences up to position -135 no longer allows expression in adipocytes, thus revealing a fat cell specific element (FSE) between -176 and -135. This region contains the palindromic sequence TTTCGAAA which is footprinted by nuclear extracts from adipocytes but not from adipoblasts. Similarly, an oligonucleotide containing this palindrome leads to an adipocyte specific gel-shift. In addition to the FSE, the FSP27 5'flanking sequences contain a non-fat cell specific enhancer element further upstream that increases the expression from a heterologous promoter in a differentiation independent fashion.

E 112 DEVELOPMENTAL AND HORMONAL REGULATION OF PORCINE G-PROTEIN α_{i-2} AND α_{i-3} SUBUNIT GENES IN RENAL CELLS. Louis Ercolani, Brian W. Soper, Dennis A. Ausiello, and Eliezer J. Holtzman. Mass. General Hosp. and Harvard Med. School, Boston, Massachusetts
G-protein α_{i-2} and α_{i-3} subunits are differentially expressed during the development of LLC-PK_1 cells and are targeted to different membrane domains[1]. To examine the regulation of these genes, DNA segments encoding the promoter-enhancer regions of each α_i subunit were isolated from an EMBL-3 porcine genomic library. Although the coding areas in Exon 1 of each gene were 79% identical, no sequence identity was found in their 5' flanking segments. A cyclic AMP binding site "CREB", an "AP2" binding site, and a "TGTGG" sequence were found for α_{i-2}, whereas "E2A" and "E4" and binding sequences were found for α_{i-3}. These DNA segments were fused to a promoterless firefly luciferase gene and transfected into LLC-PK_1 cells to quantify gene transcription. In transient transfection assays transcription of an α_{i-2}, α_{i-3}, and a steroid responsive angiotensinogen gene were not influenced by 10^{-7} M corticosterone. However, following complementation of these cells with a human glucocorticoid receptor, 10^{-8} M corticosterone increased α_{i-3} and angiotensinogen gene transcription by 3-fold and 12-fold, respectively, while inhibiting α_{i-2} gene transcription by 50%. In clonal LLC-PK_1 cells stably transfected with α_{i-2} and α_{i-3} genes, transcription was 7-fold greater in non-polar cells at 10-30% confluence, compared to fully polarized confluent cells. These findings demonstrate that the transcription of α_{i-2} and α_{i-3} subunit genes in renal cells are independently influenced by hormonal and developmental processes.

1. Ercolani, L. , Stow, J.L., Boyle, J.F., Holtzman, E.J., Lin, H., Grove, J.R., and Ausiello, D.A. 1990, Proc. Natl. Acad. Sci. USA, 87:4635-4639

E 113 *IN VIVO* AND *IN VITRO* REGULATION OF THE *HOX-7* GENE, Louis H. Ferland, Benoît Robert, Arlette Cohen and Margaret E. Buckingham. Institut Pasteur, 75724 Paris Cédex 15, FRANCE.

Hox-7, a member of the family of mouse homeobox genes, was isolated by virtue of its homology with the *Drosophila msh* gene, a distant relative of *Antennapedia*. We showed using *in situ* hybridization techniques (Robert et al., EMBO J., 8:91-100, 1989), that *Hox-7* is expressed in the neural folds and the cephalic neural crest of embryos, as well as in the developping heart valves. In addition, the mandibular and hyoid arches and the limb buds are strongly labelled, with expression restricted to the most distal part of these structures. In the limb, the apical ectodermal ridge and the underlying mesoderm, which defines the "progress zone", are intensely labelled, suggesting a role for *Hox-7* in limb morphogenesis.

The overall structure of the *Hox-7* gene is similar to that of most known *Hox* genes, with 2 exons separated by an intron of approximately 2 kb; the homeobox is near the beginning of the second exon. Sequence comparison between our genomic clone and a previously published cDNA sequence of mouse *Hox-7* (Hill et al., Genes & Devel., 3:26) shows a number of differences, two of which are located in the coding sequence (second exon, 3' from the homeo box), and result in a shift of the reading frame. As a consequence, the amino acid sequence deduced from our genomic clone diverges radically in the C-terminal portion of the protein.

The expression of *Hox-7* has been examined in various cell lines (derived from embryonic carcinoma cells) in order to establish a system for the study of the regulatory sequences governing the expression of this gene, in transfection experiments. In parallel, the regulation of *Hox-7* expression is being investigated *in vivo*, using transgenic animals technology. Finally, in the view that retinoic acid, a potent morphogen known to be involved in the definition of the limb structures, has receptors that are expressed at the same sites as *Hox-7* in the developping limb (e.g. in the interdigital zone of programmed cell death), we are particularly interested in the involvement of retinoic acid in the expression of this *Hox* gene.

E 114 ANDROGEN POSITIVE AND NEGATIVE REGULATION OF SEXUALLY DIFFERENTIATED GENE EXPRESSION IN THE LARYNX OF *XENOPUS LAEVIS*, Leslie Fischer, Diana Catz, and Darcy Kelley, Department of Biological Sciences, Columbia University, New York, NY 10027

The larynx of *Xenopus laevis* is composed primarily of two sexually dimorphic tissues, muscle and cartilage. Androgens regulate the normal male development of the larynx, and can masculinize female larynges if supplied exogenously. We investigated the expression of two potential androgen target genes: androgen receptor (AR), and a male specific myosin heavy chain (mHC) by Northern blot analysis. Specifically we assayed how expression levels of these genes responded to fluctuations in hormone levels. Juvenile frogs of both sexes were implanted with dihydrotestosterone (DHT) pellets for 3 weeks. Laryngeal tissue was collected either immediately following treatment, or 1 week after pellet removal. Total RNA was extracted from laryngeal muscle and cartilage separately. In addition, age-matched male frogs were castrated, and whole laryngeal RNA collected 3 weeks later. DHT treatment *decreases* AR mRNA levels in a tissue and sex specific manner. Cartilage is more affected than muscle in both sexes. For muscle, males are more affected than females. One week following pellet removal, AR mRNA levels decline further in female muscle, increase in male muscle, and remain unchanged in laryngeal cartilage. DHT treatment *increases* mHC mRNA levels in a sex specific manner. DHT treatment has no effect on mHC expression in male laryngeal muscle, but dramatically increases mHC expression in female laryngeal muscle. One week after androgen withdrawal, mHC message levels rise slightly in males, and decline in females. Castration has no apparent effect on AR or mHC mRNA levels. Thus androgens may direct larynx development by coordinate positive and negative regulation of gene expression. [Supported by NS 19949 and NS 23684]

E 115 FUNCTIONAL CHARACTERIZATION OF THE RAT GROWTH HORMONE SILENCERS, Sylvain, L. Guérin, René Roy and Pierre Gosselin, CHUL Research Center, Québec, (Canada), G1V 4G2.

Cell-specific regulation of the rat growth hormone gene expression has been shown to be determined by both positive and negative regulatory mechanisms. Most of these effects are mediated by the binding of a variety of trans-acting factors to distinct regulatory sequences located within the rGH promoter and 5'-flanking sequences. We previously demonstrated that two 25bp-long silencer sites, apart from each other of 200bp and located 305 and 520bp upstream from the rGH start site, are involved in the cell-specific repression of the rGH gene in non-pituitary cell-lines. A detailed footprinting analysis of both silencer sequences provided the evidence that two distinct trans-acting proteins interact with these negative regulatory elements. The first of these factors, designated SBP_1, binds to a DNA region highly homologous to the DNA binding site recognized by the well known transcription factor NF-1 and located in the 5' portion (position -309 and -526) of both rGH silencers. The second protein, designated SBP_2, binds immediately 3' from SBP_1 (position -298) in the rGH silencer-1 region. Gel shift analysis using double-stranded oligonucleotides of similar length containing either the SBP_1 or the SBP_2 recognition site indicated that the former protein has a molecular weight slightly larger than SBP_2. In addition, the specifically retarded band detected for each DNA/protein complex using nuclear extracts prepared from non-pituitary cells is missing in extracts prepared from pituitary GH4C1 cells suggesting that both proteins are either missing or modified in GH expressing cells. When inserted upstream from an heterologous promoter (mouse p12 promoter), only silencer-2 could function in a cell-specific manner, silencer-1 repressing CAT gene expression in both pituitary (GH4C1) and non pituitary (COS-1) cells. These observations suggested that additional regulatory elements present in silencer-2 but missing in silencer-1 are needed to fully achieve a significant level of tissue-specific regulation. As determined by transient transfection assays, SBP_1 proved to be essential to the mechanism of gene repression while the function of SBP2 is still unclear.

E 116 POST-TRANSCRIPTIONAL CONTROL OF c-myc PROTO-ONCOGENE BY GLUCOCORTICOIDS IN HUMAN T LYMPHOMA CELLS. A. Gulino, M. Maroder, S. Martinotti, A. Vacca, I. Screpanti, E. Petrangeli and L. Frati, Dept. of Experimental Medicine, University of Rome and University of L'Aquila, Italy.

Because of the role played by c-myc in haematopoietic cell growth, the previously described decreased expression of this oncogene by glucocorticoids is suggested to represent a step in the cascade of events leading to the hormone-induced inhibition of lymphoid cell proliferation. We studied the mechanisms involved in the regulation of human c-myc by glucocorticoids in T lymphoblastic leukemic cells. A significant decrease (50%) of the steady state levels of c-myc mRNA was observed as early as 3 h after dexamethasone (DEX) treatment of CEM-1.3 human lymphoma cells, reaching less than 5% values, with respect to untreated cells, 24 h after hormone administration. Nuclear run-on experiments showed no modifications of the transcriptional rate from the first exon. However a slight decrease (15%) of the transcript elongation from the first exon/first intron boundary was observed in DEX-treated cells. Using actinomycin D to block gene transcription, a significant increase in the rate of c-myc mRNA decay was observed after DEX treatment. Cycloheximide was able to overcome the DEX-induced downregulation of c-myc. Our data suggest that DEX inhibits human c-myc gene primarily at the post-transcriptional level, through the synthesis of hormone-induced regulatory protein(s) controlling c-myc transcript stability.

E 117 RAT BRAIN CREATINE KINASE GENE REGULATION: TISSUE SPECIFIC EXPRESSION IN TRANSGENIC MICE AND *TRANS*-ACTIVATION BY THE HUMAN CYTOMEGALOVIRUS IE2 GENE PRODUCT, Patricia P. Harlow, Johann P. Hossle, Grace M. Hobson, Mark T. Mitchell, Anamaris M. Colberg-Poley and Pamela A. Benfield, Central Research and Development Dept., E.I. duPont de Nemours & Co., Experimental Station, Wilmington, DE. 19880-0328

The rat brain creatine kinase gene (CKB) is expressed in many tissues at varying levels, but the highest level of expression occurs in brain. We show that a construct consisting of the 2.9 kb rat CKB gene along with 0.2 kb of 5' flanking and 0.5 kb of 3' flanking region is expressed at its highest level in the brain of transgenic mice. Thus, this 3.7 kb of DNA contains sufficient information for the tissue specific expression of the CKB gene. During permissive infection human cytomegalovirus (HCMV) increases the activities of certain cellular enzymes, including CKB, by elevating RNA levels. We show that the HCMV immediate early gene product, IE2, but not IE1, elevates transcription from rat CKB promoter constructs in nonpermissive HeLa cells cotransfected with various HCMV IE constructs. In the presence of IE2, transcription from the ckb promoter, assayed by primer extension, was observed not only from the normal rat start site utilized *in vivo*, but also from an upstream start site. In normal tissues so far examined the upstream site is not utilized, however, *in vitro* it is utilized with liver, but not brain or HeLa nuclear extracts. Using various mutations of the CKB promoter, we are determining whether IE2 has a specific DNA sequence target.

E 118 TRANSFORMATION OF HUMAN Ah RECEPTOR TO A FORM BINDING TO A DIOXIN RESPONSIVE ELEMENT: INHIBITION BY SODIUM MOLYBDATE. Patricia A. Harper, Allan B. Okey, and *Michael S. Denison. Division of Clinical Pharmacology and Toxicology, Hospital for Sick Children Toronto, Ontario, Canada, and *Department of Biochemistry, Michigan State University, East Lansing, Michigan.

The mechanism of induction of cytochrome P450IA1 is similar to the induction of specific response genes by steroid hormones. Transformed AhR:ligand complexes bind to *cis*-acting dioxin-response-elements (DRE) adjacent to the 5'-end of the cytochrome P450IA1 gene resulting in an increased rate of gene transcription.

We used a gel retardation assay to investigate the interaction of transformed human cytosolic Ah receptor (AhR) from the colon carcinoma cell line LS180, with a complementary pair of synthetic oligonucleotides containing the sequence 5'-GATCCGGAGTTGCGTGAGAAGAGCCA-3'.

We observed a striking difference in the effect of molybdate on transformation of human cytosolic AhR as compared to other species. Inclusion of 20 mM sodium molybdate prevented transformation of human AhR:ligand complexes to a form which will bind to a DRE oligonucleotide in vitro. The time of addition of molybdate was important; if added before or during ligand binding to the receptor, no receptor:DNA complexes were observed, whereas addition after transformation or during DNA binding had no effect. In rodents, molybdate does not interfere with transformation of the AhR. From analysis of AhR:ligand complexes by sucrose gradient centrifugation, sodium molybdate appears to stabilise the Ah receptor in a 9 S multimeric complex.

These data are consistent with the proposed model that the multimeric 9 S form of the Ah receptor is inactive in DNA binding and that ligand mediated transformation of Ah receptor is irreversible.

E 119 **HORMONAL REGULATION OF PLASMINOGEN ACTIVATOR AND PLASMINOGEN ACTIVATOR-INHIBITOR GENE EXPRESSION IN RAT HEPATOMA CELLS,** Heaton JH, and Gelehrter TD, Department of Human Genetics, University of Michigan Medical School, Ann Arbor, MI 48109-0618

Plasminogen activators (PAs) are membrane-associated serine proteases that convert the inactive zymogen plasminogen to plasmin. Plasmin, the major fibrinolytic enzyme in blood, also participates in a number of physiologic functions involving protein processing and tissue remodelling and may play an important role in tumor invasion and metastasis. PA activity is regulated in part by a specific PA-inhibitor, PAI-1.

Incubation of HTC rat hepatoma cells with dexamethasone (Dex), decreases tPA activity by inducing a 4-fold increase in PAI-1 mRNA; paradoxically, tPA mRNA is increased modestly but transiently. In contrast, incubation with 8-bromo-cAMP (cA) increases tPA activity >50-fold, primarily the result of a 90% decrease in PAI-1 mRNA; tPA mRNA is increased only 2-fold. Thus, separately Dex and cA each regulates tPA activity primarily by regulating PAI-1 mRNA accumulation. Dex and cA, in combination, cause a >100-fold increase in tPA activity, the result of both an 80-90% decrease in PAI-1 mRNA and a 10- to 20-fold increase in tPA mRNA. Inhibition of protein synthesis by cycloheximide does not prevent the Dex-induced increase in PAI-1 but does block the cA-induced decrease. Inhibition of RNA synthesis by actinomycin D prevents both the Dex and cA regulation of both PAI-1 and tPA mRNAs.

Nuclear run-on experiments show that Dex increases both PAI-1 and tPA gene transcription. cA appears to have little effect on PAI-1 and only slightly increases tPA transcription. Dex and cA, in combination, also have little effect on PAI-1, but significantly increase tPA gene transcription. Determination of message stability reveals that PAI-1 mRNA has a half-life of 4-5 hours in cells incubated with or without Dex. cA, however, significantly increases the rate of PAI-1 mRNA decay ($t_{1/2}$ = 1.5h).

Our results suggest that Dex regulates PAI-1 and tPA primarily by increasing gene transcription and has little, if any, effect on message stability. The synergistic effect of Dex and cA on tPA mRNA is also mediated by a direct increase in transcription. In contrast, the cA-induced decrease in PAI-1 mRNA appears to be mediated primarily by increasing the rate of message decay.

E 120 GLUCOCORTICOID RESISTANCE IN THE DDT1MF-2 CELL VARIANT IS RESTRICTED TO THE GROWTH INHIBITION AND p29 INDUCTION RESPONSES, William J. Hendry III and Lawrence E. Cornett, Departments of Medicine, Biochemistry/Molecular Biology and Physiology/Biophysics, University of Arkansas for Medical Sciences, Little Rock, AR 72205

At least two glucocorticoid-dependent responses (growth inhibition and induction of p29 protein synthesis) that are characteristic of the wild-type DDT1MF-2 hamster smooth muscle tumor cell line (DDT-WT) are known to be lacking in a clonal variant (DDT-GR). Further comparisons of the two cell lines have shown the following: 1) According to ligand-binding assays and immunoprecipitation/Western blot analysis, glucocorticoid receptors were less abundant in DDT-GR cells, but the same proportion underwent glucocorticoid-dependent nuclear accumulation as in DDT-WT cells; 2) Glucocorticoid-enhanced expression of the ß2 adrenergic receptor gene was observed to the same extent (~2-fold) at the mRNA as well as membrane protein level in both DDT-WT and DDT-GR cells; 3) The synthesis of several proteins other than p29 were glucocorticoid regulated in an identical manner in DDT-WT and DDT-GR cells; 4) All the above responses that were shared by DDT-WT and DDT-GR cells as well as growth inhibition and p29 induction which are restricted to the DDT-WT cell could be blocked by the anti-glucocorticoid, RU 486. Together these data indicate that DDT-GR cells contain enough functional glucocorticoid receptors to normally regulate the expression of some if not most of the appropriate set of responsive genes. The highly selective nature of the glucocorticoid-resistant phenotype in the DDT-GR cell is currently being exploited to identify those genes that are specifically involved in the anti-proliferative action of glucocorticoid on the DDT-WT cell. (Supported by NIH grants GM30669 and DK38025).

E 121 **TRANSCRIPTIONAL CONTROL OF THE *XENOPUS LAEVIS* PRO-OPIOMELANOCORTIN GENE**, Judith A. Hewitt and Y. Peng Loh, Laboratory of Developmental Neurobiology, National Institute of Child Health and Human Development, Bethesda, MD 20892.

We are interested in the mechanisms controlling the onset of pro-opiomelanocortin (POMC) gene expression in developing *X.laevis* embryos. One approach we are taking is the identification of the *cis* and *trans* elements involved in POMC gene expression. We have cloned the POMC gene and constructed reporter plasmids with 473 bp of upstream DNA fused to the chloramphenicol acetyl transferase (CAT) coding region. Transient transfection of POMC-CAT genes into mouse AtT20 corticotroph tumor cells gave moderate expression which was 2 fold induced with 5 μM forskolin, 0.3 mM IBMX. Mouse fibroblasts (L929), rat pituitary tumor (GH3) and rat glioma (C6) cells were also transfected and expressed the reporter gene. The transient transfection system does not reproduce tissue specificity, and so further experiments are required to address this issue. The POMC promoter used in these experiments did show cAMP responsiveness even though the consensus element defined for other genes is not present, and therefore this POMC promoter will be used to delineate the cAMP regulatory elements.

E 122 POSTTRANSLATIONAL MODIFICATION INDUCES STRUCTURAL AND FUNCTIONAL CHANGES OF THE INTERFERON REGULATORY FACTOR IRF-1, K. Hoffmann, M. Nourbakhsh and H. Hauser; Genetics of Eucaryotes, GBF, Ges. f. Biotechnol. Forschung mbH, D-3300 Braunschweig, F.R.G.

IRF-1 is a nuclear DNA-binding protein which specifically interacts with sequences represented in all known mammalian type I interferon promoters and within the interferon stimulated regulatory element (ISRE) of interferon regulated genes. IRF-1 is induced by a series of biological factors like viruses, dsRNA, TNF-alpha, IL-1, TPA and IFN.

We have isolated a cDNA fragment encoding IRF-1 from mRNA of mouse cells using the polymerase chain reaction and cloned it into expression vectors in order to express the protein in mammalian cells and in E.coli. Transient expression of cloned IRF-1 leads to a significant induction of type I IFN's in mammalian cell lines. Constitutive expression of IRF-1 is not tolerated. The stop of animal cell growth upon overexpression might be related to the antiproliferative effect of interferons.

IRF-1 expressed in E.coli was analysed by several in vitro techniques. Although no differences in the DNaseI footprint and methylation interference patterns between recombinant IRF-1 and partially purified IRF-1 from HeLa nuclear extract could be observed, the gel retardation pattern is quite different. This difference is due to the phosphorylation of the protein. With cellular extracts from Ltk⁻-cells and purified porcine casein kinase II we were able to phosphorylate recombinant IRF-1 in vitro. After phosphorylation recombinant IRF-1 was able to form a DNA-protein complex which shows similar gel retardation properties if compared with complexes derived from HeLa nuclear extracts. As shown in crosslinking experiments, in vitro phosphorylated recombinant IRF-1 is able to form dimers which have enhanced affinity to their target sequence motifs and are resistant to a final dephosphorylation with different phosphatases. Our studies suggest that IRF-1 from mammalian cells is constitutively phosphorylated by a casein kinase II type of protein kinase leading to a dimerisation of recombinant IRF-1 and structural changes of the molecule.

E 123 AN ACIDIC TRANSCRIPTIONAL ACTIVATION DOMAIN BINDS TO THE TATA-BOX FACTOR TFIID. C. J. Ingles[1], K. F. Stringer[1], M. Shales[1], W. D. Cress[2], S. J. Triezenberg[2], and J. F. Greenblatt[1]. [1]Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada and [2]Department of Biochemistry, Michigan State University, East Lansing Michigan.

To understand the molecular mechanisms of transcriptional activation, we used the potent acidic activation domain of the HSV-encoded polypeptide VP16 in affinity chromatography experiments. This C-terminal 79 amino acid domain of VP16 bound strongly, highly selectively, and directly to the TATA box-binding factor TFIID (Stringer et al. Nature **345**, 783-786. 1990). To address the biological significance of this VP16-TFIID interaction we used mutants of VP16 compromised in their transactivation function *in vivo* and established a correlation between the transcriptional activity of VP16 *in vivo* and the strength of TFIID-VP16 interaction *in vitro*. We estimate the K_D for the wild-type VP16-TFIID interaction to be $2x10^{-7}$ M^{-1}. Using deletion mutants of yeast TFIID (kindly provided by M. Horikoshi and R. Roeder) we have established that the integrity of the evolutionarily conserved C-terminal region of TFIID is critical for binding VP16. By analysing missense mutations in TFIID it may be possible to more precisely identify amino acids involved in the activator-TFIID contact.

E 124 CHICKEN AVIDIN GENE FAMILY; A VERSATILE MODEL SYSTEM TO STUDY THE EUKARYOTIC GENE EXPRESSION, Riitta A. Keinänen, Mika J. Wallén, Tarja A. Kunnas and Markku S. Kulomaa, Department of Biomedical Sciences, University of Tampere, SF-33101 Tampere, Finland.

Chicken avidin has two different induction mechanisms. First, it is induced in most of the chicken tissues in connection with inflammation, and secondly in oestrogen-primed chick oviduct specifically by a single steroid hormone, progesterone. Based on studies at the protein and mRNA level, the progesterone induction is suggested to be mainly transcriptional. Thus the avidin gene(s) offer a versatile model to study the eukaryotic gene expression. Five different avidin related genes (avrl-5) have been cloned. The amino acid sequence translated from their putative exons, show a high homology (70-85%) to the known sequence of egg-white avidin, but could not encode exactly its correct peptide chain. However, DNA elements known to be involved in transcriptional activation, have been found in the 5'-flanking regulatory region of avrs. Final studies to find out whether these genes are functional, and if so, under which type of induction, are in progress. In order to clone the gene which encodes the progesterone regulated egg-white avidin, different approaches including PCR are used. The cloned gene and its regulatory region will offer an additional and a specific model system to study the progesterone action in regulation of eukaryotic gene expression.

E 125 REGULATION OF AP-1 ENHANCER ACTIVITY AND C-JUN RNA LEVELS DURING THE DIFFERENTIATION OF U937 HUMAN LEUKEMIC CELLS. Andrew S. Kraft, Tino Unlap, Evans S. Adunayh and Fred Wagner, Department of Hematology/Oncology, University of Alabama, Birmingham, AL 35294

To evaluate whether activation of specific enhancer sequences is involved in controlling hematopoietic progenitor differentiation towards monocytes or neutrophils, we have studied U937 human leukemic cells which are primitive cells capable of differentiation along both pathways. The addition of phorbol esters (PMA) or diacylglycerols (DiC8) differentiates them towards monocytes while treatment with cyclic AMP (cAMP) stimulates granulocyte-like maturation. We find that the addition of either cAMP, PMA, or DiC8 stimulates increases in total cellular c-*jun* and c-*fos* mRNA. The increases induced by all three agents occurs over a similar time course with maximal changes in c-*jun* levels found at 1 hour. To study activation of enhancer sequences, U937 cells were electroporated with constructs containing multiple repeats of AP-1 followed by a CAT recorder gene. We find that PMA treatment activated AP-1 (25X), and cAMP (4X). Although a single dose of DiC8 was capable of induction of c-*jun* mRNA, no increase in Jun protein or AP-1 enhancer activity was found. However, if multiple doses of DiC8 were given every 2 hours (3 doses) sufficient to induce differentiation of these cells then Jun protein was increased and AP-1 enhancer activity was stimulated (8x). To evaluate the mechanism by which these agents increase c-*jun* transcription, U937 cells were electroporated with constructs containing various regions of the c-*jun* promoter/enhancer. Phorbol esters activate varying amounts of CAT enzyme activity from these constructs with -1.1/+740 > -132/+740 > -79/+170. Our data would suggest that induction of differentiation either towards granulocytes with cAMP or towards monocytes with PMA is associated with increases in c-*jun* mRNA and activation of AP-1 enhancer activity. We find that activation of c-*jun* transcription is not only modulated by the AP-1 site previously identified in the -79/+170 region of the promoter, but that other upstream regions play an important role in phorbol ester induction of gene transcription.

E 126 Intron exon structure of the human retinoic acid receptor gamma
Jürgen M. Lehmann, Birgit Hoffmann, Magnus Pfahl
La Jolla Cancer Research Foundation, 10901 North Torrey Pines Road, La Jolla CA 92037, USA

Retinoic acid (RA) is a vitamin A metabolite which exerts its pleiotropic effects on cell growth, fetal development, and cellular differentiation through a subfamily of nuclear retinoic acid receptors (i.e. RA receptors RAR-α , β, and γ). Here we have characterized for the first time the exon-intron structure of one of the RARs, the human RAR-γ. We show that in comparison with the steroid receptor genes, various exon-intron boundaries of the RAR occur at altered positions . Exon 1 encodes 142 bp of the 5'-untranslated region and the first 61 amino acids. This exon is unique for RAR-γ1 and distincts this isoform from RAR-γ2. Exon 2 encodes the entire B-region together with the first "zinc-finger" of the DNA-binding domain. This is in contrast to steroid receptors which encode the large A-region together with most of the B-region in a single exon. The splice site between Exon 2 and 3(i.e. Lys-111) is identical to that found in RAR-β and TR-β. This position is highly characteristic for the TR/RAR subfamily and differs from the conserved position (i.e. 8 amino acids downstream) found in steroid receptor genes. Exon 4which encodes the hinge region, is much shorter than the comparable exon of the steroid receptors. The hormone binding domain is encoded by the exons 5-7 and by parts of exon 8. The remainder of exon 8 encodes the rest of the molecule and the entire 3'-untranslated region. The exon-intron structure of the RAR-γ hormone binding domain is distinct from that found in the steroid receptors (i.e. differences in length of exons and positions of splice sites), except for the splice site within the heptad repeat which is in a conserved region. This suggests that during evolution the TR/RAR subfamily has developed independently from the steroid receptors. Noteworthy is, that the organisation of nuclear receptors in A-F domains, which is based on sequence homology, is in particular in the hormone binding domain not consistent with the exon-intron structure. The availability of exon flanking sequences will facilitate the analysis of mutations in RAR-γ associated with desease and cancer.

E 127 SEQUENCE DETERMINANTS FOR *IN VITRO* RECOGNITION OF A STEROID RESPONSE ELEMENT BY PROGESTERONE RECEPTOR, Benjamin A. Lieberman, Dean P. Edwards and Steven K. Nordeen, Department of Pathology, University of Colorado Health Sciences Center, Denver, CO 80262

The mouse mammary tumor virus (MMTV) promoter is inducible by four classes of steroid hormones which bind to distinct receptors that comprise a related subset of the steroid/thyroid receptor family. This study examines the sequence determinants that constitute a recognition sequence for the progesterone receptor. A library of substitution mutants of a known progesterone responses element (PRE) derived from MMTV was constructed using "dirty bottle" oligonucleotide synthesis. Small (36 bp) fragments of individual mutant elements were isolated, labeled and then assessed by a gel shift assay for their ability to bind progesterone receptor *in vitro* relative to a wild type MMTV PRE. Point mutations in the wild type sequence were found to have a wide range of effects on receptor recognition of the element. Certain mutations had no effect on the binding of receptor whereas other point mutants exhibited essentially undetectable levels of binding. In this way a detailed map of the sequence features required for progesterone receptor binding has been assembled. Complementary studies are also underway to assess the *in vivo* ability of each of the mutant PRE sequences to confer hormone responsiveness onto an uninducible promoter. Parallel studies on *in vitro* binding by glucocorticoid and androgen receptors are also underway. The implication of these data toward the molecular mechanics of steroid-receptor DNA interaction will be discussed.

E 128 DIFFERENCES BETWEEN E1A AND cAMP ACTIVATION OF PROMOTERS CONTAINING SIMILAR RESPONSE ELEMENTS, Mary R. Loeken and Sunanda Babu, Joslin Diabetes Center, 1 Joslin Place, Boston, MA 02215

Trans-activation of some viral promoters by Adenovirus E1A requires a binding site for the transcription factor ATF. The consensus sequence for ATF binding is identical to the consensus sequence for a cAMP response element. To determine whether E1A interacts with cellular pathways which are part of cAMP-dependent signaling, we compared the activation of the Adenovirus E2A and the neuropeptide somatostatin promoters in two cultured lines of CV-1 cells, one of which was cAMP responsive, and one of which was not. E1A activation of the E2A promoter containing only an ATF site was fully functional in both cell types. E1A did not, however, activate the somatostatin promoter. Conversely, cAMP did not activate either the E2A promoter or the E4 promoter, which also contains ATF sites. A plasmid expressing the catalytic subunit of cAMP dependent protein kinase (PKA) was transfected over a 10,000-fold range. Low level expression of PKA stimulated the somatostatin promoter, while high level expression inhibited it and suppressed basal activity of the E2A promoter. This latter effect required binding sites for the transcription factor EIIF, rather than ATF. There was no effect of PKA on activity of the E4 promoter. These results suggest that, in CV-1 cells, E1A stimulation of transcription does not involve interaction with cAMP dependent pathways. In addition, phosphorylation may inactivate, as well as stimulate, transcription. Gel shift analysis demonstrated that different factors bind to the ATF and cAMP response elements of the respective promoters. The response of particular promoters to stimulation by various signals may involve not only specific members of a transcription factor family, but also differential activation pathways of these transcription factors.

E 129 TRANSIENT INDUCTION OF INSULIN ENHANCER BINDING FACTORS DURING β-CELL DIFFERENTIATION/DE-DIFFERENTIATION, AND EVIDENCE FOR DIFFERENTIAL EXPRESSION OF THE TWO RAT INSULIN GENES, K. Lund, P. Serup, N. Blume, H.V. Petersen, B. Michelsen, and O.D. Madsen, Hagedorn Research Laboratory, Gentofte, Denmark.

A glucagon producing rat cell line, NHI-6F, undergoes differentiation by in vivo passage to express both the endogenous insulin genes as well as a stably transfected human insulin gene. During this differentiation nuclear factors, the appearance of which is tightly coupled to the insulin producing phenotype, can be revealed to specifically bind the rat I insulin gene enhancer as monitored by gel mobility shift assays. DNaseI footprinting shows that protection patterns mainly change in the distal E-1 region, and at the 5′ border of E-4. Identical results were obtained using nuclear extracts from a control insulin producing cell line, RINm-5AH. During de-differentiation by prolonged in vitro culturing of NHI-6F tumor isolates specific binding to the rat I enhancer was gradually lost. In this process preferential expression of the rat I gene was observed, the expression of the rat II and human insulin genes being extinguished more rapidly. These results were obtained using highly specific antisera recognising C-peptides from rat I, rat II, and human proinsulins, respectively. Double-labeling experiments showed that while most cells expressed the rat I C-peptide only, few cells co-expressed rat II or human C-peptide with the rat I C-peptide. Interestingly, cells expressing the human C-peptide were found to be a sub-population of those positive for the rat II C-peptide. On account of the human insulin gene bearing more resemblance to the original rat II insulin gene from which the rat I gene, including 520 bp 5′ to the coding sequence, was derived, we conclude that sequences further upstream from position -520 may be involved in β-cell specific insulin gene expression. Also, we propose that the appearance of nuclear factors binding the enhancer regions E-1 and E-4 may reflect events of normal islet cell differentiation during pancreas ontogeny.

E 130 STUDIES ON THE STRUCTURE AND FUNCTION OF THE HUMAN S-ADENOSYLMETHIONINE DECARBOXYLASE GENE PROMOTER

Svetlana C. Maric, Jorma Palvimo, Anne Crozat, and Olli A. Jänne, The Population Council and The Rockefeller University, 1230 York Avenue, New York, NY 10021

S-Adenosylmethionine decarboxylase (AdoMetDC) is an important enzyme in polyamine biosynthesis. Mammalian AdoMetDC protein is highly conserved among different species and has a rapid rate of turnover. AdoMetDC activity and AdoMetDC mRNA concentration are tightly regulated by a variety of stimuli, such as steroid hormones, in a tissue-specific manner.

To study factors regulating AdoMetDC promoter activity, we have isolated and sequenced approximately 1.5 kb of 5'-flanking DNA of the human AdoMetDC gene. This sequence contains DNA motifs for binding of various transcription factors/regulatory proteins, including AP-1, CRE and SP-1 sites. In addition, there is similarity to a half palindromic sequence of the putative androgen-response element, ARE. In the initial gel retardation experiments, we have used the proximal promoter region to the AdoMetDC gene. The results show that three to four different proteins complexes in nuclear extract of kidney, liver and spleen interact specifically with this DNA fragment.

In transfection studies using the bacterial CAT gene as the reporter, the 1.5-kb AdoMetDC 5'-flanking region was shown to be fairly strong promoter in both 3T3 and HepG2 cells. Further studies are under way to characterize functionally important regulatory elements of the human AdoMetDC gene.

E 131 TRANSCRIPTIONAL ACTIVATION OF HUMAN TUMOR NECROSIS FACTOR IS REGULATED BY NEGATIVE AND POSITIVE CIS-ACTING ELEMENTS

David F. Mark and Ayesha Siddiqui, Department of Microbial Chemotherapeutics and Molecular Genetics, Merck Sharp & Dohme Research Laboratories, P. O. Box 2000, Rahway, NJ 07065

Tumor Necrosis factor (TNF-α) is an important monokine responsible for pleiotropic effects. These include mediation of an inflammatory response which may ultimately lead to systemic injury, shock and death. TNF-α gene expression is responsive to a number of stimuli at different stages including PMA (phorbol myristate acetate). The cis-acting sequences involved in PMA inducible TNF-α transcription were determined by promoter deletion analysis. The 615 bp region upstream of transcription initiation site was fused upstream of the bacterial CAT-gene, and was used to generate sequential 5' deletions by using naturally occuring restriction enzyme sites. These promoter deletions were used to transiently transfect the human monocytic cell line U937. Promoter deletion analysis of TNF-α promoter identified both a repressor and an activator sequence involved in PMA responsiveness of the gene. The repressor site has been localized to a 226 bp region of the promoter since deletion of these sequences resulted in a five fold increase in the induced expression of TNF-α. The cis-acting sequence responsible for PMA activation has been narrowed down to a 23 bp region. Possible roles of the repressor and activator sequences are discussed.

E 132 TISSUE-SPECIFIC, DEVELOPMENTAL AND HORMONAL CONTROL OF EXPRESSION OF A SURFACTANT PROTEIN A (SP-A):hGH FUSION GENE IN TRANSGENIC MICE,

Carole R. Mendelson, Robert E. Hammer, Margaret E. Smith, Shanna Maika and Joseph L. Alcorn, Dept Biochem & Howard Hughes Medical Institute, UT Southwestern Med Ctr, Dallas, TX 75235

Surfactant, a developmentally-regulated lipoprotein synthesized in pulmonary type II cells, acts to reduce surface tension and prevent alveolar collapse. Expression of the gene encoding the major surfactant protein, SP-A, is lung-specific and is initiated in fetal lung tissue only after 70% of gestation is completed. SP-A gene transcription in fetal lung *in vitro* is stimulated by glucocorticoids and cAMP. In the present study, transgenic mice were used to map regions of the rabbit SP-A gene involved in tissue-specific, developmental and hormonal regulation of expression. A fusion gene comprised of 4.0 kb of 5'-flanking DNA from the rabbit SP-A gene linked to the human growth hormone (hGH) structural gene was introduced into mice and was found to be expressed exclusively in lung. hGH mRNA was first detectable in lungs of transgenic fetuses on day 16 of gestation and was increased markedly by day 18-19 in concert with endogenous SP-A gene expression. To analyze effects of cAMP and glucocorticoids on transgene expression, lung explants from 16 day transgenic fetal mice were cultured in the absence or presence of dexamethasone (Dex) and Bt_2cAMP added alone or in combination. In accordance with its stimulatory effects on SP-A gene expression, Bt_2cAMP caused a 5-6 fold increase in hGH production by the fetal lung explants. By contrast, Dex caused a dose-dependent inhibition of transgene expression and reduced the cAMP induction of hGH levels by *>65%, suggesting the presence of a negative glucocorticoid regulatory element. These findings further suggest* that elements within the 4 kb 5'-flanking sequence of the SP-A gene are sufficient to direct tissue-specific, developmental and cAMP regulation of expression; however, elements within or downstream of the SP-A structural gene are required for glucocorticoid stimulation of SP-A gene expression in fetal lung.

E 133 EXPRESSION OF PRO-OPIOMELANOCORTIN GENE AND ITS REPRESSION BY GLUCOCORTICOIDS IN A CELL FREE TRANSCRIPTION SYSTEM, Mai Nguyen and Jacques Drouin, Laboratoire de génétique moléculaire, Institut de recherches cliniques de Montréal, Montréal (Québec) CANADA, H2W 1R7

The gene encoding pro-opiomelanocortin (POMC) is expressed predominantly in the anterior and intermediate lobes of pituitary. However, glucocorticoids specifically inhibit transcription of the POMC gene in the anterior pituitary. We have developped a cell-free transcription system to investigate specificity of POMC gene transcription as well as repression by glucocorticoids. A 543 bp 5'-flanking fragment of the gene is sufficient to confer specific transcription of POMC gene in AtT-20 pituitary tumor cell extracts as compared to L or Hela cell extracts. In agreement with *in vivo* experiments, 5'-end deletion analysis revealed two promoter regions required for *in vitro* transcription of POMC gene in AtT-20 extracts. Treatment of AtT-20 cells with 10^{-7} M dexamethasone (DEX) resulted in a specific inhibition of POMC transcription *in vitro*. Furthermore, addition of purified glucocorticoid receptor (GR) to AtT-20 extracts resulted in decreased POMC transcription and this GR-dependent inhibition can be reversed in the presence of MMTV GRE oligonucleotides which bind to the purified GR with high affinity. Further studies are in progress to define the mechanism of GR-dependent inhibition as well as to understand cell-specific activation.

E 134 HISTONE H1 MAY FUNCTION AS A NUCLEAR G-PROTEIN: EVIDENCE FOR ITS ROLE IN MEDIATING ACTION OF NUCLEAR RECEPTORS, J. Oikarinen[1], R.-M. Mannermaa[1], P. Nilsson[2], and T. Grundström[2], [1]Collagen Research Unit, Biocenter and Department of Medical Biochemistry, University of Oulu, Kajaanintie 52A, SF-90220 Oulu, Finland, [2]Department of Applied Cell and Molecular Biology, University of Umeå, S-90187 Umeå, Sweden

The DNA in eukaryotes is packed into nucleosomes, in which two turns of DNA are twined around a core structure consisting of histones H2A, H2B, H3 and H4, and H1 binds externally to the hinge/spacer region. H1 acts as a repressor by triggering aggregation of nucleosomes into inactive solenoid structures. It interacts with A+T-rich regions through SPKK-motifs, although it may also recognize a more specific sequence through its HD-like globular domain (Ristiniemi and Oikarinen, JBC 264, 2164-2174, 1989; Mannermaa and Oikarinen, BBRC 168, 254-260, 1990). The concensus recognition sequence for H1 may resemble that of CTF/NF-I (Nilsson et al., NAR 17, 4061-4075, 1989).

We have demonstrated that nucleotide triphosphates such as GTP inhibit binding of H1 to specific sequences, while nucleotide diphosphates such as GDP stimulate. The γ phosphate is of crucial importance in triggering H1 conformation, as shown using non-hydrolyzable GTP analogs, GDP analogs and AlF_4^-. Nucleotide triphosphates are hydrolyzed and the phosphate may be incorporated to exogenous proteins.

The molecular structure of H1 displays similarity to G_α proteins, and certain regions of H3 and H4 display homology to G_β and G_γ respectively. It is therefore suggested that H1, H3 and H4 comprise a nuclear G protein involved in the action of nuclear receptors.

E 135 GLUCOCORTICOID RECEPTOR DIMERIZATION IS REQUIRED FOR EFFICIENT DNA-BINDING. William Cairns, Carol Cairns, Ingemar Pongratz, Jan-Åke Gustafsson, Lorenz Poellinger and Sam Okret
Dept. of Medical Nutrition, Karolinska Institute, Huddinge University Hospital, Novum F60, S-141 86 Huddinge, Sweden

Glucocorticoid hormones regulate specific gene activation through interaction with and activation of an intracellular glucocorticoid receptor (GR). The hormone-receptor complex recognises specific DNA sequences (GREs) in target genes and modulates the transcriptional activity of these genes. Functional GREs are palindromic, each half of the palindrome recognizing one receptor molecule. We were interested to see whether receptor dimerization occured in cytosol (crude receptor preparations) prior to DNA-binding and if this is a requirement for efficient DNA-binding. Centrifugation of cytosolic GR by density gradient centrifugation into a 4S monomeric and 6S multimeric complex showed that the 6S peak bound specific DNA sequences much more efficiently than the 4S peak. Gel retardation assays with full and half palindromic GREs resulted in GR-GRE complexes of identical mobilities. Taken together our results suggests that GR dimerization occurs prior to DNA binding and is required for efficient interaction of the GR with its GRE.

E 136 STIMULATION OF TRANSCRIPTION BY TRANSFORMING GROWTH FACTOR-BETA1 (TGF-β1): POSSIBLE INVOLVEMENT OF TWO DIFFERENT TRANS-ACTING FACTORS
Andrea Riccio and Vincenzo Paolo Pedone, Centro di Endocrinologia ed Oncologia Sperimentale del CNR, Dipartimento di Biologia e Patologia Cellulare e Molecolare, II Facoltà di Medicina, University of Naples, Italy.
TGF-β1 is a multi-functional peptide with stimulatory or inhibitory effects on cell growth.TGF-β1 also stimulates fibroblast cells to accumulate extracellular matrix proteins, by increasing their synthesis and decreasing their degradation.We have analysed the transcriptional induction of human Plasminogen activator inhibithor type-1 (PAI-1) gene after TGF-β1 treatment of cultured cells.The effect is observed with either TGF-β1 growth-stimulated (fibroblasts) or growth-inhibited (hepatocites) cells.The analysis of hepatoma and fibroblast cells transiently transfected with PAI-1-CAT fusion genes has allowed the identification of a 70bp fragment, which is necessary for TGF-β1 regulation in the PAI-1 promoter and can confer TGF-β1 inducibility if fused to an heterologous promoter.DNAse I footprinting and gel mobility shift data, obtained with crude nuclear extracts or partially purified proteins indicate the presence of two different binding sites for nuclear factors in the 70bp region, one of which binds NF-1.Interestingly two homologous sequences, one of them binding NF-1, can be found in the regulatory region of another TGF-β1-inducible gene.Point mutants are being used to establish the role of each site in the regulatory mechanism.

E 137 SYNERGISTIC ACTIVATION OF THE RAT GROWTH HORMONE PROMOTER BY HORMONE RECEPTORS AND THE PITUITARY-SPECIFIC TRANSCRIPTION FACTOR.
Fred Schaufele and John D. Baxter, Metabolic Research Unit, University of California, San Francisco CA 94143
Many tissue-specific promoters, including that of the rat growth hormone (rGH) gene, are activated not only by tissue-specific but also by tissue-general transcription factors. This suggests that the architecture of a tissue-specific promoter allows tissue-general information to be utilized only in a particular cellular environment. In order to investigate the mechanisms by which tissue-general factors activate a promoter tissue-specifically, we have been transfecting the rGH promoter into heterologous cell types along with combinations of vectors expressing the pituitary-specific, Pit-1 transcription factor and the glucocorticoid (GR) and thyroid hormone (TR) receptors. The rGH promoter is minimally activated by the expression of either the Pit-1, TR or GR transcription factors. In contrast, co-expression of any two of these factors results in a substantially stronger transcriptional activation. Co-expression of all three factors yields tha maximal activation of the rGH promoter. Interestingly, all of the transcriptional stimulations observed are dependent upon culturing cells in the presence of activators of protein kinase A and/or protein kinase C.

E 138 A PROTEIN SHARING FUNCTIONAL PROPERTIES WITH NF-κB BINDS TO THE STEROID-DEPENDENT REGULATORY ELEMENT OF THE OVALBUMIN GENE,
Lora A. Schweers and Michel M. Sanders, Department of Biochemistry, University of Minnesota, Minneapolis, MN 55455
The chicken ovalbumin gene is controlled at the level of transcription by four classes of steroid hormones. A steroid-dependent regulatory element (SDRE) from -900 to -732 is required for this regulation. To delineate more precisely sequences that are required for steroid inducibility. Exonuclease III 3' deletion mutants were made in the SDRE of a plasmid containing 900 bp of ovalbumin 5' flanking sequence fused to the chloramphenicol acetyltransferase (CAT) gene. The SDRE mutants were tested for hormone inducibility by transfection into steroid-responsive primary oviduct cell cultures and expression levels measured by CAT assay. A region from -793 to -759 was identified that is required for maximal induction by steroids. Footprinting and methylation interference assays showed that oviduct nuclear proteins bind to sequences from -776 to -768. A region overlapping this binding site from -773 to -764 is identical to a region of the β-interferon gene regulatory element (IRE). Since these sequences in the β-interferon gene bind NF-κB, experiments were performed to determine if the ovalbumin protein was related to NF-κB. A known NF-κB binding site from the immunoglobulin κ-gene did not compete for the binding activity at the SDRE. Treatment of oviduct nuclear extracts with cycloheximide induced binding to both oligomers, however tryptic digest of the DNA/protein complexes produced digestion products with differing molecular weights. Insertion of the κ-gene oligomer into the SDRE of a 3' deletion mutant that was not steroid inducibile restored a 3.0 fold increase in steroid inducibility, while a fragment containing the immunoglobulin heavy chain enhancer had no effect. These data suggest that the protein binding to the SDRE has functional properties that are similar to NF-κB but that it is a distinct protein.

E 139 GLUCOCORTICOID REGULATION OF CORTICOTROPIN-RELEASING HORMONE GENE EXPRESSION, Audrey F. Seasholtz, Mental Health Research Institute and Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48109. Corticotropin-releasing hormone (CRH) is the major hypothalamic releasing factor in the mammalian stress response. CRH stimulates the synthesis and secretion of ACTH from the anterior pituitary which in turn stimulates the production and release of glucocorticoids from the adrenal cortex. CRH secretion is regulated by catecholamines, opioid peptides, acetylcholine, serotonin, glucocorticoids, angiotensin II, and interleukin I, but the effects of most of these compounds on CRH transcription is unknown. However, the endogenous CRH gene has been shown to be negatively regulated by glucocorticoids in the hypothalamus while it is positively regulated by glucocorticoids in human placental trophoblasts. In order to more systematically evaluate the role and mode of action of glucocorticoids and other potential transcriptional regulatory agents on CRH expression, we have utilized CRH gene transfer experiments in well characterized cell lines. The glucocorticoid regulation of the rat and human CRH genes (or CRH-chloramphenicol acetyltransferase (CRHCAT) fusion genes) is particularly intriguing since both positive and negative glucocorticoid regulation have been observed in different cell lines. We are currently using deletion analysis to localize the positive and/or negative cis-acting transcriptional control elements important for glucocorticoid regulation of CRH (or CRHCAT) expression in these cell lines. Additionally, we are using DNase I footprint analysis to examine protein binding sites in the CRH 5' flanking DNA sequences using nuclear extracts from a number of cell lines. The data obtained from these gene transfer and biochemical studies will hopefully allow us to better understand the molecular mechanisms involved in glucocorticoid regulation of CRH gene expression. This work was supported by grants to A.F.S. from the University of Michigan Horace H. Rackham School of Graduate Studies, Medical School Biomedical Research Support Grant (NIH RR05383), and Michigan Memorial-Phoenix Project.

E 140 DNA-BINDING AND THE MOLECULAR FORM OF THE ESTROGEN AND PROGESTERONE RECEPTORS ARE INTERRELATED, D.F. Skafar[1] and J.P. Bond[2], [1]Dept. of Physiology, Wayne State Univ., Detroit, MI 48201 and [2]Dept. of Chemistry, Cornell Univ., Ithaca, NY 14853.
The estrogen (ER) and progesterone (PR) receptors have homologous DNA-binding domains. Surprisingly, the ER had a 15-fold higher affinity for calf thymus DNA than did the PR (180 mM KCl, >6 nM receptor, pH 7.4) as measured by isocratic elution. Also, hormone-binding had no effect on the affinity of the PR for DNA or the number of ion pairs between the PR and DNA, whereas hormone-binding doubled the affinity of the ER for DNA and increased the number of ion pairs between the ER and DNA from ≈8 to ≈13. However, the PR and the monomeric ER each made 2-3 ion pairs with DNA. These results suggested the ER was a dimer and the PR a monomer at the same receptor concentration. The dimerization constant of the PR was measured using the decrease in the Hill coefficient of progesterone binding from 1.2 to 1 as the PR concentration was decreased. The value obtained, 7 nM, was over 10-fold greater than that reported for the ER, 0.3 nM. This indicates the major form of the ER and PR could differ in vitro and possibly in vivo. Interestingly, the presence of DNA favored the monomeric form of the ER and PR. Analysis of equilibrium binding models indicated that the effect of DNA on the molecular form of a protein depended on the quarternary structure of the DNA-binding site. If the DNA sequences bound by each subunit must be next to each other, increasing the DNA concentration will eventually dissociate the protein to monomers. If the DNA sequences can be distant or on different fragments, increasing the DNA concentration may favor the dimer. This suggests that the molecular form of the PR and ER and their DNA-binding properties are highly interrelated. Supported by NSF grant DCB 8716044.

E 141 RETINOID REGULATED EXPRESSION OF THE TISSUE TRANSGLUTAMINASE GENE, Joseph P. Stein*, Janice M. Andrews*, Margaret Saydak', James Basilion', and P. J. A. Davies', *Department of Pharmacology, SUNY Health Science Center, Syracuse, NY, and 'Department of Pharmacology, Univ. of Texas Medical School, Houston, TX

Retinoids are a class of polyisoprenoid lipid molecules that are structurally and functionally related to vitamin A. Retinoic acid, in particular, has been shown to exert a profound effect on the process of embryonic morphogenesis and on the growth and differentiation of both normal and transformed cells. Despite their physiological importance, progress in understanding the mechanisms of action of retinoids has been hampered by a lack of indices of the direct effects of retinoic acid on gene expression. We have shown that the transcription rate of the tissue transglutaminase (TGase) gene is increased by retinoic acid in myeloid cells, and so use this system as a model for retinoid regulation of gene expression. TGase cDNA clones have been isolated from various mouse, human, and chick tissues. Sequence analysis and nuclease protection experiments indicate that two TGase mRNAs are transcribed, most likely from alternative promoters of a single gene. The 5' portion of the mouse TGase gene has been cloned from an adult Balb/c mouse library, and one putative promoter region that contains several half-response elements has been identified. Experiments to identify a functional retinoid response element are underway. In addition, we are investigating the relationship between the expression of various retinoic acid receptors (RARs) and the induction of TGase expression. Preliminary experiments with stably transfected 3T3 cell lines that express different RARs indicate that probably all RARs are capable of driving expression of the TGase gene. Our studies in these two areas should lead to an understanding of the molecular mechanism by which retinoic acid regulates specific gene expression.

E 142 REGULATION OF THE EXPRESSION OF THE RETINOIC ACID RECEPTOR GENES
Henry M. Sucov and Ronald M. Evans, Gene Expression Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037

The vitamin A derivative retinoic acid (RA) exerts its effects via a family of high affinity nuclear receptors. As an approach towards understanding the developmental and physiological utilization of retinoic acid, we have initiated an analysis of the regulation of the RA receptor genes. Promoters for the three RAR genes, and the two related RXR genes, have been isolated and fused to CAT vectors. The expression of these reporter constructs have been studied in transfection assays as a consequence of chemical or environmental perturbation, and by cotransfection with expression plasmids encoding known transcriptional activators.

E 143 FUNCTIONAL INTERACTION BETWEEN REGULATORY ELEMENTS OF THE RAT PRO-OPIOMELANOCORTIN GENE. Marc Therrien and Jacques Drouin. Laboratoire de génétique moléculaire, Institut de recherches cliniques de Montréal, Montréal (Québec) CANADA, H2W 1R7
The pro-opiomelanocortin (POMC) gene encodes the precursor to ACTH, the endorphins and the melanotropins. This gene is mainly expressed in corticotroph and melanotroph cells of the anterior and intermediate lobe of the pituitary, respectively. We previously localized the rPOMC promoter to a 543 bp 5'-flanking DNA fragment of the gene using transfection and transgenic mice experiments. Functional analyses of internal and 5' deletion mutants in POMC-expressing cells have revealed three domains within the rPOMC promoter: proximal, central and distal. Whereas the proximal domain functions independently, the distal and central domains are active only when they are together. To identify regulatory elements within those functional domains, we have used *in vitro* binding experiments, fine deletion mapping and linker scanning mutagenesis. At least nine different regulatory elements appear to contribute to promoter activity. Three of these elements, which bound distinct nuclear proteins in gel retardation assays, are duplicated in different promoter domains. The other three elements bind different nuclear proteins and are unrelated to each other. *In vitro* binding assays showed that COUP-TF and AP-1 bind two of these elements, while the other elements show limited homology, if any, to known regulatory elements. Mutagenesis experiments indicated that each of these elements contributes equally to promoter activity, suggesting that their combinatorial interaction within the rPOMC promoter is necessary for full transcriptional activity. We are currently investigating the role of these regulatory elements in the pituitary-specific rPOMC gene expression.

E 144 ALTERATIONS IN FACTORS BINDING TO THE PERIPHERIN PROMOTER DURING NGF-INDUCED NEURONAL DIFFERENTIATION, Mary Ann Thompson, Deirdre Lawe, and Edward Ziff, Department of Biochemistry, New York University Medical Center, New York City, NY 10016.

We are studying the transcriptional regulation of the peripherin gene, which is induced when nerve growth factor (NGF) stimulates PC12 cells to differentiate into neurons. The peripherin gene, encoding a neuronal-specific type III intermediate filament protein, is induced at the time during which the cells begin to develop neuronal characteristics (12 hr to 2 days). Deletion mapping of the peripherin promoter coupled to a CAT reporter gene indicates that there are two positive regulatory elements necessary for full induction by NGF: a distal element greater than 2290 bp upstream from the transcriptional start site, and a proximal element within 110 bp of the start site. In addition, there is a negative regulatory element (situated between nt -179 and -111), which may function to keep basal peripherin expression at a low level. DNA mobility shift assays performed using a ^{32}P-labelled DNA probe containing the negative regulatory element revealed 2 retarded bands representing DNA binding activity which is present in nuclear extracts from uninduced PC12 cells but not in extracts from PC12 cells treated for 1 week with NGF. This data is consistent with the hypothesis that positive factors induced by NGF may derepress the promoter in part by hindering the binding of a repressor to the negative element.

Inspection of the peripherin promoter sequence revealed 2 "E box" sequences (CANNTG) separated by 4 bp at -468 to -453. Since this motif is recognized by transcription factors of the B-HLH family (which have been implicated in differentiation of other cell types), binding of proteins in undifferentiated and differentiated PC12 extracts to this sequence was assessed by DNA mobility shift assay. Synthetic oligonucleotides with mutations in one or the other E box were used to demonstrate that protein binding to one E box is present only in undifferentiated extracts, whereas protein binding to the other E box predominates in extracts from differentiated PC12 cells. The function of this sequence in the context of the full-length promoter, as assessed by mutagenesis, will be reported.

E 145 REGULATION OF HIV-1 GENE EXPRESSION BY CELLULAR PROLIFERATION INDUCED BY PLATELET DERIVED GROWTH FACTOR, Sandra Tong, Dale Yuzuki, and B. Matija Peterlin, Howard Hughes Medical Institute and SF VAMC, University of California, San Francisco, CA 94143

Transcriptional mechanisms that control cellular genes newly expressed in activated T cells also activate the HIV1 regulatory region, or long terminal repeat (LTR). T cell activation leads to release of interleukin (IL)-2 and surface expression of its receptor, which then allows proliferation of T cells through autocrine stimulation. Because the signals generated by perturbation of the IL-2 receptor (IL-2R) are not clearly defined, we have examined the response of the HIV1-LTR to cellular proliferation induced by the platelet-derived growth factor (PDGF) and its receptor. A plasmid construction containing the HIV1 LTR upstream from a reporter gene, the chloramphenicol acetyl transferase (CAT) gene was used in transient transfections in a mouse embryo fibroblast cell line, Swiss3T3, which serves as a model for PDGF-responsive cells. The HIV1 LTR responds 6-fold to stimulation with serum, PDGF, or phorbol myristate acetate (PMA). This stimulation was observed in the absence or presence of the HIV1 regulatory protein, tat-1, which amplifies HIV-1 LTR directed gene expression several hundred fold in human cell lines. As an indicator of stimulation by serum, PMA, and/or PDGF, a plasmid construction with the fos enhancer and promoter, which contain responsive elements to each of these stimuli, upstream from the CAT reporter gene was transfected and responded 5-fold to each of the stimuli. Thus, the HIV1 LTR responds to cellular proliferation as induced by a specific growth factor, PDGF, which may serve as a model for cellular proliferation events in activated T cells.

E 146 POSITIVE AND NEGATIVE REGULATORY SITES IN HUMAN UROKINASE PROMOTER, Pasquale Verde, Claus Nerlov, Anna Caracciolo, Fabrizia Pergola and Francesco Blasi, International Institute of Genetics and Biophysics, Via G. Marconi 10, 80125, Naples, Italy, and Institute of Microbiology, University of Copenhagen, Oster Farimagsgade 2A, Copenhagen K, Denmark.

We have characterized regulatory sequences and DNA-protein binding sites in the human urokinase promoter. A putative AP-2 binding site, close to the TATA box, is required both for cAMP-mediated induction by FSH (Follicle Stimulating Hormone) in mouse Sertoli cells, and for TPA induction in HeLa cells. In HepG2 cells, not containing AP-2 factor, response to TPA is conferred by a distal element (around -2000), essential for enhancer basal activity. By in vitro binding studies and transfection of point-mutated constructs, we have shown that this functional TRE (TPA Responsive Element) results from cooperative interaction between an AP-1 binding site and an adjacent site for PEA3, recently identified as the product of c-ets proto-oncogenes. The 90 bp region on the 3' of the PEA3/AP-1 element contains multiple protein binding sites, required for basal enhancer activity; we have localized in the same region target sequences for glucocorticoid negative regulation. We are currently testing the hypothesis of interference between glucocorticoid receptor and positive regulatory sites.

E 147 MOLECULAR BIOLOGY AND ANDROGEN REGULATION OF HUMAN PROSTATIC PROTEINS: PAP AND PSA, Pirjo Virkkunen, Pirkko Henttu and Pirkko Vihko, Biocenter and Departement of Clinical Chemistry, University of Oulu, Kajaanintie 50, SF-90220 Oulu, Finland.

We have cloned and sequenced the cDNAs coding for two androgen regulated human prostatic proteins, prostate specific antigen (PSA) (Henttu and Vihko 1989, Biochem. Biophys. Res. Comm. 160:903-910) and prostatic acid phosphatase (PAP) (Vihko et al. 1988, FEBS Lett. 236:275-281). The regulation of PAP and PSA biosynthesis by steroid hormones was studied in LNCaP cell line derived from a lymph node metastasis of prostatic carcinoma. Administration of R1881, a synthetic androgen, in charcoal dextran stripped fetal calf serum stimulated the growth of these cells and enhanced the secretion of PSA protein and the steady state level of PSAmRNA in a dose dependant manner, while PAP secretion as well as PAPmRNA level were diminished. Estradiol and progesterone were able to elicit similar effects as R1881. Cycloheximide treatment of cells for 7 hours increased PAPmRNA levels 3 fold and PSAmRNA levels 2 fold over the levels measured for control and androgen treated cells. By 24 hours the cycloheximide induction had changed to repression of both mRNA levels, suggesting that ongoing protein synthesis is required for the regulation of the mRNA levels. In order to elucidate the regulation of PAP biosynthesis at the DNA level, we are clarifying the structure of the PAP gene with the aid of our cDNA clones.

E 148 Jeffrey W. Voss+, Laura Wilson* and Michael G. Rosenfeld+. Howard Hughes Medical Institute +Eukaryotic Regulatory Biology Program and Center for Molecular Genetics School of MedicineUniversity of California, San Diego 9500 Gilman Drive La Jolla, California 92093-0648.** Two members of the POU-domain family of transcriptional activators, Pit-1 and Oct-1, are co-expressed in cells of the anterior pituitary gland. The pituitary-specific developmental regulator, Pit-1, and the widely expressed Oct-1 protein can bind to elements of the rat growth hormone and prolactin genes that confer tissue specific gene regulation. We demonstrate that Pit-1 and Oct-1 can each associate independently, or together as a heteromeric complex to these elements. When expressed in a heterologous cell line both Pit-1 and Oct-1 can induce the expression of reporter genes linked to the prolactin gene regulatory sequence. However, the effect of these proteins on the expression of the growth hormone gene differs remarkably. Pit-1, causes a modest increase in growth hormone gene expression whereas Oct-1 has little or no effect on the expression of the wild type growth hormone gene. Surprisingly, mutations in sites recognized by other trans-acting factors render the growth hormone gene responsive to Oct-1 but have no detectable effect on the response of the growth hormone gene to Pit-1. These observations may be pertinent to an understanding of the mechanisms that underlie the restriction of hormone expression to specific pituitary cell lineages.

E149 AGP/EBP is necessary for maximal glucocorticoid induction of the rat alpha-1 acid glycoprotein gene in HTC-JZ cells.

Paul (Mickey) Williams, Thomas Ratajzcak, Diego DiLorenzo^, Sheng Chung Lee* and Gordon M. Ringold
Syntex Research, 3401 Hillview AV, Palo Alto, CA 94304
^Istituto di Chimica, Ospedale Civile di Brescia, Brescia, Italy
*Institute of Biological Chemistry Academia Sincia, Tapei,Taiwan

The rat alpha-1 acid glycoprotein gene encodes an acute phase reactant protein whose expression is induced by glucocorticoid hormones. Previous results mapped an 80 base pair region capable of conferring glucocorticoid induction to a heterologous promoter. We have examined this 80 base pair region of the AGP promoter using footprint and gel shift assays. The results show a factor can bind to two sites of shared homology. Western blot, northern blot and "super-shift" assays show AGP/EBP (a member of the closely related C/EBP family of proteins) is present in the rat hepatoma cell line, HTC-JZ and binds to both sites. We have introduced three base substitutions in each site which prevent binding of AGP/EBP. When these substitutions are placed in the AGP promoter the glucocorticoid induction is reduced to ~30% of the wild type promoter when transfected into HTC-JZ cells. This indicates that AGP/EBP is necessary for maximal glucocorticoid induction of the AGP gene in these hepatoma cells.

E 150 CHANGES IN THE LEVEL OF TRα ISOFORM SPECIFIC RNAs DURING NEONATAL BRAIN DEVELOPMENT. Ken N. Wills, Xiao-kun Zhang, and Magnus Pfahl. La Jolla Cancer Research Foundation, La Jolla, CA 92037

Thyroid hormone receptors (TRs) are nuclear proteins which can regulate gene expression through specific DNA sequences. Alternative splicing of the TRα and TRβ gene transcripts can create individual protein products with distinct functions. In the rat, at least three mRNA species can be generated by differential processing of the TRα transcript. Only one of the isoforms, TRα-1, is a transcriptional activator, while the regulatory roles of the carboxyterminal variants TRα-2 and TRα-2v remain unclear. It is well known that thyroid hormones have critical functions in the control of normal development. In this study, we have used polymerase chain reaction (PCR) amplification of total RNA to compare TRα-1, TRα-2, and TRα-2v mRNA levels in the brainstem, cerebellum, cerebrum, midbrain, and olfactory bulbs of developing neonatal brains in rats. The patterns of the TRα isoform messages suggest that control of individual isoform message levels may play an important role in the control of hormone dependent development. All three receptors are co-expressed during development, with TRα-2 generally maintaining the highest level of expression and TRα-1 the lowest. TRα-1 message is generally increased in hypothyroid tissues while TRα-2 is not. To elucidate the regulatory role of the "inactive" TRα isoforms TRα-2 and TRα-2v, we have analysed their function as gene activators and modulators of phorbol ester stimulated gene activity, a major novel function for "active" thyroid hormone receptors recently discovered by us.

E 151 RECONSTITUTION AND ANALYSIS OF HUMAN GLUCOCORTICOID RECEPTOR FUNCTION IN YEAST, Anthony P H. Wright and Jan-Åke Gustafsson, Centre for Biotechnology and Department of Medical Nutrition, Karolinska Institute, Huddinge University Hospital, NOVUM F60, S-141 86 Huddinge, Sweden

The glucocorticoid receptor is a member of a large family of structurally related receptors which mediate the transcriptional response to steroid and thyroid hormones, vitamins A and D and some as yet unknown ligands in mammalian cells. It has been shown that several of these receptors function as ligand dependent transactivators of gene expression in yeast cells when an appropriate reporter plasmid is provided. However, unlike some other receptors the GR shows an abnormal ligand specificity profile for transactivation. We have shown that a truncated GR, from which the N-terminal half has been removed, exhibits a specificity profile similar to that of the endogenous receptor. Further data evaluating the role of the N-terminus in the abnormal specificity profile of the GR in yeast will be presented. In mammalian cells the amount of transactivation from two adjacent glucocorticoid responsive elements (GREs) is about 10-fold that from a single element. We have shown that the same is true for the GR expressed in yeast. The role of different receptor domains in the synergistic response of two GREs will be presented.

E 152 REGULATION OF STEROID HYDROXYLASE GENE EXPRESSION: IN VITRO TRANSCRIPTION IN ADRENOCORTICAL CELL EXTRACTS. Ulrich M. Zanger, Johan Lund, Evan Simpson and Michael R. Waterman, Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, Texas 75235.

Regulation of steroid hormone biosynthesis in steroidogenic tissues involves the transcriptional activation of genes encoding steroid hydroxylase P450s and adrenodoxin by peptide hormones via cyclic AMP. Using chimeric gene constructs and transfection into Y1 adrenocortical tumor cells, we have previously identified in the 5'-regulatory regions of the bovine genes CYP17 and CYP22A sequence elements, which confer cAMP-responsiveness to the β-globin promoter. To investigate functionally the relationship between these cAMP responsive sequences (crs) and the well documented consensus cAMP response element CRE, we have now developed an *in vitro* transcription assay based on Y1 cell extracts. Analysis of serial 5'-deletion mutants demonstrated that the same crs identified by transfection also activate transcription of a reporter gene *in vitro*. Depletion of protein factors from Y1 cell extracts by CRE sequence specific affinity chromatography specifically abolished *in vitro* transcriptional activation by CRE, but not by crs or the SV40-enhancer. In depleted Y1 extracts, CRE-dependent transcription could be restored with bacterially expressed CREB, which did however not significantly influence the transcription of crs- or SV40-enhancer-activated reporter genes. We conclude, that activation of transcription by cAMP responsive sequences found in these steroid hydroxylase genes is mediated by nuclear factors which are different from those with high affinity for consensus CRE-binding sites.

Signalling

E 200 HIGH-LEVEL EXPRESSION OF THE OVINE GROWTH HORMONE RECEPTOR IN TRANSFECTED CHO CELLS, Timothy E. Adams, Rodney J. Fiddes and Malcolm R. Brandon, Centre for Animal Biotechnology, School of Veterinary Science, The University of Melbourne, Parkville, Victoria 3052, Australia

The mechanism(s) by which signal transduction is mediated by the growth hormone (GH) receptor, and the subsequent activation of GH-responsive genes remains poorly understood. We have cloned and sequenced full-length cDNA clones encoding the ovine GH receptor. The ontogeny of receptor expression is developmentally regulated, with GH receptor mRNA transcripts being first detected in foetal liver just prior to parturition. Furthermore, tissue-specific expression of the receptor in hepatic and non-hepatic tissues results from alternative splicing within the 5' untranslated region of the mRNA, indicating utilisation of multiple promoters. Stably transfected Chinese hamster ovary (CHO) cell lines have been established that express the GH receptor cDNA under the regulatory control of a human metallothionein IIA promoter/SV40 enhancer. Scatchard analysis of one cloned line, R9.5, reveals high-level expression of cell-surface GH receptors (> 400,000 molecules/cell) that bind ligand with high-affinity (0.2nM). The ability of these cells to mediate a number of cellular responses typical of GH (i.e. induction of c-*fos* expression, stimulation of protein synthesis) is currently being examined.

E 201 THYROTROPIN-RELEASING HORMONE (TRH) ACTION ON THE PROLACTIN PROMOTER IS MEDIATED BY THE POU PROTEIN PIT-1, **Carter Bancroft[1], Guo-zai Yan[1], and Wayne T. Pan[1,2], Departments of Physiology and Biophysics[1] and Microbiology[2], Mount Sinai School of Medicine, New York, NY 10029**

Little is known about mechanisms involved in TRH regulation of prolactin (PRL) gene transcription. We have characterized TRH response elements (TRHRE's) in the promoter region of the rat PRL gene, and the gene-proximal protein that transmits the TRH signal to these elements. Exposure of GH_3 rat pituitary cells to TRH yielded a large, specific stimulation of transient expression of (-204)PRL-CAT. 5'-deletion analysis implied that positions -174/-113 and -75/+38 each contain a TRHRE. GH_3 cell extracts footprint four sites, termed 1P-4P, on the PRL promoter region. The more proximal TRHRE was identified as element 1P at -63/-39, since two copies of 1P transferred a TRH response to either (-39)PRL-CAT or (-39)mMT-CAT. The distal TRHRE may be element 3P, at -167/-144, since two copies of this element also transferred a TRH response to (-39)PRL-CAT. Pit-1 activates pituitary cell-specific PRL gene expression by binding to site 1P. For wild-type and mutant 1P oligos, a strong correlation was observed between pit-1 binding and ability to transfer a TRH response, directly implicating pit-1 as the gene-proximal protein that transduces the TRH signal to site 1P. Pit-1 also bound avidly to element 3P (and more weakly to elements 2P and 4P), suggesting that pit-1 may also transduce a TRH signal to element 3P. These results show that the cell-specific activator pit-1 is also a mediator of peptide hormone regulation of the prolactin gene.

E 202 FUNCTIONAL DOMAIN EXCHANGE BETWEEN HUMAN β2 ADRENERGIC RECEPTOR AND YEAST ALPHA FACTOR RECEPTOR, Anne Bell, Jennifer Reasoner, Paul Sheppard and Andrzej Sledziewski, ZymoGenetics, Inc., 4225 Roosevelt Way NE, Seattle, WA 98105

Both yeast and mammalian cells have surface receptors coupled to G-protein signal transduction pathways. These receptors are structurally similar, containing ligand binding domains, G-protein docking sites and regions involved in down regulation in a seven membrane spanning design. We are interested in functional dissection of these receptor elements.

Human β2 adrenergic receptor (β2-AR) and the mating pheromone receptor (STE2) from *Saccharomyces cerevisiae* are two such receptors which are functionally coupled to G-protein signal transduction pathways. These receptors share similar structural characteristics, although they respond to different ligands. Morphological and metabolic changes occur in yeast cells expressing STE2 when challenged with the yeast mating pheromone α factor. We have expressed β2-AR in yeast and, although it maintains its relative affinities for its various ligands at levels similar to β2-AR expressed in mammalian cells, we are unable to detect G-protein coupling or functional response to its ligands in yeast. WE have constructed a β2-AR/STE2 hybrid in which the domain believed to be the G-protein binding region of STE2 was inserted in place of the corresponding element of β2-AR. We are also attempting to modify the C-terminus of β2-AR which is believed to be involved in receptor down regulation. These hybrids will be expressed in our yeast system in order to study how they respond to challenges with yeast mating pheromone and catecholamines.

E 203 17ß-ESTRADIOL INHIBITS IL-6 PRODUCTION BY BONE MARROW STROMAL CELLS AND OSTEOBLASTS: A POTENTIAL MECHANISM FOR THE ANTIOSTEOPOROTIC EFFECTS OF ESTROGENS, G. B. Boder,* G. Girasole, H. G. Derigs, H. S. Boswell and S. C. Hanolagas, *Lilly Research Laboratories; Department of Medicine, Indiana University; The Walther Oncology Center.
Bone-active cytokines are produced by stromal and bone cells in the bone marrow microenvironment. Prompted by this and evidence for an important role of Interleukin-6 (IL-6) in osteoclast recruitment and bone resorption, the effects of 17ß-estradiol on IL-6 were examined in: a) murine bone marrow-derived stromal preadipocytes, b) normal human bone osteoblasts from two young females and one male, and c) two non-transformed osteoblast cell lines from rats. In all these cell types, IL-6 production was stimulated as much as 10,000-fold in response to synergistic effects of recombinant Interleukin-1 (IL-1) and tumor necrosis factor (TNFα). Addition of physiologic concentrations of 17ß-estradiol in the cultures exerted a potent and dose-dependent inhibition of the IL-1-, TNF- and IL-1+TNF-induced production of bioassayable IL-6; as well as a decrease of the steady-state levels of the IL-6 mRNA. This evidence suggests, for the first time, a potent mechanism by which estrogens might exert at least part of their antiresorptive influence on the skeleton.

D 204 POSSIBLE INVOLVEMENT OF NF-KB IN TAT ENHANCEMENT OF GENE EXPRESSION DIRECTED BY HETEROLOGOUS TRANSCRIPTIONAL REGULATORY ELEMENTS, Timothy A. Coleman, John Lucas, Joe Kenny, William Blue and John J. Kopchick, Edison Animal Biotech. Center, 201 Wilson Hall, Ohio University, Athens, OH 45701

The HIV-I trans-activator TAT is required for efficient viral gene expression and replication. TAT functions by interacting with viral RNA at a target sequence, TAR. Recently, it has been demonstrated that maximal TAT transactivation is observed only when TAR is present in conjunction with the HIV-LTR NF-KB and Sp1 DNA sequences. Previous studies have shown that TAT can augment gene expression driven by the human cytomegalovirus immediate early (hCMVIE) transcriptional regulatory element (TRE). In the present study, we set out to determine whether HIV-I TAT could activate other heterologous viral or cellular TREs and, if so, whether similar cis-acting DNA sequences are involved. Utilizing co-transfection studies with a TAT expression vector, or a stable cell line constitutively expressing TAT, we corroborate the ability of TAT to augment hCMVIE TRE directed gene expression. We further demonstrate that TAT can activate the RSV-LTR and SV-40 Early TREs. Deletion analysis of the SV-40E TRE maps the loss of TAT activation to a position that includes an NF-KB site. These results suggest a possible role for NF-KB mediated TAT activation of heterologous TREs.

E 205 TISSUE-DEPENDENT EXPRESSION OF ALTERNATIVE α_1-ADRENERGIC RECEPTOR GENE TRANSCRIPTS IN THE SPRAGUE DAWLEY RAT, Lawrence E. Cornett and Robert E. McGehee, Jr., Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, Little Rock, AR 72205.
Northern blots of total cellular and poly [A$^+$] RNA isolated from various rat tissues which were probed with a full-length cDNA encoding the hamster α_1-adrenergic receptor resulted in detection of two transcripts, 3.3 kb and 2.7 kb. The relative amounts of the two mRNAs varied considerably among the five tissues (liver, renal cortex, spleen, brain and heart) studied. However, the ratio of the two transcripts remained relatively constant in the same tissue taken from animals at different developmental ages. Currently, the physiological significance of multiple α_1-adrenergic receptor gene transcripts is unclear. However, both the 3.3 kb and 2.7 kb mRNAs were found to be associated with hepatic polysomes which suggests that these mRNA species are translated into protein. Finally, using non-overlaping 5′ and 3′ cDNA probes, large sequence differences were not evident between the 3.3 kb and 2.7 kb mRNAs, although the 3′-probe hybridized to a 4.0 kb mRNA in addition to the two smaller transcripts in poly [A$^+$] RNA isolated from renal cortex, but not other tissues. Our results suggest that α_1-adrenergic receptor gene expression in the rat is under complex regulatory control that in part is tissue-dependent. (Supported by NIH GM30669 and AHA, Arkansas Affiliate).

E 206 IDENTIFICATION OF G-PROTEINS PREFERRED BY α_2-ADRENERGIC RECEPTOR (α_2-AR) SUBTYPES, Isabelle Coupry, Emir Duzic, Stephen M. Lanier, Cell.and Mol. Research Lab, Cardiac Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114

α_2-AR subtypes are expressed in a tissue-specific manner and receptor activation can elicit a variety of cell responses utilizing pertussis toxin (PT) sensitive G-proteins(Giα1-3, Go). The preferred G-protein for each receptor subtype is not known. To address this issue we stably expressed rat genomic clones encoding α_2-AR subtypes (RG10, RG20) in cell phenotypes expressing different PT-sensitive G-proteins. The shape of agonist competition curves and level of agonist-stimulated GTPγS binding served as indices of R-G interaction.

In NIH-3T3 cells expressing RG20 (Bmax=2000 fmol/mg), agonist competition curves were biphasic (K_iH=10 nM, K_iL=1 uM) and Gpp(NH)p sensitive. In contrast, agonist competition curves in RG10 transfectants (Bmax= 1050 fmol/mg) were monophasic (K_i=700 nM). As Western analysis reveals that NIH-3T3 cells express Giα2>Giα3, but not Goα or Giα1 these data suggest that RG20 but not RG10 interacts with Giα2/Giα3. To identify the preferred G-protein for RG10, we cotransfected NIH-3T3 cells with both RG10 and Goα. In transfectants expressing RG10 (600-3000 fmol/mg) and Goα, agonist competition curves were shifted to the left by 100-fold (K_iH=3 nM, K_iL=220 nM)) as compared to cells transfected with RG10 alone. The high affinity state for agonist (~50% of total sites) was Gpp(NH)p and PT sensitive.

These data indicate that RG10 preferentially interacts with Go. As both RG10 and Goα are expressed primarily in brain and Goα modulates ion channel activity, these data suggest that RG10 is the α_2-AR subtype mediating (through Go) agonist activation/inhibition of ion channels in neuronal tissue.

E 207 NORMAL HUMAN KERATINOCYTES SYNTHESIZE NERVE GROWTH FACTORS (NGF) IN A GROWTH REGULATED MANNER AND INDUCE THE EXPRESSION OF NGF RECEPTORS IN MELANOCYTES: A NOVEL PARACRINE CIRCUIT. Michele De Luca*, Eddi Di Marco*, Pier Carlo Marchisio+, Sergio Bondanza*, Antonella Melchiori*, Adriano T. Franzi^, and Ranieri Cancedda*. *Ist.Naz.le Ric.sul Cancro, Genova, +Dip. Scienze Biom. e Oncol. Umana, Univ. di Torino, ^Ist. di Anat. Umana Norm., Univ. di Genova. ITALY

Normal human keratinocytes cultured in vitro reconstitute a stratified squamous epithelium resembling the in vivo epidermis. Keratinocytes specifically induce regulated melanocyte growth and are able to physiologically organize melanocytes in the basal layer of the in vitro reconstituted epidermis. In addition melanocytes maintain differentiated functions, have a star-shaped morphology with several long dendrites and transfer melanosomes into basal keratinocyte cytoplasm. The molecular basis of this mechanism is not known. We now report that i) normal human keratinocytes synthesize and secrete nerve growth factor (NGF), ii) NGF synthesis is limited to the basal layer of the epidermis, depends upon the growth phase of the keratinocyte colonies and is regulated by corticosteroids, iii) keratinocytes induce the expression of NGF receptors on the plasma membrane of melanocytes, iv) NGF induces melanocyte migration but has no clear effect on melanocyte growth, dendritic arborization and melanization. Since NGF synthesis is regulated by the keratinocyte proliferation level and induce melanocyte migration, we postulate that this novel paracrine regulatory mechanism might have a crucial role in repigmentation of the skin during wound healing.

E 208 **CONTROL OF THYROID FUNCTION, GROWTH AND DIFFERENTIATION.** Dumont, J.E., Roger, P.P., Reuse, S., Maenhaut, C., Raspé, E., Verjans, B., Van Sande, J., Lamy, F., Lecocq, R., Lefort, A., Erneux, C., Boeynaems, J.M., Pereira, A. Institute of Interdisciplinary Research, Free University of Brussels, School of Medicine, Campus Erasme, Route de Lennik 808, B-1070 Brussels, Belgium.

The human and dog thyroid cells are controlled by 4 major pathways : the TSH cyclic AMP cascade which activates function, proliferation and differentiation expression; the Ca^{++} phosphatidylinositol cascade which modulates function positively and negatively, which activates proliferation and represses differentiation expression; the growth factor tyrosine kinase pathway which has no effect on function, activates proliferation and inhibits differentiation expression; the insulin tyrosine kinase pathway which is required for growth and for some differentiation expression. The cross signalling of these pathways in the human and dog thyroid cell and the differences between the two species are described. For instance while TSH activates the two first cascades in man it activates only the first in dog. In human cells the TSH phosphatidylinositol cascade can be induced and repressed thus proving its independence of the cyclic AMP cascade. Both cascades are inhibited by iodide through an unknown intermediate XI. A putative XI, an iodoaldehyde has been purified and characterized from in vivo iodide treated thyroids. The cloning of TSH receptors of both species has allowed their expression in permanently transfected cell lines (CHO, NIH, $3T_3$). The respective actions of the receptors on the various cascades in these cell lines is described.

E 209 THE HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 TAT REGULATORY PROTEIN CONTAINS AN ARG-GLY-ASP (RGD) CELL ADHESION SITE, Catherine F. Farrell[1], David A. Brake[1], Brian D. Hellmig[2], Jeffrey M.Stadel[3] and Christine Debouck[1], Departments of Molecular Genetics[1], Protein Biochemistry[2] and Pharmacology[3], SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406.

Human immunodeficiency virus type 1 (HIV-1), a member of the retrovirus family, is the causative agent of AIDS and related disorders. HIV-1 encodes an 86-amino acid long regulatory protein termed tat which greatly increases viral gene expression and replication. Interestingly, tat contains the tripeptide Arg-Gly-Asp (RGD) sequence within its carboxyl-terminal portion and this sequence is highly conserved among HIV-1 isolates. RGD sequences are found in fibronectin, vitronectin, collagen,... and constitute extracellular matrix attachment sites used for integrin-mediated cell adherence. We have now obtained convincing evidence that this RGD sequence constitutes a cell attachment site for tat. Purified recombinant tat protein was shown to bind to various cell types including human T-lymphocytivc and monocytic cell lines which are replication-competent for HIV-1. The specificity of this cell attachment was further demonstrated using RGD-containing peptides, anti-tat monoclonal antibodies and mutant tat proteins which contain amino acid substitutions within the RGD sequence. We have also used ^{125}I-labelled tat protein to demonstrate specific binding of tat to cells. Further studies aimed at the molecular characterization and elucidation of the physiological relevance of a cell adhesion site on this regulatory protein are in progress.

E 210 PUTATIVE G PROTEIN-COUPLED RECEPTOR EXPRESSED IN ERYTHROID CELLS FROM A LARGE OPEN READING FRAME LOCATED 3' TO THE HUMAN BETA GLOBIN GENE CLUSTER Elise A. Feingold*, Bernard G. Forget[+] and Arthur W. Nienhuis*, *Clinical Hematology Branch, NHLBI, NIH, Bethesda, Maryland 20892 and [+]Dept. of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut 06511

A large open reading frame (ORF) located ~95 kb 3' to the human beta globin gene cluster encodes a 311 amino acid putative protein that is related to members of the family of G protein-coupled receptors. A computer search of known protein sequences revealed that this putative protein is most homologous to the rat 5HT1c serotonin receptor, sharing a 21% identity. Comparison of the ORF to a number of members of this family indicates the presence of many highly conserved residues throughout the entire length of the sequence. Hydropathy analysis indicates the presence of at least seven hydophobic regions that could represent seven transmembrane spanning domains, the hallmark of this family of receptors. Messenger RNA transcripts from this ORF have been detected by the polymerase chain reaction (PCR) in both K562 and HEL erythroleukemia cell lines and in K562 cells by RNAse protection. PCR analysis of RNAs from a number of different mouse tissues revealed that the homologous mouse ORF is expressed almost exclusively in bone marrow and fetal liver. DNA sequence analysis of the human ORF predicts the absence of introns in the coding portion of the mRNA. RNAse protection analysis of the 5' end of the ORF, however, suggests the presence of an intron in the 5' untranslated region. Therefore, it appears likely that this ORF encodes a novel G protein-coupled receptor that is expressed in erythroid cells.

E 211 THE HUMAN NEUROKININ A RECEPTOR 5' UNTRANSLATED SEQUENCE CONTAINS NEGATIVE REGULATORY ELEMENTS, Levi A. Garraway, Craig Gerard, and Norma P. Gerard, Departments of Biological Chemistry and Molecular Pharmacology, Medicine and Pediatrics, Harvard Medical School, Boston, MA 02115.

To investigate the transcriptional control of tachykinin receptor genes, we constructed chimeric plasmids containing 5' flanking sequences of the human neurokinin A receptor (NK-2) gene fused to the bacterial chloramphenicol acetyltransferase (CAT) gene. The NK-2 receptor 5' untranslated region (5'UTR) (-3000 to -1) caused >60% repression of CAT activity relative to control (promotorless) CAT activity when transfected into COS-7 cells; NK-2 receptor 5'UTR-mediated repression was also observed when transfected into DDT1-MF2 hamster smooth muscle cells. Addition of retinoic acid 8 hr prior to harvest caused partial reversal of the repression. When a CAT plasmid containing a truncated NK-2 receptor 5'UTR sequence (-550 to -1) was transfected into COS cells, the repression was reduced to 40% relative to control. Sequence analysis of the NK-2 receptor 5'UTR reveals four 8-bp motifs between -766 and -523 homologous to an 8-bp common sequence which exists in the multiple negative regulatory elements of the human calpain gene. Taken together, these data suggest that negative transcriptional regulation, possibly mediated by multiple consensus elements, may play an important role in NK-2 receptor gene expression.

E 212 GROWTH HORMONE REGULATION OF SEX SPECIFIC AND PHENOBARBITAL-INDUCIBLE CYTOCHROMES P-450 IN ADULT RAT HEPATOCYTES CULTURED ON THE BASEMENT MEMBRANE MATRIX MATRIGEL.Guzelian, P.S., Li, D., Schuetz, E.G., Division of Clinical Toxicology and Environmental Medicine, Departments of Internal Medicine and Pathology, Medical College of Virginia, Richmond, Virginia
Studies of hypophysectomized rats suggest that growth hormone alters the expression of liver-specific genes such as the sexually dimorphic pair of cytochrome P-450 isozymes, male-specific P-450h and female-specific P-450i, and the xenobiotic inducible P-450p and P-450b/e. We tested the effects of growth hormone in a system for primary monolayer culture of adult rat hepatocytes on a laminin-rich extracellular matrix (matrigel), which permits sustained expression of both constitutive and inducible liver genes in a chemically defined medium. Cultures of freshly isolated hepatocytes prepared from untreated male rats contained readily detectable quantities of immunochemically assayed P-450h protein and hybridizable P-450h mRNA but contained neither P-450i protein nor P-450i mRNA. Additions of physiologic concentrations of growth hormone, but not of prolactin, promptly induced P-450i protein and P-450i mRNA in male hepatocyte cultures required as little as 24 hr of exposure, and use of matrigel rather than type I collagen Growth hormone treatment also induced the level of mRNA for insulin-like growth factor I, whereas the amount of mRNA for the male-specific urinary protein a2m-globulin was unaffected. Treatments with estrogens, androgens, glucocorticoids, or growth hormone induced P-450h mRNA or P-450h immunoreactive protein in cultures of female hepatocytes. When cultures were treated with phenobarbital, there was rise in mRNAs for the cytochromes P-450b/e and P-450p and activated transcription of P-450b/e and P-450p genes in nuclei isolated from the cultures. These effects were abolished completely by co-incubation with growth hormone but not with insulin-like growth factor I. We conclude that the somatogenic effects of growth hormone acting alone and directly on the hepatocyte in culture are sufficient to "feminize" the cytochrome P-450 phenotype and to selectively repress xenobiotic induction of some liver drug metabolizing enzymes..

E 213 NOVEL COUPLING OF ANGIOTENSIN II AND BETA-ADRENERGIC RECEPTORS TO ADENYLATE CYCLASE IN RAT FETAL FIBROBLASTS. M. Cecilia Johnson and Greti Aguilera,
Section on Endocrine Physiology, NICHD, NIH, Bethesda, MD 20892
We have recently demonstrated the presence of unique angiotensin II (AII) receptors in skeletal muscle and connective tissue in late gestation mammalian fetuses. In order to investigate the role of these receptors on cellular function, we analyzed the AII receptor coupling to adenylate cyclase and their interaction with beta-adrenergic receptors and bacterial toxins in cultured rat fetal skin fibroblasts. In the presence of phosphodiesterase inhibitors, AII increased cAMP accumulation, with a threshold concentration of 1 nM and maximum stimulation of 60% at 100 nM AII (n=34). The beta-adrenergic agonist, isoproterenol (ISO), increased cAMP production to a maximum of 27-fold with 1 uM ISO, and this effect was potentiated by 50% with 100 nM AII (n=35). The synergism between AII and ISO was not prevented by 18 hr preincubation of the cells with 1 ug/ml pertussis toxin (PTx), indicating that the potentiation was not due to interaction with Gi (n=4). In addition, PTx pretreatment resulted in 30% inhibition of cAMP production stimulated by ISO alone (n=5), indicating that in contrast with traditional systems, the beta-adrenergic receptor coupling to adenylate cyclase in fetal fibroblasts is not under tonic inhibition by Gi. On the other hand, direct activation of Gs by 3 hr preincubation with cholera toxin, increased cAMP accumulation 80-fold, an effect also potentiated by AII (n=3), suggesting that AII may have a facilitatory effect on the coupling of Gs to the catalytic subunit of adenylate cyclase. This novel interaction between the fetal AII and beta-adrenergic receptors with adenylate cyclase is probably due to developmental changes in expression of the components of the system, and may reflect dynamic changes in function of these regulatory hormones during ontogeny.

E 214 The level of mRNA for cellular retinol binding protein in Sertoli cells is regulated by serum factors, TPA and cAMP. Kroepelien,C.F., Hansson,V. and Eskild, W. Institute of Medical Biochemistry, University of Oslo, P.O. Box 1112 Blindern, 0317 Oslo 3, Norway.

The mRNA level for cellular retinol binding protein (CRBP) in primary cultures of Sertoli cells is subject to positive regulation by foetal calf serum (FCS) and the phorbol ester TPA. Negative regulation of CRBP mRNA levels is exerted by cAMP. Foetal calf serum (10%) induces CRBP mRNA levels significantly already after 3 hrs and a maximum level (10 - 56 fold) is seen after 12 hrs. The effect is reversible. Maximum levels of CRBP mRNA are maintained for 48 hrs in the presence of FCS. The serum induction required an active protein synthesis. When the cells were incubated with TPA a maximum induction (6 fold) was reached after 6 hrs in the presence of 1µM TPA followed by a decline. High levels of cAMP in Sertoli cells have been shown to decrease the level of CRBP mRNA. When the cells were incubated in the presence of the cAMP analogue dibutyryl cAMP and 10% FCS, we observed a time and concentration dependant decrease in the effect of FCS on the level of CRBP mRNA. We are presently trying to identify the active substances in serum which are responsible for the induction of CRBP mRNA. In addition we are investigating the 5′ region of the CRBP gene, in order to identify cis - elements and transcription factors active in the regulation of CRBP mRNA.

E 215 A NOVEL MEMBER OF THE G PROTEIN-COUPLED RECEPTOR FAMILY IS EXPRESSED IN BURKITT'S LYMPHOMA BUT NOT IN EPSTEIN BARR VIRUS IMMORTALIZED B CELLS, Martin Lipp, Ingrid Wolf, Thomas Emrich and Thomas Dobner, Institut für Biochemie, Ludwig-Maximilians-Universität, Karlstrasse 23, D-8000 Munich 2, FRG.

Burkitt's lymphoma (BL) are characterized by constitutive expression of the proto-oncogene *MYC* caused by specific reciprocal chromosomal translocations involving the *MYC* locus and one of the immunoglobulin loci. However, deregulation of *MYC* alone is insufficient to establish the malignant phenotype of BL. We started to identify additional genes which are switched on or off and thus might affect cellular differentiation, growth control or transformation. The tumorigenic Burkitt cell line BL64 carrying a variant t(2;8) translocation has been compared to the non-tumorigenic lymphoblastoid cell line IARC549 derived from the same patient, and to the T-cell line L735 by subtractive hybridization. Differential sreening of two subtracted cDNA libraries yielded several cDNA clones which were found to occur more abundant or specifically in BL64 cells in comparison to IARC549, L735, or non-B-cells. A clone which detected a transcript of 4.0 kb in RNA from BL cells, but not from LCL cells and any other cell line tested was used to isolate full length cDNA. The DNA sequence revealed an open reading frame of 372 aa including seven hydrophobic regions. Although a sequence comparison to known proteins did not show significant homologies some conserved residues within putative transmembrane domains indicate that this protein might represent a novel member of the G protein-coupled neurotransmitter and hormone receptor family. Our finding suggests that this receptor might participate in a regulating interaction between the neuroendocrine or endocrine systems and the immune system.

E 216 ROLE OF LARGE ANIONS (FATTY ACIDS), CYTOKINES AND NEURO-ENTERO PEPTIDES IN IMMUNE CELL GROWTH, W.S. Lynn, D. Mathews, and J.C. Wallwork, Department of Preventive Medicine and Community Health, UTMB, Galveston, TX 77550.

Anions which chronically elevate intracellular Ca^{2+}, activate the apoptotic endonuclease, and kill murine or human T cells include palmitic acid, linoleic acid and oleic acid. Anions which inhibit the effects of the above anions include μ3 linolenic acid, μ3 eicosapentanoic acid, ascorbic acid, nicotinic acid, indate, chromate, gallinate, borate, aurintricarboxylic acid, arginine and glutamate.

Peptide anions, which also activate the above Ca^{2+}-dependent apoptotic process in T cells, include IL-1, TGFβ, TNFα, somatostatin, and interferon γ. Peptides which block the above toxic peptides include IL-2, growth hormone, prolactin, LH, endorphin, releasing factors for LH and TSH, bombesin, CCK, and peptide Y. These latter peptides also block the cytotoxic effects of the above toxic fatty acids. Anions which have been shown to block the toxic effects of IL-1 and TNFα on T cells include ascorbic acid, μ3 linolenic acid, borate, chromate and arginine.

These observations suggest that the beneficial effects on age-related symptoms (glycosuria, proteinuria, hypertension, autoimmune inflammation, neoplasia and cell death) observed *in vivo* with restriction of excess calories (palmitic acid) or by replacement of the dietary sources of palmitic acid with the μ3 unsaturated fatty acids result from better control, i.e., inhibition of apoptosis in the immune system.

E 217 INHIBITION OF CASEIN KINASE II ACTIVITY IN 3T3 PREADIPOCYTES BY ANTISENSE cDNA. Herman Meisner and Michael P. Czech, Dept. of Biochemistry and Molecular Biology, Univ. Massachusetts Medical School, Worcester, Ma.01605

Casein kinase II is a protein serine/threonine kinase that is activated by different external stimuli, including insulin, EGF, and phorbal esters, and therefore qualifies as a signal transducer. The enzyme, which is an $\alpha_2\beta_2$ heterotetramer, is found in the cytosol and nucleus. Many nuclear proteins including several proto-oncogenes (myb, myc) are phosphorylated in vitro and in vivo at acidic amino acid residues by this kinase. Whether phosphorylation causes an activation of these substrates is largely unknown. The purpose of the present study is to reduce casein kinase II activity by antisense cDNA, in order to determine whether phosphorylation of putative substrates is modified. A mouse brain cDNA library in λGT10 was screened with a human β subunit cDNA, and a 970 nt. cDNA encoding the mouse β subunit was cloned and sequenced. The deduced amino acid sequence is identical to the rat and human β subunit sequence, and 94% similar at the nucleotide level. Antisense cDNA was made from 200 bp. restriction fragments from the 5' and 3' regions, including coding and non-coding portions, and transfected into 3T3L1 preadipocytes by the calcium phosphate method. Casein kinase II activity in several clones representing cells transfected with 3' antisense cDNA, but not 5' antisense cDNA, was reduced 30-40% compared to sense cDNA controls. RNAse protection verified that antisense β subunit mRNA was present in these cells, at a concentration similar to endogenous β subunit mRNA. These cell lines should be valuable model systems for examining the role of casein kinase II in signal transduction systems.

E 218 CLONING AND NUCLEOTIDE SEQUENCING OF THE MURINE ß3-ADRENERGIC RECEPTOR GENE, Clara Nahmias, Jean-Marc Elalouf, A. Donny Strosberg and Laurent J. Emorine, CNRS at ICGM, 22 rue Mechain 75014 Paris, France.

Recently, a new human ß-adrenergic receptor gene, referred to as "ß3", has been isolated (Emorine et al. Science 245, 1118-1121, 1989). The corresponding polypeptide is 50.7% and 45.5% homologous to the human ß1AR and ß2AR, respectively, and displays all the hallmarks of a G protein-coupled receptor. Analyses of the ß3AR expressed in eukaryotic cells reveal ligand binding properties, and a pattern of agonist-induced activation of adenylyl cyclase, similar to those of "atypical" ßARs which in rodents mediate the effects of catecholamines in various metabolic processes. However, several pharmacological differences exist between the human ß3AR and the atypical ßAR of rodents, and may be attributed either to species differences or to the existence of a family of highly homologous ß3ARs.

To further address this question, we undertook the cloning and nucleotide sequencing of the murine equivalent of the human ß3-adrenergic receptor gene. The murine gene encodes a polypeptide of 388 residues that displays 82% amino acid homology with the human ß3AR, and even higher homology in regions involved in ligand binding and signal transduction. Southern blot analyses using as probes several regions of the gene reveal in each case a single hybridizing fragment in the mouse genome, therefore indicating that the murine ß3 gene is unique. The pharmacology of the murine ß3AR expressed in eukaryotic cells is under investigation.

E 219 **TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL REGULATION OF lck mRNA DURING T CELL ACTIVATION. EFFECTS OF CYCLOHEXIMIDE, A PROTEIN SYNTHESIS INHIBITOR.**
F. Paillard, and C.Vaquero.U152 INSERM, ICGM, Hopital Cochin. 22, rue Mechain, 75014 Paris, France.

Full activation of T lymphocytes mediated via the TcR/CD3 complex with anti-CD3+PMA transiently down-modulated lck mRNA coding for the p56lck tyrosine kinase, whereas full activation mediated via the CD2 molecule did not.
In order to study the mechanisms of regulation responsible for this down-modulation we measured the transcriptional level as well as the half-life of lck mRNA during anti-CD3+PMA activation. Transcription of the lck gene strongly decreased at 3 h post-anti-CD3+PMA activation. In addition, lck mRNA half-life was transiently decreased with minimal level observed 1 h post-activation.Thus, lck mRNA down-modulation is due to the decrease of both transcription and mRNA stability.

We analysed the effects of cycloheximide (CHX) on lck mRNA constitutive expression and on anti-CD3+PMA-mediated lck mRNA down-modulation.
First, CHX alone superinduced lck mRNA. This superinduction was entirely due to a strong stabilization of lck mRNA since CHX strongly increased lck mRNA half-life, whereas CHX decreased its transcription. This suggests that a labile destabilizator and a labile transcriptional activator are involved in the constitutive expression level of lck mRNA.
Second, CHX inhibited the down-modulation of lck mRNA mediated by anti-CD3+PMA. This inhibition was entirely due to the stabilization of lck mRNA since CHX reincreased the stability of the mRNA that had been decreased after anti-CD3+PMA activation whereas surprisingly, CHX decreased the transcriptional level of lck gene.

Taken together the results obtained when CHX was either used alone or in presence of anti-CD3+PMA activation allowed us to approach (1) the nature of the positive and negative regulatory factors involved in the constitutive expression of lck mRNA and (2) the effects of anti-CD3+PMA activation signal on the fate of these factors.

E 220 **PCR-BASED CLONING STRATEGY FOR G PROTEIN-COUPLED RECEPTORS,** M. Parmentier, F. Libert, A. Lefort, J. Perret, C. Gerard, C. Maenhaut, J. Van Sande, C. Mollereau, D. Eggerickx, J.E. Dumont and G. Vassart. I.R.I.B.H.N., ULB Campus Erasme, Free University of Brussels, Brussels, Belgium.

A strategy for homology cloning exploiting the polymerase chain reaction has been devised to clone new members of the family of genes encoding G protein-coupled receptors. It involves the use of degenerate primers corresponding to sequences of transmembrane segments of known receptors belonging to this gene family. Clones encoding three known receptors and four new putative receptors were thus obtained from thyroid cDNA (1). The same strategy was used with different sets of primers and with human genomic DNA or cDNA libraries as starting material. Fourteen new putative receptor clones were thus obtained. Among these, one (now known as the FSH receptor) had sequence characteristics different from all the others. Used as a probe in the screening of a thyroid cDNA library, it led to the cloning of the thyrotropin receptor cDNA (2). G protein coupling of the TSH receptor is presently under investigation in stable transfectants, while eight other putative receptors are tested with a panel of ligands in order to assign them a function.
(1) Libert et al. Science 244: 569 (1989), (2) Parmentier et al. Science 246: 1620 (1989).

E 221 SIGNAL TRANSDUCTION PATHWAYS IMPLICATED IN THE ACTION OF PARATHYROID HORMONE ON OSTEOBLASTIC CELLS, Nicola C. Partridge, Timothy C. Lorenz, Donald K. Scott, Cheryl O. Quinn, Pediatric Research Institute, St. Louis University School of Medicine, St. Louis Missouri 63110

Parathyroid hormone (PTH) treatment has been shown to result in generation of the second messengers cAMP, Ca^{++}, and diacylglycerol in osteoblastic cells. Agents which mimic these second messengers were tested for their effects on collagen synthesis, collagenase secretion and induction of collagenase gene transcription in the rat osteosarcoma cell line, UMR 106-01. The cAMP analogue, 8BrcAMP, demonstrated a dose-dependent inhibition of collagen synthesis with a maximum inhibiton at 10^{-4} M, more than the greatest inhibition achieved by PTH (10^{-7} M). Both 8BrcAMP (10^{-3} M) and PTH (10^{-8} M) produced an 8-10 fold enhancement of collagenase gene transcription. The calcium ionophore, ionomycin (10^{-7} M), produced an inhibition of collagen synthesis, to even greater levels than observed with PTH. However, ionomycin had no effect on collagenase secretion or gene transcription. The protein kinase C activator, phorbol myristate acetate, had no effect on collagen synthesis or collagenase transcription. We conclude that activation of protein kinase A is the major regulatory pathway for PTH action in UMR cells, although there may be contributions of the calcium-calmodulin dependent protein kinases in regulating expression of certain genes.

E 222 PROTEOLYTIC ACTIVITY OF THE HORMONE-BINDING SUBUNIT OF THE ESTROGEN RECEPTOR Giovanni A. Puca, Nicola Medici, Ciro Abbondanza, Vincenzo Nigro, Saverio Minucci, Ignazio Armetta, Bruno Moncharmont and Anna M. Molinari
Istituto di Patologia generale ed Oncologia, I Facoltà di Medicina e Chirurgia, S. Andrea delle Dame 2, I-80138 Napoli, Italy

The estradiol receptor belongs to a super-family of nuclear receptors. It acts as ligand-inducible transcriptional activator by interacting with specific enhancer DNA sequences. Binding to DNA does not depend on estradiol interaction, and is not affected by antiestrogens; however, estradiol, not its antagonists, is required for the transcriptional activation function associated with the hormone binding domain of the estrogen receptor. We found a serine active site in the purified hormone binding subunit of estrogen receptor located in its hormone-binding domain. This site was covalently-labeled with radioactive diisopropylfluorophosphate and was able to hydrolyze synthetic peptide substrates containing phenylalanine at the carboxyl-terminal. This indicated that the hydrolytic activity was chymotripsin-like. The estradiol binding and hydrolytic activity co-migrated, when the purified estrogen receptor underwent a preparative SDS-PAGE. Hampered by diisopropylfluorophosphate and aprotinin inhibitors, the enzyme activity is specifically and totally induced by estradiol. Others hormones and antiestrogens were ineffective. The estradiol-dependency of the hydrolytic activity leaves us to ponder the role of this latter in hormone-dependent activation of gene transcription.

E 223 EXPRESSION OF THE HUMAN apoE GENE ALTERS SIGNAL TRANSDUCTION IN MOUSE ADRENAL CELLS, Mary E. Reyland, Margaret M. Prack and David L. Williams, Department of Pharmacological Sciences, State University of New York at Stony Brook, Stony Brook, NY 11794

Adrenocortical cells produce corticosteroids from cholesterol; *in vivo* studies suggest an inverse relationship between steroidogenesis and expression of the lipid binding protein, apolipoprotein E (apoE). We have previously reported that expression of the human apoE gene in mouse Y1 adrenocortical cells results in up to a 100-fold suppression of basal and ACTH or cAMP stimulated steroidogenesis. To investigate how apoE suppresses steroidogenesis in Y1 cells, we have analyzed the expression of two cAMP regulated steroid hydroxylases, P450-side chain cleavage ($P450_{scc}$), the rate limiting enzyme in steroidogenesis, and p45011ß-hydroxylase ($P450_{11\beta\text{-OH}}$), required for the terminal step in corticosterone production. By northern blot analysis, basal expression of $P450_{scc}$ mRNA was reduced dramatically (> 10 fold) in the apoE expressing cell clones (Y1-E cells) as compared to the parent cell line (Y1-P), or a control cell line transfected only with the neomycin resistance gene (Y1-G418). In the presence of 1 mM dBcAMP, $p450_{scc}$ mRNA increased 2-3 fold in the Y1-P and Y1-G418 cells, but not in the Y1-E cell lines. The reduction of $P450_{11\beta\text{-OH}}$ mRNA was even more striking; no $P450_{11\beta\text{-OH}}$ mRNA was detectable in the Y1-E clones under basal conditions or following incubation with 1 mM dBcAMP. The decrease in expression of these cAMP regulated genes is not due to a deficiency in protein kinase A expression, since cAMP-dependent protein kinase activity *in vitro* was similar for the Y1-P, Y1-G418 and the Y1-E cell lines. In contrast, total cell protein kinase C (PKC) activity *in vitro* was increased eight fold in the Y1-E clones as compared to the Y1-P and Y1-G418 cell lines. The relationship between PKC and suppression of cAMP mediated events required for steroidogenesis is currently under investigation.

E 224 ESTRADIOL INDUCES CATHEPSIN D AND DOWN-REGULATES IGF-II/M6P RECEPTOR IN BREAST CANCER CELLS : A SATURATION MECHANISM TO EXPLAIN DEROUTING AND SECRETION OF LYSOSOMAL ENZYMES. H. Rochefort, F. Capony, F. Vignon and M. Mathieu, INSERM U148, and University of Montpellier, 60 rue de Navacelles, 34090 Montpellier France.

In human breast cancer cells, the lysosomal enzyme cathepsin D (Cath D) which shares a common receptor with IGF-II, is synthesized and secreted in excess. We have previously shown that the binding interactions between IGF-II and Cath D on IGF-II/M6P receptor is associated to inhibition of IGF-II mitogenic action in breast cancer cells (Mol. Endocrinol, in press, sept. 1990). We have now studied the regulation of this receptor by estradiol and its consequence in lysosomal enzyme derouting. In three estrogen receptor positive cell lines (MCF7, T47D, ZR75-1), estradiol dose-dependently and specifically decreased the steady state levels of IGF-II/M6P receptor mRNA, while it markedly stimulates the synthesis and secretion of Cath D. Western ligand blot assay with (^{125}I) Cath D confirmed a delayed decrease (max. 6 days) of receptor protein concentration.

We propose that estrogens facilitate saturation of IGF-II/M6P receptor system which results in derouting and hyper-secretion of pro-cathepsin D and other lysosomal pro-enzymes which are not regulated at the gene level.

E 225 CLONING AND CHARACTERIZATION OF A PHOSPHOTYROSINE PHOSPHATASE GENE EXPRESSED IN MOUSE EMBRYO. Michel L. Tremblay, Bedrich Mosinger, Ulrich Tillmann and Heiner Westphal. Laboratory of Mammalian Gene and Development, NICHD, National Institutes of Health. Bethesda, MD 20892.

Several tyrosine-specific protein kinases have been identified as playing important roles in mammalian development. Recently, the isolation of phosphotyrosine-specific phosphatases (PTPases) has provided new directions into the study of tyrosine phosphorylation during embryogenesis. Using oligonucleotide primers derived from the human T-cell PTPase (Cool et al, PNAS 86:5257-5261,1989), we have isolated by PCR, two human pseudogenes of this PTPase. We have screened a 11.5 day-old cDNA library using our two pseudogenes as probes and identified the mouse homologue of the human T-cell PTPase. A similarity greater than 90 % was found between the human and the mouse gene. We have used this clone in northern analysis and in situ hybridization to study its expression during mouse development. A mRNA of 1.3 kb was found in specific tissues. However, the highest expression was found in testis with the presence of a second message of 0.9 kb. These results and a further characterization of this enzyme will be presented.

E 226 THE 10.4K AND 14.5K PROTEINS CODED BY REGION E3 OF ADENOVIRUS: DOWN-REGULATION OF THE EGF RECEPTOR AND PROTECTION OF SOME TRANSFORMED MOUSE CELL LINES AGAINST LYSIS BY TUMOR NECROSIS FACTOR, W. Wold[1], A. Tollefson[1], R. Stewart[1], S. Yei[1], T. Ranheim[1], P. Krajcsi[1], L. Aquino[2], C. Carlin[1], and L. Gooding[2], [1]Institute for Molecular Virology, St. Louis Univ. Medical Center, St. Louis, MO 63110; [2]Dept. of Microbiology and Immunology, Emory Univ. School of Medicine, Atlanta, GA 30322

The E3-10.4K and E3-14.5K proteins are cytoplasmic membrane proteins. The 14.5K is O-glycosylated and phosphorylated on Ser. The 10.4K co-immunoprecipitates with 14.5K, suggesting that 10.4K and 14.5K exist in a complex *in vivo*. Also, 10.4K affects the posttranslational processing of 14.5K. Thus, 10.4K and 14.5K probably function in concert.

We have reported that 10.4K stimulates endosome-mediated internalization and degradation of the EGF receptor (EGF-R) in adenovirus (Ad)-infected cells. We now report that both 10.4K and 14.5K are required for this effect. Also, as judged by immunofluorescence using anti-phosphotyrosine antibody, both 10.4K and 14.5K appear to stimulate tyrosine-phosphorylation of proteins. If this phosphorylation is effected via activation of the protein tyrosine kinase activity of EGF-R, then Ad infection may mimic EGF signal transduction. More than 20 virus mutants have been constructed with in-frame deletions and Cys->Ser conversions in the 91 a.a. 10.4K protein. Analysis of mutants indicated that a.a. 1-60 and 83-91 are essential for down-regulation of EGF-R, but that a.a. 61-81 are nonessential.

We have also reported that the E3-14.7K protein protects nearly all mouse cell types examined against lysis by TNF. We now report that, in the absence of 14.7K, both 10.4K and 14.5K are required to protect C127 and several other mouse cell lines against TNF cytolysis. This was shown by infection of cells with 14.5K/14.7K or 10.4K/14.7K double mutants. Mouse fibroblasts such as C3HA or NIH3T3 are not protected by 10.4K/14.5K.

These results raise the possibility of a relationship between adenovirus effects on EGF-R and TNF protection in some cell types.

Gene Regulation II

E 300 **THE 5'-FLANKING REGION OF THE RABBIT SURFACTANT PROTEIN A (SP-A) GENE CONTAINS A NEGATIVE GLUCOCORTICOID REGULATORY ELEMENT (GRE)**, Joseph L. Alcorn, Robert D. Gerard, Margaret E. Smith and Carole R. Mendelson, Dept Biochem, UT Southwestern Med Ctr, Dallas, TX 75235

Surfactant protein A (SP-A), the major surfactant protein, is synthesized in pulmonary type II cells and is developmentally and hormonally regulated in fetal lung tissue. We have found that glucocorticoids and cAMP analogues stimulate SP-A gene transcription in fetal rabbit lung in organ culture; when fetal lung explants are treated with glucocorticoids and cAMP in combination, an additive effect is observed. To begin to analyze the genomic regions that mediate the cAMP and glucocorticoid induction of SP-A gene transcription, a fusion gene comprised of 1.7 kb of 5'-flanking DNA from the rabbit SP-A gene linked to the human growth hormone (hGH) structural gene was subcloned into a replication defective human adenovirus vector and used to infect human type II cells in monolayer culture. Treatment of the infected cells with Bt_2cAMP (1mM) resulted in a 5-fold increase in hGH production as compared to control cells. Unexpectedly, the addition of dexamethasone (Dex, 10^{-7} M) caused a 90 % decrease in the hGH production as compared to controls. Dex also markedly attenuated the cAMP stimulation of hGH production; Dex treatment caused a 90% decrease in hGH production as compared to that observed with cAMP alone. The inhibitory effect of Dex was dose-dependent, with an apparent K_d of 1.5×10^{-10} M. These results suggest the presence of a GRE in the 5'-flanking region of rabbit SP-A that confers negative regulation of fusion gene expression and antagonizes the stimulatory effect of cAMP. Since endogenous SP-A gene transcription is postively regulated by glucocorticoids, our findings suggest that other GRE(s) exist within the SP-A structural gene or in the 3'-flanking region that confer the stimulatory effects of glucocorticoids on SP-A expression.

E 301 GLUCOCORTICOID REPRESSION OF THE HUMAN GLYCOPROTEIN HORMONE α-SUBUNIT GENE INVOLVES DIRECT INTERACTIONS OF THE GLUCOCORTICOID RECPTOR WITH OTHER TRANSCRIPTION FACTORS Joachim Altschmied, Claudia Stauber, Ingrid E. Akerblom, Jesse L. Marron and Pamela L. Mellon, Regulatory Biology Laboratory, The Salk Institute, La Jolla, CA 92037

Though the transactivation properties of steroid receptors and their cognate DNA elements have been characterized with many inducible genes, the mechanisms by which steroids negatively regulate gene expression have proven more elusive. We have found that the promoter of the human glycoprotein α-subunit gene is repressed by glucocorticoids in a placental cell line using transient expression. Glucocorticoid inhibition was conferred by a DNA fragment containing a duplicated cAMP responsive element (CRE), which is crucial both for cAMP induction and for placental-specific expression of this gene. This region of the promoter also contains several *in vitro* binding sites for the glucocorticoid receptor (GR). Detailed mutational analysis of the promoter demonstrated that steroid repression does not require these GR binding sites. Parallel studies using GR mutants identified the DNA binding domain as indispensable for inhibition of transcription.

The glucocorticoid receptor is known to interact with the fos protein to confer steroid repression to promoters containing a binding site for the heteromeric fos/jun protein complex. This interaction was functionally mapped to the DNA binding domain of GR. Like fos, the CRE binding protein CREB, which is crucial for expression of the α-gene in placenta, is a member of the leucine zipper protein family. We are currently expressing CREB and the glucocorticoid receptor DNA binding domain in *E.coli* to study specific interactions between these proteins. Preliminary results indicate that in the presence of excess glucocorticoid receptor, CREB binding to its cognate DNA element is inhibited providing a model for steroid repression of the human glycoprotein α-subunit gene.

E 302 SITE DIRECTED MUTAGENESIS OF THE DNA BINDING DOMAIN OF CREB Ourania Andrisani, John S. Williams and Jack E. Dixon, Department of Biochemistry and Walther Cancer Institute, Purdue University, West Lafayette, IN 47907

The 43 Kda CREB phosphoprotein mediates signals of the cAMP transduction pathway and is required for transcription of the rat somatostatin gene. The CREB protein belongs to the leucine zipper family of transcription factors. Adjacent to the leucine zipper motif, required for dimerization, is the DNA contact region of the protein, composed of a stretch of basic amino acid residues. In order to understand how the basic domain of the CREB protein is involved in the recognition process towards the CRE binding site, we have carried out site directed mutagenesis of the CREB basic domain. We have analyzed the CREB DNA binding mutants by gel retardation, *in vitro* competition and methylation interference assays.

We will present the characterization of DNA binding mutants of the CREB protein, displaying reduced affinity and relaxed specificity towards the CRE binding site.

E 303 REGULATION OF THE HUMAN ß-GLOBIN GENE, M. Antoniou, F. Grosveld, E. deBoer, N. Dillon, P. Fraser, D. Greaves, O. Hanscombe, J. Hurst, M. Lindenbaum, S. Philipsen, S. Pruzina, D. Talbot and D. Whyatt, Laboratory of Gene Structure & Expression, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK.

The human ß-globin gene family is located on the short arm of chromosome 11 and contains five functional genes. These are arranged in the same order as they are expressed during development, i.e. 5'-ϵ-γ_G-γ_A-δ-ß-3' over a distance of 55kb. The embryonic ϵ-globin gene is active when the yolk sac is the hematopoietic tissue, the γ-globin genes are active in the liver during the foetal stage, and the δ- and ß-globin genes in the adult stage bone marrow (for review, see Collins & Weissman, 1984). Each gene contains a number of tissue- and developmental stage-specific regulatory regions and the entire locus is controlled by the so-called Dominant Control Region (DCR). This DCR consists of four strong hypersensitive regions (HSS) upstream of the ϵ-globin gene. Addition of these regions confers copy number dependent expression on the human ß-globin gene in murine erythroleukaemia cells and transgenic mice, at levels comparable to the endogenous mouse globin genes. We describe a deletional analysis of three of these hypersensitive regions and show that 200-300bp fragments are sufficient to direct copy number dependent, integration site independent expression of the human ß-globin gene. Biochemical analysis in vitro and mutagenesis experiments in vivo show at least two erythroid specific proteins (NF-E1 and NF-E2) and one non- erythroid protein to be essential for the function of this region. Addition of the DCR to globin genes also results in altered developmental expression patterns of an individual gene. By using multiple globin genes, we show that the combination and order of genes is important for their expression. A model for the regulation of this multigene locus will be presented.

E 304 ESTROGEN RECEPTOR GENE EXPRESSION IN NORMAL HUMAN BREAST: EVIDENCE FOR DOWN-REGULATION BY ESTROGEN, Sue A. Bartow, Marie T. Boyd, Richard H. Hildebrandt, Department of Pathology, University of New Mexico School of Medicine, Albuquerque, NM 87131

Estrogen is thought to stimulate proliferation of normal human breast epithelium. This stimulation presumably is mediated through DNA binding of the estrogen receptor protein, complexed with the hormone. However, unlike human breast cancer cells, normal human breast epithelium is only rarely positive for nuclear associated estrogen receptor protein by immunohistochemical techniques. We have studied the expression of the estrogen receptor gene in normal human breast at the mRNA transcript level by northern analysis. In general, the gene transcripts were elevated in the breast tissue of infants, children, luteal phase of the menstrual cycle, and post-menopausal women - periods of either low circulating estrogen levels, or simultaneously high levels of progesterone. Transcripts of the gene were low in perimenarchal girls, women in the follicular phase of the menstrual cycle, and late pregnancy - times of high circulating estrogen levels. Estrogen receptor mRNA transcript levels thus do not parallel increased epithelial proliferation, i.e., perimenarche and luteal phase of the menstrual cycle. Rather, epithelial proliferative activity appears to follow periods of high levels of both estrogen receptor gene activation and circulating estrogen. These results are consistent with down-regulation of the estrogen receptor gene by estrogen in normal human breast tissue.

E 305 **Induction of the Mouse Mammary Tumor Promoter by Steroid Hormone Receptors is Modulated by OTF-1,** Ulf Brüggemeier, Martha Kalff, Sabine Franke, Claus Scheidereit and Miguel Beato, Institut für Molekularbiologie und Tumorforschung, Emil- Mannkopffstr. 2, 3550 Marburg, FRG

Steroid hormones induce transcription from the mouse mammary tumor virus promoter by complex mechanisms requiring binding of hormone receptors to the hormone responsive elements (HRE) of the long terminal repeat region. Here we show that the MMTV promoter contains two degenerated octamer motifs immediately upstream of the TATA- box that together bind OTF-1 (Oct-1, NFIII) with a similar affinity as the octamer consensus sequence. In transfection experiments, mutation of these octamer motifs interferes with the hormonal response of the MMTV promoter. *In vitro*, these mutations do not influence basal transcription, but completely abolish the stimulatory effect of purified progesterone receptor. Progesterone receptor bound to the HRE facilitates binding of OTF-1 to the two octamer motifs. Thus OTF-1 is a natural mediator of progesterone induction of the MMTV promoter and acts through cooperation with the hormone receptor for binding to DNA.

E 306 INDEPENDENTLY BINDING PROTEINS SYNERGISTICALLY ACTIVATE THE TISSUE-SPECIFIC EXPRESSION OF THE HUMAN GLYCOPROTEIN α-SUBUNIT GENE, Marita Büscher, Angelo M. Delegeane, Joachim Altschmied, Jesse L. Marron and Pamela L. Mellon, Regulatory Biology, The Salk Institute, La Jolla, CA 92037

The placenta-specific transcription of the α-subunit gene of the chorionic gonadotropin is controlled by two synergistically acting DNA elements. One of these elements contains two tandemly arranged copies of the consensus sequence for cAMP responsive elements (CRE). Though the αCREs are inducible enhancers, they are also strictly required for placenta-specific expression. However, they appear to have no independent tissue-specificity. The tissue-specific expression is mediated by a second DNA element (trophoblast specific element, TSE), which is located just upstream of the CREs. Although the TSE has only little enhancer activity on its own it becomes a strong trophoblast-specific enhancer when combined with the CREs. The synergistic action of the TSE and αCREs to create placenta-specific expression is dependent on the proximity of the two elements, suggesting that the corresponding proteins might bind cooperatively. The αCREs are bound by an ubiquitous transcription factor, CREB, whereas TSE is bound by a placenta-specific nuclear protein, TSEB. We have purified TSEB to near homogeneity and determined by amino acid sequencing that it represents a novel protein. Using purified TSEB and CREB we performed in vitro DNA binding studies demonstrating that TSEB binds independently of CREB. Thus, the synergistic activation via TSE and CRE is not due to cooperative binding of the corresponding proteins.

E 307 TRNASCRITIONAL CONTROL OF HBSAG GENE EXPRESSION BY INSULIN IN HUMAN HEPATOMA CELL LINE. Chen-Kung Chou and Jaw-Ji Yang, Department of Medical Research, Veterans General Hospital, and Institute of Genetics, National Yang-Ming Medical College, Shih-Pai, Taipei, Taiwan, Republic of China.

Human hepatoma Hep 3B Cell has integrated HBV DNA in its chromosome and continuously secreted HBV surface antigen. The HBsAg production in Hep 3B cells can be suppressed by insulin. The suppression of HBsAg production by insulin was paralleled with the decrease in HBsAg mRNA (J. Biol. Chem. 264, 15304-15308,1989). In order to understand the regulatory mechanism of insulin action on HBsAg gene expression, the nuclear run on assay and S1 mapping experiment were performed. We identified the correct initiation site of HBsAg transcript in the control and the insulin treated Hep 3B cells. The transcritional rate of HBsAg gene was suppressed by insulin after 3 hrs treatment. Our results strongly suggested that the suppressive effect of insulin may mediate through the putative insulin response element in the HBsAg promoter.

E 308 IDENTIFICATION OF HUMAN DNA SEQUENCES RECOGNIZED BY RETINOIC ACID RECEPTORS. Patricia Costa-Giomi[1], Marie-Pierre Gaub[2], Pierre Chambon[2] and Patricio Abarzúa[1]. [1] Department of Oncology, Roche Research Center, Nutley, New Jersey 07110, USA and [2] LGME CNRS/U184 INSERM, Université Louis Pasteur, Strasbourg, France.

Retinoic acid (RA) can markedly influence growth and differentiation of normal and malignant cells and act as a morphogen during human development. Several nuclear receptors for RA have been identified. RARs belong to the steroid/thyroid hormone receptor superfamily and current evidence suggests that they can act as ligand-inducible transcriptional activators.

As a first step in understanding retinoid regulation of gene expression and identifying genes regulated by retinoids we are attempting to identify discrete human DNA sequences recognized by RARs. The approach taken has been described by K. W. Kinzler and B. Vogelstein (Nucl. Acid. Res. 17: 3645, 1989) and involves immunoprecipitation of the DNA/receptor complex followed by PCR amplification of the bound DNA. Several clones have been isolated by colony hybridization and sequenced. Computer analysis has shown that several of the clones contain small stretches of sequence homology. The most prevalent element contains two copies of an imperfect direct repeat of the motif AAG(G/A)TCA. This repeat is related to the thyroid hormone responsive element (TRE) consensus sequence AGGT(C/A)A. Using gel retardation assays we are looking at DNA-protein interactions between RARs and these cloned human fragments. Specific DNA-protein complexes are detected with at least one of the fragments isolated. This fragment contains the sequence CAAGGGCAGGAGAAGGTCAG which shares 45% homology with the RA responsive element from the RARß gene. Functional assays are in progress to determine whether this sequence can confer RA responsiveness when inserted upstream of the HSV thymidine kinase promoter.

E 309 PROBING THE STRUCTURAL DOMAINS OF THE GLUCOCORTICOID RECEPTOR THAT ARE INVOLVED IN DETERMINING THE SPECIFICITY OF HORMONE BINDING, Mark Danielsen, Xiayuan Liang, and Jacqueline Jonklaas, Department of Biochemistry and Molecular Biology, Georgetown University Medical School, 3900 Reservoir Rd., N.W., Washington, DC 20007.

Deletion analysis of the mouse glucocorticoid receptor has shown that the hormone binding site lies within the C-terminal half of the receptor. Characterization of hormone binding deficient receptors from mouse lymphoma cells resistant to glucocorticoids has revealed that residues throughout this region are required for high affinity binding of glucocorticoids. The hormone binding domain of the androgen receptor is highly homologous to the equivalent domain from the glucocorticoid receptor, yet they bind steroids with different specificity. To determine which amino acids in the hormone binding domains of these two receptors are functionally homologous, and which are involved in determining the specificity of hormone binding, we have subjected the glucocorticoid receptor to homolog scanning mutagenesis. Regions of the glucocorticoid receptor hormone binding domain (approximately 15 amino acids each time) were replaced systematically by the homologous sequences from the androgen receptor. The results reveal that some regions of the hormone binding domains of the two receptors are indeed functionally homologous and are not involved in hormone binding specificity. The remaining regions are candidates for specificity determinants and these regions are undergoing extensive mapping.

E 310 SELECTIVE HIGH AFFINITY LIGANDS FOR RETINOIC ACID RECEPTOR (RAR) SUBTYPES, Michel Darmon, Marie-Thérèse Cavey, Bernard Martin, Chantal Delescluse, Bruno A. Bernard, Uwe Reichert, and B. Shroot, Centre International de Recherches Dermatologiques Galderma (CIRD Galderma), Sophia Antipolis, 06565 Valbonne, France.

Biological effects of retinoids are mediated through their binding to closely related nuclear receptors (RARs) belonging to the steroid-thyroid nuclear receptor family. RARs are able to modulate the transcription of specific genes by binding to DNA responsive elements. In situ hybridization has shown that the distribution of each RAR in various tissues of the developing embryo and in the adult is not uniform. Thus, the identification of synthetic retinoids which would behave as selective ligands for each type of RAR would be invaluable tools for studying the biological roles of each RAR type. Moreover, from a pharmacological point of view, such selective compounds may possess a higher therapeutic index and a lower teratogenic risk. To approach this issue, we set up two complementary assays: 1) an in vitro binding assay allowing the determination of Kd values of retinoids for the RARα, RARβ, and RARγ receptors, and 2) a functional transcription assay of the potential of retinoids to transactivate a reporter gene through their binding to one RAR type. The binding assay uses nuclear extracts of cos7 cells transfected with the expression vectors RAROα, ROβ, or ROγ. The transcription assay measures the induction by retinoids of the synthesis of Chloramphenicol Acetyl Transferase (CAT) in HeLa cells cotransfected i) with the chimeric receptors RARα-ER.CAS or RARβ-ER.CAS and the reporter gene vit-tk-CAT, or ii) with the expression vectors for the natural receptors used in the binding assay and a TRE-tk-CAT reporter. Selective agonists for RARα, RARβ, and possibly RARγ receptors were identified.

E 311 RETINOIC ACID AND THYROID HORMONE RESPONSE OF THE HUMAN ALCOHOL DEHYDROGENASE GENE *ADH3*: IMPLICATIONS FOR CONTROL OF RETINOIC ACID SYNTHESIS, Gregg Duester, M. Scott McBride, Mary Lou Shean, Mark J. Stewart and Perri P. Harding, Department of Biochemistry, Colorado State University, Fort Collins, Colorado 80523.

Retinoic acid regulation of one member of the human class I alcohol dehydrogenase (ADH) gene family was demonstrated, suggesting that the retinol dehydrogenase function of ADH may play a regulatory role in the biosynthetic pathway for retinoic acid. Promoter activity of human *ADH3*, but not *ADH1* or *ADH2*, was shown to be activated by retinoic acid in transient transfection assays of Hep3B human hepatoma cells. Deletion mapping experiments identified a region in the *ADH3* promoter located between -328 and -270 base pairs (bp) which confers retinoic acid activation. This region was also demonstrated to confer retinoic acid responsiveness on the *ADH1* and *ADH2* genes in heterologous promoter fusions. In a 34 bp stretch from -318 to -285 bp, the *ADH3* retinoic acid response element (RARE) contains three direct repeats of the sequences TGACC or TGAAC which constitute the critical nucleotides of RAREs present in other genes. A block mutation of the TGACC sequence located at -289 to -285 bp eliminated the retinoic acid response. As assayed by gel shift DNA binding studies, the RARE region (-328 to -270 bp) of *ADH3* specifically bound the human retinoic acid receptor β (RARβ) and was competed by DNA containing a RARE present in the gene encoding RARβ. Further, our results indicate that thyroid hormone does not activate the *ADH3* promoter, but does down-regulate the observed retinoic acid activation of *ADH3*. Since ADH catalyzes the conversion of retinol to retinal, which can be further converted to retinoic acid by aldehyde dehydrogenase, these results suggest that retinoic acid activation of *ADH3* constitutes a positive feedback loop regulating retinoic acid synthesis. Also, this data suggests that retinoic acid positive feedback control of its own synthesis is modulated by the negative action of thyroid hormone at the level of *ADH3* transcription.

E 312 XF1 AND XF2 ACT THROUGH XENOBIOTIC RESPONSE ELEMENTS TO MODULATE TRANSCRIPTIONAL ACTIVATION OF THE CYTOCHROME P450IA1 GENE BY THE DIOXIN (Ah) RECEPTOR), John B. Fagan, Fahri Saatcioglu, David J. Perry, Margaret E. Richardson, and David S. Pasco, Molecular Biology Laboratory, Maharishi International University, Fairfield, Iowa, 52556

We have reported that dioxin (TCDD), as well as many other potent environmental contaminants and carcinogens, induce the expression of the cytochrome P450IA1 gene by both transcriptional and post-transcriptional mechanisms (J. Biol. Chem., 263, 8671, 1988). The dioxin receptor, or Ah receptor, when complexed with polycyclic aromatic compounds, is a DNA-binding protein that interacts sequence-specifically with xenobiotic response elements (XREs). This interaction is thought to be central to the mechanism by which polycyclic compounds activate P-450IA1 gene transcription. We (J. Biol. Chem., 265, 9251, 1990) and others have mapped these interactions in detail. We have also demonstrated that Zn^{+2} is an essential cofactor for the sequence-specific DNA-binding activity of the dioxin receptor (J. Biol. Chem., 265, 9251, 1990). In the process of characterizing dioxin receptor-XRE interactions, we have identified two additional hepatic nuclear factors, XF1 and XF2, that bind sequence-specifically to the XREs of the P-450IA1 gene. We have purified these factors several thousand fold by ammonium sulfate precipitation, heparin agarose chromatography, DEAE-Sephadex chromatography and sequence-specific DNA affinity chromatography. The molecular weights of XF1 and XF2 were found to be about 115,000 and 80,000, respectively. Biochemical, cell biological and genetic evidence clearly distinguishes these factors from the dioxin receptor, yet the molecular interactions of XF1 and XF2 with XREs (mapped by three independent methods) overlap substantially with those of the dioxin receptor. This suggests that the binding of these factors to XREs may compete with binding of the dioxin receptor to the same sites and may function to modulate the transcriptional regulatory function of this receptor. Transfection experiments will be presented that support this hypothesis, suggesting that XF1 and XF2 act through XREs to down-regulate P-450IA1 transcription. Supported by NIH Grant RO1-CA 38655.

E 313 Functionally Distinct Isoforms of the CRE-BP DNA Binding Protein Mediate Activity of a T Cell Specific Enhancer. *Katia Georgopoulos#*, Bruce A. Morgan*, and David D. Moore#**. #Department of Molecular Biology, Massachusetts General Hospital, and *Department of Genetics, Harvard Medical School, Boston, MA 02114.

Expression of the CD3δ gene of the TCR complex is regulated by a T cell specific enhancer. Activity and specificity of this enhancer are mediated by an element designated δA that interacts with nuclear factors found predominantly in mature T cells. Using this enhancer element as a probe, we have isolated three cDNA clones encoding three distinct protein isoforms, products of differential splicing and alternate promoter usage of the CRE-BP gene. These DNA binding proteins share a C-terminus containing basic and leucine zipper domains but diverge at the N terminus of the molecule. Their activities as transcription regulators differ: CRE-BP2 is a potent activator, CRE-BP3 is a weak activator and CRE-BP1 can function as a competitive inhibitor. This family of proteins is expressed in many tissues, but the levels of the three isoforms vary between cell types. Expression of a mutant CRE-BP isoform which can dimerise but not bind DNA appears to repress the activity of the enhancer in T cells. We propose that the activity of the δA enhancer is modulated by differential expression of the CRE-BP isoforms. Formation of homo and heterodimers between the active and inactive CRE-BP isoforms may control the levels of activity of the δA enhancer. Within the T lineage, postranslational modifications of the active isoforms or interactions with other T cell specific factors may further regulate the activity of the CD3 δ enhancer.

E 314 ISOLATION OF PARTIAL cDNA CLONE FOR A NOVEL MEMBER OF THE POU DOMAIN FAMILY EXPRESSED IN DEVELOPING MOUSE PLACENTA. Brian T. Greuel, Teresa Calzonetti, and Janet Rossant. Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Ave., Toronto, Ont., M5G 1X5, Canada.

We have used the polymerase chain reaction to screen cells of the trophoblast lineage in mice for the expression of novel genes encoding POU domain-containing factors. Others have shown that members of the POU domain gene family are expressed in a developmentally-regulated or tissue-specific manner. For example, Pit-1 potentially activates the expression of the prolactin and growth hormone genes in the anterior pituitary during embryogenesis and postnatal development of the mouse. The genes for these hormones are closely related to the placental lactogen gene family whose members are expressed highly in trophoblast. This raises the interesting possibility that Pit-1 or a related POU-domain gene might be expressed in the trophoblast and play a critical role in the regulation of this lineage. Partially degenerate oligonucleotides corresponding to the consensus sequences for segments of POU-specific subdomain B and the POU homeodomain were used to amplify cDNA following first strand synthesis from total RNA of 13.5 day mouse placenta. DNA fragments of approximately 320 bp were gel purified and subcloned in the plasmid vector pGEM7zf+. A partial cDNA clone for a novel member of the POU domain family, named P-3, was thus obtained and found to be most similar to the Oct-3/Oct-4 gene in the POU domain family. The two genes are approximately 70% identical by nucleotide sequence and 60% identical by amino acid sequence within the cloned region of the P-3 gene. Isolation of full-length clones and characterization of the expression pattern of this gene are in progress. Supported by MRC of Canada.

E 315 GLUCOCORTICOIDS DOWNREGULATE PHORBOL ESTER-INDUCED C-JUN AND C-FOS GENE EXPRESSION ON THE TRANSCRIPTIONAL LEVEL BY INTERFERING WITH AP-1 BINDING

Ralf Hass; Marion Brach and Donald Kufe ;*Clin.Pharmacology, Dana-Farber Cancer Institute, Boston, MA 02115*

Recent studies have shown that phorbol ester (TPA)-induced monocytic differentiation of human U-937 myeloid leukemia cells is associated with the rapid activation of c-jun and c-fos early response gene expression. The present work demonstrates that the glucocorticoid dexamethasone (DEX) inhibits TPA-induced increases in c-jun and c-fos mRNA levels. These findings were associated with a partial block in the appearance of the TPA-induced monocytic phenotype. Previous studies have demonstrated that c-jun and c-fos gene expression in myeloid leukemia cells is regulated by protein kinase C (PKC). The finding that DEX had no effect on TPA-induced activation of PKC suggested that this glucocorticoid inhibits signals downstream to this enzyme. Nuclear run-on assays showed that: 1) the expression of c-jun and c-fos is transcriptionally induced by TPA; 2) the TPA-induced expression of c-jun and c-fos does not require protein synthesis; and 3) the TPA-induced expression of both genes is inhibited by DEX at the transcriptional level. In order to further define the effects of DEX at the molecular level, we prepared a series of deleted c-jun promoter fragments linked to the chloramphenicol acetyltransferase (CAT) gene. Transient expression of these constructs in TPA-treated cells demonstrate increases in CAT activity in the region (-94 to -20) of the promoter that contains the AP-1 binding site. This induction of CAT activity by TPA was sensitive to DEX. Taken together, these findings suggest that DEX down-regulates TPA-induced transcription of the c-jun gene by inhibiting activation of the AP-1 site. Further studies are needed to determine whether this effect is related to inactivating Jun/AP-1 or to the induction of a protein that blocks binding to the AP-1 site. Other promoter studies are needed to determine how DEX blocks TPA-induced c-fos gene expression.

E 316 THE CORTICOTROPIN RELEASING HORMONE (CRH) GENE CAN BE TRANSCRIPTIONALLY ACTIVATED BY THE POU-DOMAIN PROTEIN, Brn-2.

Xi He, Renee Gerrero, Donna Simmons*, Larry Swanson* and Michael G. Rosenfeld, Eukaryotic Regulatory Biology Program, School of Medicine, UCSD, La Jolla, CA 92093-0648, *Neural Systerms Laboratory, La Jolla, CA 92037

Because Brn-2, a member of the POU-domain gene family, is expressed in paraventricular hypothalamic neurons producing corticotropin releasing hormone(CRH),we examined the potential role of Brn-2 in the activation of corticotrpin releasing hormone gene expression. Using a method involving protein nitrocellulose transfer and random oligonucleotide selection, we were able to determine the specific-DNA binding activity of Brn-2 and further its recognition concensus sequence. Inspection of the 5'-flanking region of the CRH gene revealed several sequences highly homologous to the identified Brn-2 binding concensus, and DNaseI footprinting analysis proved that these sequences were indeed the binding sites for Brn-2. Transfection assay showed that Brn-2 specifically activated CRH promoter in heterologous cell lines. These data demenstrate that Brn-2 is a transcription regulator and suggest that the POU-domain gene family can participate in the regulation of neuro-specific genes in the brain.

E 317 A RETINOIC ACID RESPONSE ELEMENT WITH DIFFERENTIAL RECEPTOR SPECIFICITY,

Birgit Hoffmann, Jürgen Lehmann, Xiao-Kun Zhang, Thomas Herrmann, Matthias Husmann, Gerhart Graupner and Magnus Pfahl,Cancer Research Center, La Jolla Cancer Research Foundation , La Jolla, CA 92037

The morphogen retinoic acid (RA) mediates complex pattern of gene expression during development and homeostasis. The three different nuclear receptors for RA (retinoic acid receptors ;RARs) described so far belong to the superfamily of the steroid/thyroid hormone receptors. These regulatory proteins contain a characteristic highly conserved DNA binding domaine that encodes two "zinc-finger" structures and a less well conserved hydrophobic ligand binding domaine. Interaction of the receptor with specific DNA sites - response elements- near sensitive promotors is essential for the regulatory effects these proteins exert on gene transcription. While responsive elements for steroid and thyroid hormone receptors have been well characterized, much less is known on the DNA sequences that are directing the RA response. The high homologies observed between the DNA binding domaines of thyroid hormone receptors (TRs) and RARs are of functional significance since RARs utilize TREs as specific response elements. This suggested that RARs and TRs regulate gene expression via a common group of responsive elements. To obtain a better understanding on how the retinoic signal is mediated in complex biological programs, we analyzed a retinoic responsive promotor: the RARβ/ε promotor we find that the activity of this promotor is under strict retinoic acid control. A retinoic acid receptor specific element is located next to a TATA box. The response element is bound by RARs with high affinity in the absence and presence of ligand. Other receptors also bind to the response element but do not show any effect on the transcriptional regulation of the promotor.

E 318 **LUTEINIZING HORMONE RELEASING HORMONE (LHRH) INCREASES THE TRANSCRIPTION OF LUTEINIZING HORMONE (LH) SUBUNIT GENES α AND ß,** Kimberly A. Howes, Charles H. Hensel, and Russel J. Reiter, Department of Cellular & Structural Biology, University of Texas Health Science Center, San Antonio, TX 78284-7769
LHRH regulates the biosynthesis of both LH peptide subunits, α and ß. While the α subunit peptide is common to LH, follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH) and chorionic gonadotropin (CG), functional specificity is determined by the ß subunit peptide of each of these hormones. LHRH has been reported to regulate the mRNA levels for both subunits. However, the molecular mechanisms of this regulation remain unclear. The mechanism whereby LHRH regulates LH subunit expression was assayed utilizing a primary cell culture system prepared from male rat anterior pituitaries. Treatment of pituitary cultures with 10^{-7}M- 10^{-10}M LHRH induced up to 8 fold and 4 fold increases in LHß and α RNA levels, respectively, within 30" of treatment, as measured with a sensitive RNA solution hybridization and RNAse protection technique. Treatment with the transcriptional inhibitor Actinomycin D eliminated the 10^{-8}M LHRH-induced increase in α and LHß RNA levels. This result indicates that LHRH can regulate the transcription of both α and LHß genes.

E 319 AN ATF-LIKE FACTOR WHOSE DNA BINDING ACTIVITY IS MODULATED BY ITS PHOSPHORYLATION STATE, Helen C. Hurst and Ian G. Cowell, ICRF Oncology Group, Hammersmith Hospital, London W12 0HS, U.K.
We are interested in how transcription factor activity may be controlled and are currently studying two factors whose binding activity is modulated by their phosphorylation state:
P4 is a 'bzip' protein of the ATF/CREB family. A clone was obtained from a placental cDNA expression library by screening with an oligomerized ATF binding site from the adenovirus E4 promoter. Unlike other ATF-like factors, *in vitro* synthesised P4 shows a strong preference for binding to the E4 site and is reminiscent of the E1a-inducible cellular factor E4F described by Nevins and colleagues. P4 binds as a dimer and forms a very stable protein DNA complex. Dephosphorylation of P4 causes a dramatic decrease in binding activity. Preliminary evidence shows binding can be restored by incubation with fresh reticulocyte lysate and we are trying to identify a specific kinase responsible for these observations.
P3A and P3B are members of a family of proteins obtained by differential splicing of transcripts from the 'ets related gene' (erg). The particular transcripts represented by the clones P3A and P3B have not previously been described. These erg proteins can bind sites recognised by ets proteins and the optimum erg binding site is presently being determined. Unlike P4, the P3 proteins show increased binding activity on being treated with phosphatase.
We are examining whether either P3 or P4 proteins alter in phosphorylation state upon serum stimulation of quiescent cells. Furthermore, we are also investigating whether P4 activity can be modulated by adenovirus E1a protein.

E 320 REGULATION OF THE RETINOIC ACID RECEPTOR β PROMOTER BY RETINOIC ACID RECEPTOR ISOFORMS, Matthias Husmann and Magnus Pfahl, Cancer Center, La Jolla Cancer Research Foundation, La Jolla, CA 92037
Retinoic acid receptors (RARs), are regarded to be crucial for the regulation of gene expression by RA. The search for genes directly regulated by RARs resulted in the recent discovery of a highly RA responsive element in the promoter of one of the RARs themselves, RARβ. This finding for the first time allows to analyze in detail the regulation of an authentic RA responsive element (RARE). The existence of various RAR isoforms and their different tissue distribution imply different functions of the RARs. In particular, RARγ and RARβ show non-overlapping spatiotemporal expression patterns. The mechanisms leading to these patterns and their biological significance remain elusive. In the present study, we show that RARγ suppresses specifically and with high efficiency the RA dependent activation of the RARβ RARE. Therefore, negative regulation of RARβ by RARγ may contribute to the almost exclusive expression patterns of the two RARs and thus be an important way of regulating developmental processes and maintaining established tissue patterns.

E 321 REPRESSION OF THE HUMAN GLYCOPROTEIN HORMONE α-SUBUNIT GENE BY GLUCOCORTICOIDS: RECEPTOR INTERACTIONS WITH TRANSCRIPTIONAL ACTIVATORS,
J. Larry Jameson, V. Krishna K. Chatterjee, Laird D. Madison, and Sara Mayo. Thyroid Unit, Massachusetts General Hospital, Boston, MA 02114

Expression of the glycoprotein hormone α gene is repressed by glucocorticoids in JEG-3 cells. The degree of glucocorticoid (GR) mediated repression was impaired by a variety of deletional and site-directed mutations between -171 and -111 bp, a region that includes both cell-specific and cAMP response elements (CREs). To further localize a negative glucocorticoid response element (nGRE) sequence, a series of overlapping α promoter DNA sequences between -170 to +29 bp were tested using avidin-biotin-complex DNA (ABCD) binding assays, but each failed to bind GR, whereas a control GRE avidly bound receptor. The absence of high affinity GR binding sites in the α promoter suggested that mutations that affected GR inhibition may have eliminated recognition sites for transactivators which are themselves targets for the GR rather than altering specific nGRE sites in the DNA sequence. To examine this possibility, GR repression was studied using chimeric transcription factors. The transcription activating domains of several different proteins were linked to the DNA binding domain of Gal 4 and transcription was driven by the Gal 4 recognition site (UAS). GR markedly repressed transactivation by Gal4-CREB and to a lesser degree, the Gal4-thyroid hormone receptor and Gal4-VP16 chimeric proteins. Thus, inhibition occurs in the absence of either the CRE or the proximal α promoter. These results support a mechanism in which GR mediated repression in JEG-3 cells occurs by receptor interference with the transactivating potential of enhancer binding proteins or associated transcription factors.

E 322 SLICING VARIANTS OF PIT-1. Kristin Konzak and David D. Moore, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114.

Rat growth hormone (GH) and prolactin (Prl) are activated by a common transcription factor, Pit-1, and are jointly expressed in somatolactotrophs. These cells further differentiate and give rise to mature somatotrophs and lactotrophs whose cell specific products are, respectively, GH and Prl. We have isolated two Pit-1 cDNAs that encode proteins of distinct molecular weight. PCR was used to confirm the presence of transcripts corresponding to both types of cDNAs in rat pituitary tumor cells. Both versions transactivate GH and Prl in transient transfection experiments, but one has only one third the activity of the other. With the GH promoter, transactivation by both versions is dominant over the silencer described by our laboratory. Pit-1 activates the GH promoter 3-10x in nonpituitary cells, compared to 50-2500x activation of the Prl promoter. This stronger effect on Prl is a consequence of both a lower basal expression and a 2-50x higher absolute level of induced transcriptional activity compared with GH.

E 323 THE ESTROGEN RECEPTOR BINDS IN THE MAJOR GROOVE OF ITS RESPONSE ELEMENTS, Nicholas J. Koszewski and Angelo C. Notides, Department of Biophysics, University of Rochester Medical Center, Rochester, NY 14642

The binding of highly purified estrogen receptor to the estrogen response elements (ERE) of the chick vitellogenin II gene was analyzed by ethylation interference. Binding to DNA fragments containing either a perfect ERE (-625 relative to gene start site) or an imperfect ERE (-353) produced specific bands in gel retardation assays. Interference experiments with ethylated DNA resulted in the exclusion of a cluster of phosphate residues within each half of the palindromic perfect ERE on both the coding and non-coding strands, consistent with the binding of a dimer. The footprint pattern also displayed a 5' stagger indicating that each monomer of the estrogen receptor dimer is binding in the major groove of the DNA. Experiments carried out using the imperfect ERE were identical to the perfect ERE with the exception that fewer contact points were observed. This may, in part, explain the observed differences in the binding affinity of the receptor for the imperfect ERE as compared with the perfect ERE.

E 324 CROSS-TALK OCCURS BETWEEN THE RETINOIC ACID (RA) AND PROTEIN KINASE C (PKC) PATHWAYS IN A HUMAN TERATOCARCINOMA (TC) CELL, J. Kurie, W. Miller, M. Burchert, C.F. Chiu, and E. Dmitrovsky, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.
We investigated whether cross-talk between the RA and PKC pathways occurs in NTera-2 clone D1 (NT2/D1), a multi-potential human TC line. We found in these cells that phorbol ester treatment (TPA 100 ng/ml) causes up-regulation of the fos and jun family members c-fos, fra-1, c-jun, jun-B, and jun-D within 2 hours while RA-treatment selectively up-regulates c-fos prior to an increase of the RA nuclear receptor-ß (RAR-ß). This up-regulation of c-fos by RA is abrogated by a dose of TPA prior to RA which depletes total cellular PKC activity to 15% of untreated cells. This suggests that c-fos induction by RA is mediated through PKC activation. Further evidence for cross-talk was found when NT2/D1 cells were transiently treated with TPA (100 ng/ml for 2 hours) to stimulate PKC prior to RA-treatment. This revealed an augmented up-regulation of RAR-ß and accelerated morphologic differentiation compared to RA-treatment alone. Preliminary data directly measuring total cellular PKC activity in RA-treated NT2/D1 cells demonstrate PKC activation after RA-treatment. Transient transfection experiments are underway to further investigate the mechanism whereby PKC modulates the effects of RA. Taken together, these data indicate that PKC activation is an early event which contributes to RA actions in NT2/D1 cells.

E 325 LOCALIZATION OF INSULIN RECEPTOR PROMOTER ACTIVITY, James R. Levy, Geoffrey Krystal, Carrie Klett, and Victoria E. Hug, Department of Research, McGuire VAMC, Richmond, VA 23249
The 5' flanking region of the insulin receptor gene is characterized as GC rich, with multiple transcriptional initiation sites and no TATA or CAAT boxes. We have previously demonstrated that most of the promoter activity is located within 578 b.p. and 877 b.p. upstream from the translational initiation site. To dissect this activating region more closely, 5' deletion mutants of the insulin receptor promoter, attached immediately upstream from a chloramphenicol acetyl transferase (CAT) gene in the plasmid pCAT3M, were prepared by Exo III/S1 nuclease digestion. All plasmid constructs were sized and then cotransfected into Hep G2 cells with a plasmid (pCH110) containing a lac Z gene driven by the SV40 early promotor. All CAT activities were normalized to ß-galactosidase activity. When expressed as a fraction of the full length (1823 b.p.) 5' flanking region, the insulin receptor promoter activity of the following constructs were demonstrated: 0-1823 b.p. = 100%, 0-820 b.p. = 94%, 0-760 b.p. = 94%, 0-720 b.p. = 125%, 0-640 b.p. = 58%, 0-620 b.p. = 45%, 0-578 b.p. = 6% (n=3). In summary, most of the activation of the insulin receptor promoter occurs between 640-720 b.p. (a segment which contains potential Ap-2 binding sites) and between 578-620 b.p. (a segment which has 4 potential Sp1 binding sites). This study will permit further characterization and localization of protein/DNA binding interactions and their physiologic significance in regulating insulin receptor gene transcription.

E 326 ESTROGEN-RESPONSIVE ELEMENTS IN HUMAN AND RAT C-FOS PROMOTER. Maggi A., Vegeto E., Pollio G. and Di Lorenzo D. Milano Molecular Pharmacology Lab, Inst. Pharmacol. Sci., Univ. of Milan, P.za Durante 11 - 20131 Milano, Italy
Recent studies performed in rat demonstrate that estrogen (E) administration results in increased levels of c-fos mRNA in tissues target for the steroid. Further determinations performed in a human cell line indicate that the expression of c-fos is not responsive to E. In order to determine whether this discrepancy had to be ascribed to the diverse experimental model used or to a specie-specific effect, c-fos transcription was examined in parallel in a murine and in a human cell line both expressing the E receptor (GH_3 and T47D, respectively). The c-fos mRNA content was quantitated by slot blot analysis. c-fos mRNA resulted to be induced by E only in the murine cell line. Sequence analysis indicated in the rat 5' flanking region the presence of the palindrome 5'GGTCT...AGACC3' at -207/-219 bp from the transcription start site. This sequence is highly homologous to the estrogen responsive element identified in the Xenopous vitellogenin gene and in the prolactin gene (5' GGTCA...TGACC). In the human c-fos promoter, however, only the half palindrome is present at -212/-224 bp. To examine whether the differential effect of E on c-fos induction could be explained by the diverse affinity of E receptor for the two promoters, gel shift experiments were performed. Such experiments indicate that the E receptor has the same affinity for the human and murine putative EREs.
It is concluded that the differential effect of E on the expression of c-fos in the human and rat GH_3 cell lines might be explained by the presence of cell specific factors which play an important role in the specie-specificity of the expression of proto-oncogene.

E 327 RETINOID X RECEPTORS: A NEW FAMILY OF RETINOID RESPONSIVE TRANSCRIPTION FACTORS, David J. Mangelsdorf, Richard Heyman, Jacqueline A. Dyck, Uwe Borgmeyer, Estelita S. Ong, and Ronald M. Evans, The Howard Hughes Medical Institute, Gene Expression Laboratory, The Salk Institute, P.O. Box 85800, San Diego, CA 92186.
We have discovered a new subfamily of nuclear receptors (termed RXRs) which respond to retinoids, but which are only distantly related to the previously characterized retinoic acid receptors (RARs). The new receptors have several isoforms located at genetically distinct loci. They are capable of transactivating through cis elements similar to the RARs, but show a different rank potency and dose dependency to retinoids. Northern analyses of the RXRs indicate that each isoform has a unique pattern of expression in adult tissue and is temporally and spatially expressed in the embryo. These studies suggest a role for RXRs in adult physiology and embryonic development. Binding experiments demonstrate that the RXR protein has a low affinity for [^{3}H]retinoic acid and, taken together with the transactivation studies, suggest the RXR ligand is a metabolite or structural analog of retinoic acid.

E 328 CO-PURIFICATION OF FACTORS BINDING TO THE MAJOR LATE INITIATION SITE AND DOWNSTREAM SEQUENCE ELEMENTS OF THE SV40 LATE PROMOTER, Janet E. Mertz, Steven Wiley, Richard Kraus, Elizabeth Murray, and Karla Loritz, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706

The SV40 late promoter contains three sequence elements necessary for transcription initiation at nt 325, the major cap site. One maps approximately 30 bp upstream, one spans the major late initiation site, and one maps approximately 30 bp downstream of the major initiation site. We have purified fractions from Hela cell nuclear extracts by affinity chromatography with a 17 bp oligonucleotide homologous to the sequences surrounding the major late initiation site. The resulting fraction contained an activity that protected this sequence from DNAse I cleavage in the absence of any upstream binding activity. Surprisingly, this extensively purified fraction also protected the SV40 major late downstream element from DNAse I cleavage. The purified fraction protected neither the initiation site nor the downstream sequence elements of the murine DHFR promoter. SDS-PAGE of the fraction showed three dominant proteins whose functions in sequence specific binding and transcription are currently under investigation. We conclude the following: i) The cellular factors that recognize the genetically significant initiation site and downstream elements of the SV40 major late promoter may interact by protein-protein association; ii) These combined activities may enhance stable binding to the promoter; and iii) Binding of these factors can occur in the absence of upstream binding activities.

E 329 ABERRANT EXPRESSION OF RETINOIC ACID RECEPTOR ALPHA (RAR-α) IN ACUTE PROMYELOCYTIC LEUKEMIA (APL). Wilson H. Miller, Jr., S. Frankel, A. Jakubowski, J. Kurie, J. Gabrilove, R. Warrell, and E. Dmitrovsky. Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.
RAR-α has been reported to play a central role in the induction of terminal differentiation by *in vitro* treatment of HL-60 human promyelocytic cells with all-*trans* retinoic acid (RA). It has also been reported that RA treatment *in vivo* will induce differentiation and clinical remission of APL in man. In the course of our clinical studies of RA in APL, we have evaluated RAR-α expression in fresh leukemic cells. RNA was extracted from mononuclear cells of bone marrow aspirates and hybridized to a cDNA RAR-α probe by standard Northern blotting techniques. Two novel bands, indicating aberrant mRNA transcripts, were seen in the leukemic cells of two patients. Both patients had the translocation t(15;17) characteristic of APL in their leukemic cells and both responded clinically to RA therapy. Up-regulation of the expression of both the normal and aberrant mRNA transcripts occurred in response to RA treatment *in vivo*. One patient with phenotypic APL lacking the t(15;17) expressed only the normal-sized RAR-α transcripts and failed to respond clinically to RA treatment. We speculate that RAR-α is involved in the abnormal differentiation seen in APL, and we are investigating whether RAR-α may be involved in the t(15;17) of APL by searching for a possible rearrangement of the RAR-α gene on chromosome 17.

E 330 FACTORS RESPONSIBLE FOR DIFFERENTIAL TATA SELECTION IN THE CKB PROMOTER, Mark T. Mitchell and Pamela A. Benfield, Central Research and Development, E.I. du Pont de Nemours & Co., Inc., Wilmington, DE 19880-0328

The brain creatine kinase gene (CKB) presents a paradox among polymerase II promoters. Although this promoter contains a consensus pol II TATA box, this is located at -60 relative to the transcription start site. At the normal position of a TATA box is the sequence TTAA, which is a very poor match to the consensus. This peculiar promoter structure has been conserved between the CKB genes of human, rat and chicken. We have previously shown that nuclear extracts from non-expressing tissues tend to recognize the upstream consensus TATA box, whereas extracts from expressing cells and tissues rely on the TTAA for minimal promoter function. We have now used human TFIID expressed in *E. coli* to directly address this differential TATA selection. Data will be presented from binding and transcription studies designed to address which factors and sequences are required for TFIID recognition of the TTAA element.

E 331 ALTERED EXPRESSION AND REGULATION OF RETINOIC ACID RECEPTOR GAMMA mRNA IN A RA-NONRESPONSIVE EMBRYONAL CARCINOMA MUTANT CELL LINE, Clara Nervi, Thomas M. Vollberg, Margaret D. George, Joseph F. Grippo* and Anton M. Jetten, NIEHS, RTP, NC 27709; ROCHE RES. CTR., Nutley, NJ 07110.

Embryonal carcinoma PCC4.aza1R cells are tumorigenic multipotent embryonic stem cells that can be induced to differentiate into mesenchymal cells by treatment with nanomolar concentrations of retinoic acid (RA). To investigate the molecular mechanism of RA induced differentiation we examined the expression of the nuclear retinoic acid receptors RARα, RARβ and RARγ in PCC4.aza1R cells and in a RA-resistent mutant cell lines PCC4(RA)$^{-}$1. The mutant cell line in addition to being resistant to RA-induced differentiation, is abnormal in the induction of the homeobox genes Hox 1.3 and Hox 1.6 genes. The inability of this mutant cell line to differentiate and the altered response of RA-inducible genes could be related to a defect in RAR expression. In PCC4.aza1R cells RA treatment induces a 10-50 fold increase in the expression of RARβ mRNA in a time- (within 2 hr, and maximally at 5 days) and concentration- (EC_{50} ≈ 10^{-9} M RA) dependent manner. RARβ mRNA is also induced by RA in the mutant embryonal carcinoma cell line PCC4(RA)$^{-}$1. PCC4.aza1R cells express two RARγ mRNA transcripts of 3.1 and 3.3 kb in size; treatment with RA for 48 hours suppresses the expression of both RARγ mRNA transcripts. In contrast to the parental cells PCC4(RA)-1 expresses only the 3.1 Kb mRNA species and this RARγ transcript is not down-regulated after treatment with RA. The altered expression of RARγ mRNA in PCC4.aza1R RA-nonresponsive mutant cell line suggest that the integrity of RARγ isoforms and the RA induced down-regulation of RARγ mRNA expression may be essential for the induction of differentiation in PCC4.aza1R cells. In ongoing experiments we are tempting to study complementations by gene transfection in these cell lines.

E 332 NEGATIVE REGULATION OF THE INTERLEUKIN-2 ENHANCER BY THE GLUCOCORTICOID RECEPTOR. Jeffrey P. Northrop (1), Leonard A. Herzenberg (2), Gerald R. Crabtree (1), and Petri S. Mattila (2,3). (1) Howard Hughes Medical Institute and (2) Department of Genetics, Stanford University, Stanford California 94305 and (3) Departments of Bacteriology and Immunology, University of Helsinki, Helsinki Finland.

Glucocorticoid hormone (GH) dependent transcriptional enhancement by the glucocorticoid receptor (GR) has been intensively studied in recent years and is known to occur through the interaction of GR with specific DNA response elements. In contrast, negative regulation of gene expression by GR is less well understood. Glucocorticoids are potent immunosuppressive agents acting primarily by inhibiting T-lymphocyte activation and lymphokine production. We have studied nuclear factors involved in the transcriptional activation of the interleukin-2 (IL-2) gene upon T-cell activation. IL-2 gene expression is inhibited by GH in sensitive T-cells. In transgenic mice carrying *c-myc* linked to a minimal IL-2 enhancer, mitogen induced expression of the transgene is inhibited by concurrent GH treatment in isolated splenocytes while a similar transgene construct driven by three copies of the binding site for the transcription factor NF-AT is not inhibited. In cotransfection experiments into GH insensitive Jurkat cells we show that the DNA binding domain of the GR must be intact while the complete N-terminus of the GR is dispensable for inhibition of the IL-2 enhancer. We have localized the site of inhibition to the proximal half of the enhancer, specifically to two AP-1 like sequences which appear to bind a similar if not identical inducible nuclear factor. Preliminary data indicate that this factor contains Fos or a Fos related antigen. Furthermore, a bacterially expressed GR DNA binding domain binds adjacent to the proximal AP-1 like sequence. These observations suggest that glucocorticoid inhibition of T cell activation is dependent upon an association of GR and AP-1.

E 333 RELATIONSHIP BETWEEN THE *DROSOPHILA ultraspiracle* LOCUS AND THE VERTEBRATE RETINOID X RECEPTOR. Anthony E. Oro*, Michael McKeown#, and Ronald M. Evans*. *Howard Hughes Medical Institute, Salk Institute, Gene Expression Lab and #Molecular Biology and Virology Lab, P.O. Box 85800 San Diego, CA 92186-5800.

The receptors for steroid, retinoid, and thyroid hormones constitute a large superfamily of nuclear regulatory proteins. In vertebrates, these molecules regulate diverse biological processes including pattern formation, cellular differentiation and homeostasis. As part of a molecular genetic approach to the hormonal control of development, we have screened the *Drosophila* genome for retinoic acid receptor homologs. None of the inserts shares a high degree of identity with the vertebrate retinoic acid receptor. However, one clone maps to the distal part of the X chromosome and thus was called XR2C. XR2C shares remarkable sequence similarity to the retinoic acid responsive transcription factor, the retinoid X receptor(RXR). Although XR2C and RXR share sequence similarity in the ligand binding domain, preliminary transfection studies indicate XR2C is not retinoic acid responsive. Genetic analysis reveals XR2C is encoded by the*ultraspiracle* locus and that chromosomes containing a genomic region encoding XR2C complement*ultraspiracle* mutants. Ultraspiracle has been previously characterized and shown to be required both in the maternal germline and zygotically for pattern formation. The discovery that the *usp* product is an RXR homolog suggests that common chemical cues may underlie morphogenic signalling in vertebrate and invertebrate systems.

E 334 COOPERATIVITY BETWEEN TWO XRES IS REQURIED TO MEDIATE TRANSCRIPTIONAL ACTIVATION BY XENOBIOTICS IN ISOLATED HEPATOCYTES, David S. Pasco, Fahri Saatcioglu and John B. Fagan, Molecular Biology Laboratory, Maharishi International University, Fairfield, IA 52556-2091

An efficient transfection system for isolated adult rat hepatocytes developed in our laboratory (Pasco and Fagan, DNA 8 (7) 535, 1989) was used to determine the cis-acting DNA elements which mediate the inducible expression of the carcinogen-metabolizing cytochrome P-450c gene (CYP1A1). Previous studies have determined that activation of CYP1A1 transcription involves the formation of a complex between the aryl hydrocarbon receptor and the inducing chemical, followed by the binding of this complex to upstream DNA elements called xenobiotic responsive elements (XREs). To determine the minimal sequence elements mediating this transriptional activation, transfection experiments were carried out using a CAT vector containing various deletions, mutations and combinations of native and synthetic sequences including two distinct XRE domains. The core domains were as follows:

XRE1 -- 5' TCTTCTCACGCAACT 3'
XRE2 -- 5' CTAGCGTGACAGCAC 3'.

Our central observation is that a single XRE domain is insufficient to mediate the transcriptional activation of this gene by xenobiotics. When either XRE1 or XRE2 was tested alone, basal expression was very low and there was no observable response to xenobiotics. When both XREs were included in the same construct, basal activity was higher and xenobiotics induced CAT expression 2- to 3-fold. Cooperativity between two XREs was also required for the enhancement of xenobiotic transcriptional activation by cycloheximide, and the orientation of these dimers influenced their responsiveness. These results suggest that responsiveness to xenobiotics requires cooperation betwen two complete XRE sequences. Supported by NIH Grant RO1-CA 38655.

E 335 A CAT ASSAY WITH AN INCREASED SENSITIVITY THAT ALLOWS WEAK PROMOTER ANALYSIS IN EITHER TRANSGENIC MICE OR ESTABLISHED TISSUE CULTURE CELL-LINES, François P. Pothier, Mariette Ouellet, Jean-Pierre Julien and Sylvain L. Guérin, CHUL Research Center, Québec, (Canada), G1V 4G2.

A number of procedures have been described for the detection of CAT activity directed by the CAT reporter gene following its introduction in either a small subset of transformed cells, using transient transfection, or in every cells of an organism, using the transgenic technology. Most are based on the preparation of a crude CAT-containing extract and its further incubation with [^{14}C] labeled chloramphenicol in the presence of acetylCoA. However, our findings clearly indicated that Triton X100, which is used in the lysis buffer of a number of different protocols for preparation of crude extracts, severely injures the efficiency of the acetylation process. Here we describe the use of a PMSF-containing buffer system much more advantageous than the currently used Tris-Triton and Tris buffers and designed to minimize protein degradation during the preparation of the cell extracts for CAT assays. A strong increase in the sensitivity of the CAT assay has been obtained when our extract preparation protocol is used in combination with the much more sensitive [^{3}H] chloramphenicol-butyryl CoA CAT assay. Extracts prepared from a number of tissues isolated from transgenic mice which have integrated the CAT reporter gene under the control of the mouse neurofilament (NFL) promoter have significantly increased CAT activity (up to 50 fold) when compared to similar experiments done with currently prepared extracts. In addition to the brain, in which the NFL gene is normally expressed, we detected significant levels of CAT activity in tissues (ovary, adrenals, and uterus) which were never shown to support CAT activity by other procedures. We tested whether the sensibility of our procedure would be as good with transiently transfected cell-lines. Plasmids containing the CAT reporter gene under the control of the mouse p12 promoter were transiently transfected in either pituitary GH4C1 or monkey kidney COS-1 cells. The hGH-normalized CAT activity detected in extracts prepared by our procedure proved to be at least 30-fold higher in both cell-types than in extracts prepared using the "Tris-Triton" procedure. Such an increase in the sensitivity of the CAT assay not only allows for the analysis of very weak promoters but it also provides an extremely powerfull tool to study the tissue-specificity mediated by such regulatory sequences. In addition, significant time saving can be achieved with much stronger promoters since relatively high levels chloramphenicol conversion can be detected in less than 30 minutes of incubation.

E 336 FUNCTIONAL ANTAGONISM BETWEEN ONCOPROTEIN C-JUN AND THE GLUCOCORTICOD RECEPTOR Roland Schüle*, Pundi Rangarajan*, Steven Kliewer*, Lynn J. Ransone+, Jack Bolado*, Na Yang*#, Inder M. Verma+ and Ronald M.Evans* *Howard Hughes Medical Institute +Molecular Biology and Virology Laboratory The Salk Institute for Biological Studies La Jolla, CA 92037 #Dept. of Chemistry University of California, San Diego La Jolla, CA 92093

We present evidence that the glucocorticoid receptor (GR) and transcription factor Jun/AP-1 can reciprocally repress each other's transcriptional activation by a novel mechanism which is independent of DNA binding. Overexpression of c-Jun prevents the glucocorticoid-induced activation of genes carrying a functional glucocorticoid response element (GRE). Furthermore, GR is able to repress transcriptional activation of the human collagenase gene or a heterologous promoter which contains the AP-1 binding site. Analysis of GR mutants in transfection studies reveals that both the ligand binding and DNA binding domains are important in the inhibition of Jun/AP-1 mediated transcriptional activation. Analysis of c-Jun mutants demonstrates that a region including the leucine zipper region of the c-Jun protein is obligatory for repression. Gel retardation analysis demonstrates that bacterially expressed c-Jun is able to prevent the formation of GR-GRE complexes. These data indicate that members of two distinct classes of transcription factors can oppose each other's activity through a mechanism likely involving protein-protein interactions.

E 337 GLUCOCORTICOID RECEPTOR REPRESSES TRANSCRIPTION OF THE HUMAN GLYCOPROTEIN α-SUBUNIT GENE IN A DNA-BINDING INDEPENDENT MANNER. Claudia Stauber, Joachim Altschmied, Ingrid Akerblom, Jesse Marron and Pamela L. Mellon. Regulatory Biology Laboratory, The Salk Institute, La Jolla, CA 90237

Transcription of the human glycoprotein α-subunit gene in the human placental cell line JEG-3 is positively regulated by the cyclic AMP-responsive element CRE and the tissue-specific element TSE (trophoblast-specific element). Cotransfected glucocorticoid receptor (GR) represses α-gene transcription 5 to 10-fold in a hormone dependent manner. It has been previously shown that this negative effect on transcription is mediated by the DNA binding domain of GR. Furthermore, four GR binding sequences have been identified by *in vitro* footprint analyses and these sequences match the GRE consensus sequence moderately. Two of these GREs partly overlap with the CREs, the others are located near the CCAAT-box and the TATA-box, respectively. When all of these GR binding sequences are mutated, we still observe transcriptional repression of the α-gene to a similar extent as obtained with the wildype promoter. Moreover, a GR mutant in which the binding specificity has been altered into an ERE binding receptor by a substitution of only 3 amino acids within the first zinc finger, can repress α-gene transcription. On the other hand, a mutant GR which has the second zinc finger replaced by the homologous finger of the thyroid receptor, exhibits no inhibitory activity, whereas if the first finger is switched, full repression can be measured. These experiments clearly indicate that DNA binding of GR to the identified binding sequences is not required for repression of α-gene transcription and that the second zinc finger plays a crucial role. Thus, repression is rather mediated by specific protein-protein interaction between GR and CREB. This conclusion is supported by *in vitro* gel shift experiments (see abstract by J.Altschmied) which show that GR is able to inhibit CREB binding to its cognate sequence.

E 338 ACTIVIN INHIBITION OF RAT GROWTH HORMONE GENE TRANSCRIPTION. R. Scott Struthers and Wylie Vale, Clayton Foundation Laboratories for Peptide Biology, The Salk Institute for Biological Studies, La Jolla, CA 92037.

Activins are homodimeric peptides structurally related to an extensive family of growth and differentiation factors that includes transforming growth factor-β. Though originally isolated from follicular fluid based on their ability to stimulate pituitary FSH secretion, activins have since been shown to exert potent effects on the function and/or proliferation of a variety of cell types, including induction of mesoderm during early *Xenopus* development. Activin-A has also been shown to act as a novel inhibitor of a complex set of intracellular responses in the somatotroph including cAMP synthesis, GH secretion, GH biosynthesis, and cell proliferation. Despite the abundance of activin's actions the underlying mechanisms remain uncharacterized.

In order to explore the possibility that inhibition of GH biosynthesis by activin is mediated by discrete cis acting sequences in the GH promoter, a GH promoter/chloramphenicol acetyl-transferase (CAT) transient transfection assay was developed in cultured somatotrophic cells. Receptors for both activin and growth hormone-releasing factor are present on these cells and were shown to be functional in regulating cAMP synthesis. Treatment with sub-nanomolar concentrations recombinant activin-A resulted in a 10-20 fold reduction in CAT enzyme activity. This inhibition of transcription does not appear to operate through previously characterized regulators of GH transcription (i.e. thyroid hormone, glucocorticoids or cAMP) suggesting the presence of an independent activin responsive element (ARE). Further, deletion mapping experiments indicate the presence of a discrete response element located within the proximal promoter region. These results suggest that inhibition of transcription can provide a possible mechanism by which activin exerts its differentiation and anti-proliferative effects.

E 339 MULTIPLE INTERACTIONS OF THE GLUCOCORTICOID RECEPTOR (GR) WITH OTHER PROTEINS AND DNA. Yu Lin Sun, Tom Schmidt†, Mona Nemer and Jacques Drouin, Laboratory of Molecular Genetics, Institut de recherches cliniques de Montréal, Montréal (Québec) CANADA, H2W 1R7 and †Department of Physiology, University of Iowa, Iowa City, IA 52242

We have characterized the *in vitro* interaction of the glucocorticoid receptor (GR) with different DNA binding sites, as well as with other nuclear proteins in order to define the molecular basis of GR's transcriptional role. Using purified rat liver GR in a gel retardation assay, oligonucleotides containing the consensus "glucocorticoid response element (GRE)" bound preferentially GR dimers as shown previously. However, significant binding of GR monomers was also observed. Mutant GREs in which only the consensus hexanucleotide TGTYCT was conserved (half GRE) preferentially bound GR monomers with significant affinity. A GR binding site present in the glucocorticoid-repressed pro-opiomelanocortin (POMC) gene bound GR with a similar apparent affinity compared to GREs. However, this sequence appeared to bind three GR subunits. In addition to binding DNA and other GR subunits, GR can interact with other proteins like HSP90 and, as we recently documented, other nuclear proteins like c-*jun*. It appears that both TPA induction and glucocorticoid repression of the collagenase gene are mediated through the TPA response element (TRE), which did not bind GR. However, addition of c-*jun* to GR significantly decreased its DNA binding activity. Cross-linking experiments suggested that this effect may depend on the formation of GR/c-*jun* complexes. The formation of these non-DNA binding complexes represents a novel and potentially widespread mechanism of transcriptional repression.

E 340 CHARACTERIZATION OF AN INDUCIBLE FACTOR (NF-GM2) THAT ACTIVATES TRANSCRIPTION OF GM-CSF GENE IN JURKAT CELLS

Akio Tsuboi, Yuko Yamaguchi-Iwai, Shoichiro Miyatake, and Naoko Arai
DNAX Research Institute, Department of Molecular Biology, 901 California Avenue, Palo Alto, California 94304-1104

Helper T cells produce a battery of lymphokines including GM-CSF when stimulated by antigens. This activation can be mimicked by the combination of phorbol ester and Ca^{2+} ionophore. A region between positions -96 and -72 upstream of the mouse GM-CSF gene (CLE2/GC box) is responsible for transcription activation induced by both phorbol ester and Ca^{2+} ionophore. This region contains two binding motifs; one is NF-GM2 binding sequence (GM2), GGTAGTTCCC, and the other is GC box sequence, CCGCCC. GM2 binding activity was detected only in the nuclear extract from stimulated Jurkat cells, whereas GC box can be recognized by constitutive factors (A1, A2 and B). We purified the inducible factor, NF-GM2, from the nuclear extract of stimulated Jurkat cells by DNA affinity chromatography. The purified NF-GM2 consists of 50 kD (p50) and 65 kD (p65) polypeptides that specifically bind to the GM2 motif. The p50, which could be eluted and renatured from a SDS polyacrylamide gel, gave DNA binding activity. The mobility of p50 complex is slightly faster than that of native NF-GM2 complex. Addition of p65 to the p50 led to the transition of p50 complex to a complex with the same mobility as that made by the native NF-GM2. Furthermore, the p65 gave rise to a faint band which migrated slower than the native NF-GM2 complex. These results revealed that p50 or p65 alone can form a protein-DNA complex; in the mixture, the p65 may associate preferentially with the p50 to make the NF-GM2 complex. Gel filtration and crosslinking analyses indicated that the NF-GM2 complex is composed mainly of $(p50\text{-}p65)_2$ tetramer, partially of p50-p65 dimer. In addition, *in vitro* transcription assay showed that the affinity-purified NF-GM2 activates the transcription from the Ig κB enhancer (GGAAAGTCCC) more effectively than that from the GM-CSF enhancer (CLE2/GC box). These results suggested that NF-GM2 is identical or highly homologous to NF-κB.

E 341 NOVEL MEMBERS OF THE STEROID HORMONE RECEPTOR SUPERFAMILY. Jonathan D. Tugwood, Isabelle Issemann and Stephen Green, Cell and Molecular Biology Department, ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire. SK10 4TJ. U.K.

The steroid hormone receptor superfamily consists of a group of structurally-related transcription factors activated by such diverse ligands as steroid and thyroid hormones, retinoic acid , and Vitamin D3.

We have isolated several new members of this receptor superfamily from a mouse liver cDNA library. One of these novel receptors is activated by a group of hypolipidaemic drugs. Data will be presented from the analysis of these newly-isolated receptors.

E 342 ACTIVATOR AND REPRESSOR FUNCTIONS OF THE HUMAN ESTROGEN RECEPTOR, Maty Tzukerman, Xiao-kun Zhang, Thomas Hermann, Ken N. Wills, Gerhart Graupner and Magnus Pfahl, Cancer Center, La Jolla Cancer Research Foundation, La Jolla, CA 92037
The conventional model for steroid hormone action has assumed that steroid hormone receptors act as transcriptional regulators only when complexed with their ligands. It has, however, become evident that most types of steroid receptors are present in the cell nucleus even in the absence of ligand. The presence of these regulatory proteins in the nucleus even in the absence of ligand suggest possible additional regulatory functions for these proteins. Here we have investigated the transcriptional regulatory functions of wild type and mutant forms of the human estrogen receptors and their interaction with a specific DNA sequence the symmetric estrogen responsive element (ERE). We find that the mutant val-400 receptor behaves like a classical steroid hormone receptor. In the presence of ligand its affinity for the ERE is increased, and its transcriptional activator function is ligand dependent. The wild type gly-400 receptor is a constitutive transcriptional activator that functions as a transcriptional activator and repressor. In the presence of agonist its activator activity is increased. The presence of antagonist increases its repressor function. Changes in these regulatory properties of ER may contribute to diseases and cancer.

E 343 ESTROGEN SUPPRESSION OF *erb*B2 GENE IN PROLIFERATING HUMAN BREAST CANCER CELLS IN VITRO AND IN NUDE MICE, Anni Wärri[1,2], Karin Majasuo[1,3], Aire M. Laine[1] and Kari Alitalo[4] and Pirkko Härkönen[3], Cancer Research Laboratory[1], Orion Corporation Farmos, 20101 Turku, Departments of Medical Biochemistry[2] and Anatomy[3], University of Turku, 20520 Turku, Cancer Biology Laboratory[4], University of Helsinki, 00290 Helsinki, Finland
Enhanced expression of the *erb*B2/HER-2/*neu* gene has been associated with an increased growth rate and a poor prognosis of human breast cancer. We have studied the relationship between *erb*B2 mRNA and protein expression and the regulation of cell growth by estrogen and antiestrogens in a human breast cancer cell line ZR-75-1 *in vitro* and in athymic nude mice. pS2 gene was used as an estrogen-stimulated marker. *erb*B2 mRNA was very low in the cells grown *in vitro* with estrogen which stimulated the cells to proliferate rapidly and induced the expression of pS2 mRNA. Upon hormone withdrawal, the expression of *erb*B2 mRNA increased to a high level, while pS2 mRNA declined to an undetectable level and cell proliferation was arrested. The biosynthesis and tyrosine kinase activity of *erb*B2 protein also increased. Upon estrogen addition, *erb*B2 mRNA decreased and pS2 mRNA was induced. The antiestrogens toremifene and tamoxifen opposed these estrogen actions. In nude mice ZR-75-1 cells formed tumors only if estrogen pellets were implanted and *erb*B2 mRNA was low and pS2 mRNA high in them. Administration of toremifene or tamoxifen or removal of estrogen pellet correspondingly increased *erb*B2 mRNA and abolished pS2 mRNA. The data show that estrogen suppression of *erb*B2 gene is associated with growth stimulation of the estrogen-dependent breast cancer cell line ZR-75-1 suggesting that *erb*B2 has important growth regulatory functions.

E 344 THYROID HORMONE INCREASES ACTIVITY OF A HEPATONUCLEAR SPECIFIC FACTOR THAT REPRESSES S14 GENE TRANSCRIPTION <u>*in vivo*</u> AND <u>*in vitro*</u>. Norman C.W. Wong and Frances E. Carr, Department of Medicine & Medical Biochemistry, University of Calgary, Calgary, AB, CANADA, T2N-4N1, and Kyle Metabolic Unit, Walter Reed Memorial Hospital, Washington, DC, USA, 20307-5001.
Previous studies demonstrated the presence of a liver specific nuclear factor, P-1 that binds to nucleotides -310 to -288 of the thyroid hormone responsive S14 gene. The close proximity of P-1 to the transcription initiation site suggests that it may play an important role in regulating expression of the gene. Recent findings indicated that the DNA binding activity of P-1 increases in response to L-triiodothyronine (T3). However, the T3-induction of P-1 DNA binding activity followed the rise in mRNA-S14, thus making it unlikely to function as a proximal stimulus that initiates S14 gene expression. To determine the role of P-1 we examined DNA binding activity of the protein in response to aging and in genetically obese and lean, Corpulent rats. In euthyroid animals, results showed an inverse correlation between P-1 and the level of mRNA-S14 raising the possibility that the protein may act to repress gene activity. To further investigate the repressor hypothesis, we have used a cell-free in vitro transcription system to assay activity of templates with or without the P-1 site attached to a 'G' free cassette (GFC). The results showed that transcription of the GFC was decreased 3 to 4-fold in the presence of the P-1 motif. Next we used transient transfections to study heterologous constructs with or without the P-1 site inserted upstream of viral TK-promoter attached to the CAT gene. Relative to Hep G2 cells transfected with TK-CAT, basal CAT activity in cells transfected with the same plasmid containing a P-1 site was reduced 3-fold. A further decrease was observed following the addition of T3. In summary, DNA binding activity of P-1 in response to age, and in obese and lean euthyroid animals, correlated inversely with expression of S14 mRNA suggesting a possible repressor role for the protein. The P-1 motif inhibited transcription of the GFC or CAT reporter DNA's in a cell-free transcription or transient transfection assays, respectively. The inhibition correlated with the level of thyroid hormone in cultured cells.

E 345 ANTAGONISM BETWEEN RETINOIDS AND PHORBOL ESTERS MEDIATED BY MUTUAL INHIBITION OF DNA BINDING BETWEEN C-JUN/C-FOS AND RETINOIC ACID RECEPTORS, Xiao-kun Zhang*, Hsin-Fang Yang-Yen+, Michael Karin+ and Magnus Pfahl*, Cancer Center, La Jolla Cancer Research Foundation, La Jolla, CA 92037 and Center for Molecular Genetics, School of Medicine, Dept. of Pharmacology, University of California, San Diego, La Jolla, CA 92093

Positive and negative control of gene expression by retinoids is mediated by nuclear receptors that belong to a large family of regulatory proteins including the steroid and thyroid hormone receptors, the vitamin D receptor and retinoic acid receptors (RAR). RA and other retinoids are potent inhibitors of collagenase gene induction by phorbol esters (TPA) and inflammatory mediators and have shown promise as therapeutic agents in rheumatoid arthritis. Transfection experiments indicate that negative effect of retinoids is mediated through the AP-1 site within the collagenase promoter and requires the presence of RARs. Further studies show that the RARs are potent inhibitors of AP-1 activity and that both c-jun and c-fos are potent repressors of RAR activity.* In vitro gel retardation assays show that this mutual repression is due to mutual inhibition of DNA binding activities. These studies therefore reveal a novel mechanism for transcriptional regulation between two major signal transduction pathways in response to extracellular stimuli.

Late Abstracts

RETINOIC ACID AND VITAMIN D_3 INCREASE THE EXPRESSION OF THE INOSITOL TRISPHOSPHATE RECEPTOR GENE IN HUMAN LEUKEMIC (HL-60) CELLS

Peter G. Bradford and Pei Hui, Department of Pharmacology and Therapeutics, School of Medicine and Biomedical Sciences, SUNY Buffalo, Buffalo, NY 14214

Treatment of human leukemic (HL-60) cells with either retinoic acid (RA) or 1,25-dihydroxyvitamin D_3 induces a program of cellular differentiation converting a population of largely promyelocytic cells into one comprised principally of polymorphonuclear cells or monocytic cells, respectively. Accompanying this differentiation process is an increased expression of the 260 kDa inositol trisphosphate receptor protein. In RA-treated HL-60 cells, the K_D for Ins(1,4,5)P_3 binding remains unchanged at 19 nM, but the maximal binding increases from 98 to 350 fmol/mg protein. After RA treatment, the mRNA encoding the $InsP_3$ receptor is increased dramatically and is shown to be a consequence of increased transcriptional start rates rather than stabilization of existing mRNA. Under similar conditions expression of housekeeping genes remains unchanged. The kinetics of mRNA expression and the dependence on protein synthesis, make it likely that the effect of RA on $InsP_3$ receptor mRNA may be secondary to RA receptor-dependent synthesis of primary transcripts.

DISTINCT MOLECULAR DETERMINANTS ARE INVOLVED IN AGONIST-INDUCED UNCOUPLING AND DOWN-REGULATION OF THE β_2-ADRENERGIC RECEPTOR, Michel Bouvier, Manon Valiquette and Hélène Bonin, Department of Biochemistry, Université de Montréal, Montréal, Québec, Canada, H3C 3J7.

Agonist mediated desensitization of transmembrane receptors is believed to play a crucial role in the regulation of cellular signalling across biological membranes. We recently documented the role of agonist-induced phosphorylation of the β_2-adrenergic receptor (β_2AR) in the desensitization of the β-adrenergic-stimulated adenylyl cyclase activity. Indeed, phosphorylation of the β_2AR, on serines and/or threonines, by the protein kinase A and the β-adrenergic receptor kinase has been shown to lead to the functional uncoupling of the receptor from the adenylyl cyclase stimulatory pathway. This uncoupling from the stimulatory guanine nucleotide binding protein (G_s) represent the first event occuring during the agonist-mediated desensitization process. However, the internalization and the down-regulation of the receptor, which also accompany the desensitization, does not require the phosphorylation of the receptor by these kinases. The internalization of several receptors (Mannose-6-PO_4, LDL, Poly-Ig) via clathrin coated vesicles involves tyrosine residues located in the carboxyl tail of these receptors. Here, we report that the substitution of alanine residues for the tyrosine-350 and -354 of the β_2AR (located at 20 and 24 amino acids from the plasma membrane in the cytoplasmic carboxyl tail) significantly delays and decreases the agonist-mediated down-regulation of the β_2AR. This mutation of the receptor also leads to a partial uncoupling of the receptor from Gs suggesting that this domain of the receptor is also involved in the interaction of the receptor with G_s. By selectively mutating only one of the two tyrosines we found that the presence of any of these two residues is sufficient to maintain a normal profile of down-regulation. These results therefore suggest that similarly to what has been reported for receptors internalized via clathrin-coated vesicles, tyrosine residues may play an important role in the internalization and the down-regulation of G-coupled receptors.

THE REGULATION OF MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I GENE EXPRESSION BY INTERFERON REGULATORY FACTOR-1. Cheong-Hee Chang, Juergen Hammer, William Fodor and Richard Flavell, Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510

To investigate the mechanism of regulation of major histocompatibility complex (MHC) class I gene expression by interferon, we have cloned, expressed and characterized the gene encoding the protein which binds to Interferon Response Sequence (IRS) of the MHC class I promoter from HeLa cells. This is identical to the Interferon Regulatory Factor-1 (IRF-1) which was previously described (Maruyama M. et al., 1989). The expression level of the construct which contains either HLA-A2 or H-2K^b promoter fused to the human β-globin gene coding sequences is increased by 3-13 folds when the IRF-1 cDNA is cotransfected transiently. The level of expression is further induced by adding interferon-γ to the cells. The mutation of IRS in H-2K^b promoter does not respond to the IRF-1 whereas the mutation in NF-κB binding site does. These data demonstrate that the IRF-1 seems to be responsible for the IFN-mediated induction of MHC class I gene expression.

TR3 RECEPTOR IS A TRANSCRIPTIONAL ACTIVATOR John Kokontis[1] & Chawnshang Chang[1,2] [1]Department of Surgery/Urology and Ben May Institute, University of Chicago, Chicago Il 60637, [2]Department of Human Oncology, University of Wiscosin, Madison, WI 53792.

TR3 receptor (Chang, et al, 1989 J. Steroid Biochem. 34, 391) is the human homolog of the mouse NUR/77 and rat NGF1-B genes. When a chimeric TR3 receptor cDNA construct containing the DNA binding domain of the androgen receptor (TR3/AR/TR3) is cotransfected with an MMTV-CAT reporter plasmid into mouse or human cells, CAT activity could be strongly induced. In mouse L cell fibroblasts, TR3/AR/TR3 receptor induction of CAT expression is dependent upon the serum (charcoal-treated) concentration in the culture medium, while in the human prostatic tumor cell line PC3, TR3/AR/TR3 receptor induction is independent of serum concentration. TR3/AR/TR3 receptor induction of CAT activity is inhibited by cotransfection of c-Jun expression vectors, similar to the recent results with glucocorticoid receptor. The converse chimeric construction (AR/TR3/AR) exhibits weak induction of CAT activity in the presence of androgen. From MMTV deletion analysis, this activation appears to be mediated by binding to a site distinct from the MMTV-HRE. Intact TR3 receptor does not induce CAT expression in this system.

INSULIN-LIKE GROWTH FACTORS AND THEIR RECEPTORS IN NEPHROGENESIS

Edward Chin, Jian Zhou and Carolyn Bondy, Developmental Endocrinology Branch, NICHD, NIH.

IGF-II is expressed in renal embryonal tumors and IGF-I has been implicated in the pathogenesis of postnatal renal hypertrophy. In order to illuminate the potential roles of IGFs in normal renal growth and differentiation, we have examined the pattern of gene expression for IGF-I, IGF-II and their receptors during embryonic and postnatal development in the rat kidney. IGF-II mRNA levels are high in primordial, nephrogenic renal vesicles and gradually diminish as these structures differentiate into nephrons. After nephron formation is completed, IGF-II mRNA is undetectable in the kidney. Messenger RNA for both type-I and type-II receptors is co-localized with IGF-II in the renal vesicles, suggesting an autocrine mode of action for IGF-II in the proliferation or epithelial transformation of the nephrogenic precursors, and a potential role for both types of receptor in this process. Type-II receptor gene expression is also seen in the developing renal medulla. With the maturation of the renal medulla, type-II receptor gene expression decreases, however, there is increasing medullary IGF-I and type-I receptor gene expression. IGF-I mRNA is confined to a sharply demarcated zone in the inner stripe of the outer medulla. Type-I receptor mRNA is also abundant in this region, but is seen in adjacent regions of the inner and outer medulla as well. These findings support the existence of an autocrine/paracrine IGF-I loop in the adult kidney which may function in renal metabolic activity as well as in compensatory hypertrophy.

CHARACTERIZATION AND PURIFICATION OF A PROTEIN COMPLEX BINDING TO A TC-MOTIF IN THE HUMAN UROKINASE PROMOTER AND TO THE NF-kB ELEMENT IN THE SV40 ENHANCER AND HIV LTR.

Stig K. Hansen, Claus Nerlov, Morten Johnsen and Francesco Blasi. Institute of Microbiologi, University of Copenhagen, Denmark.

The TPA stimulation of the human urokinase (uPA) promoter involves a TC-motif with homology to the NF-kB element in the SV 40 enhancer and HIV LTR. The DNA-binding protein complex (TRF) is different from NF-kB/KBF1 and AP-2 with respect to binding specificity and molecular weight. The constitutive binding activity has been found in both lymphoid and nonlymphoid cells. Partially purified TRF binds to a single TC-motif overlapping the NF-kB site in the SV40 enhancer and HIV LTR. Multimerized binding sites are able to confer TPA response upon a uPA minimal promoter in both HepG2 and HT1080 cells. It is possible that the identified protein complex is related to NF-kB/p50 or other rel homologous proteins.

AN 18 bp REGION OF THE ACUTE PHASE α_2-MACROGLOBULIN GENE CONFERS SYNERGISTIC INDUCIBILITY BY IL6 AND GLUCOCORTICOIDS IN HUMAN HEPATOMA CELLS. G. Hocke, D. Barry and G.H. Fey, Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

The acute phase α_2-macroglobulin (α_2M) gene contains a cis-acting DNA control element called the IL6-RE that mediates induction by IL6. Acute phase rat liver cells contain nuclear proteins that form a sequence specific complex (complex II) with the hexanucleotide core CTGGGA of the IL6-RE. IL6 treated human Hep3B hepatoma and U266 B myeloma cells contain IL6-inducible nuclear proteins capable of forming an indistinguishable, sequence specific complex II with the IL6-RE. The IL6-RE core element is sufficient to confer inducibility by I6 in Hep3B cells, suggesting an essential role for complex II in the transcriptional induction by IL6. Four tandem copies of an 18 bp sequence containing the IL6-RE were linked to an SV 40 promoter and a luciferase reporter gene. This construct was transfected into Hep3B cells. A 3.2 fold induced transcription was obtained by IL6 alone and a 22 fold synergistic induction by IL6 plus dexamethasone. Thus, the 18 bp sequence was sufficient to confer both induction by IL6 and the synergistic action of IL6 plus dexamethasone, although it lacks a consensus binding site for the glucocorticoid receptor. The same construct failed to generate an IL6 response after transfection into U266 cells, suggesting these cells lack essential components for an IL6 response other than those contained in complex II.

MOLECULAR CLONING OF TISSUE-SPECIFIC EXTINGUISHER 1, Keith W. Jones and R.E. Keith Fournier, Department of Molecular Medicine, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

Somatic cell hybrids formed by fusion of hepatoma cells with non-hepatic cells fail to express liver-specific genes, a phenomenon termed extinction. Extinction of the tyrosine aminotransferase (TAT) and phosphoenolpyruvate carboxykinase (PEPCK) genes involves a discrete genetic locus on human chromosome 17 (Hsa17) termed tissue-specific extinguisher-1 (TSE1). Using fragment-containing microcell hybrids, TSE1 has been mapped to Hsa17q23-24. To isolate TSE1, a cDNA probe was prepared from a microcell hybrid cell line which contains Hsa17 sequences including q23-24. Using solution hybridization techniques, the probe was enriched for 17q23-24-specific cDNAs by removing sequences present in a nearly isogenic microcell hybrid line which does not contain the 17q23-24 interval. The resulting subtracted probe was used to screen a human skin fibroblast cDNA library. A number of unique cDNAs which map to 17q23-24 were identified by the subtracted probe. Only one of these cDNAs displayed a concordant retention pattern to TSE1 in a panel of microcell hybrids which contain deletions in the 17q23-24 region. Sequence analysis showed this cDNA to be homologous to RIα, a regulatory subunit of protein kinase A. In order to test the possibility that RIα is TSE1, RIα-containing expression vectors were transfected into the rat hepatoma cell line FTO-2B. RIα transfectants displayed a reduced level of TAT and PEPCK gene expression in the absence of cAMP, while expression in cAMP-induced cells was restored to levels seen in induced FTO-2B cells. This observation is consistent with previous results which demonstrate that expression of TSE1-repressed genes can be restored to levels seen in hepatoma cells by treatment with cAMP. Therefore, we conclude that by using a subtractive hybridization approach we have isolated TSE1 and identified it to be RIα based on its retention pattern in a microcell hybrid panel and its ability to reproduce the TSE1 phenotype when transfected into rat hepatoma cells.

EXPRESSION AND ANALYSIS OF BINDING AND FUNCTIONAL DOMAINS OF THE THYROTROPIN RECEPTOR, Kathleen Mountjoy, Chuck Passavant, Linda Rehfuss, Linda Robbins, and Roger Cone, Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, Oregon.

Graves' disease is a major autoimmune disease of man causing a hyperthyroidism which results directly from the development of autoantibodies against the thyroid stimulating hormone receptor (TSH-R). Recently, the human TSH-R as well as the human LH/CG receptors, another member of the glycoprotein hormone receptor family, were cloned (Mol. Endo. 4:1264, 1990). Using the cDNA sequences for these receptors we have 1) constructed chimeric receptors in order to define the TSH and Graves' autoantibody binding sites on the TSH receptor and 2) expressed the amino terminal portion of the TSH receptor using the E. Coli trp E fusion system and raised high titer polyclonal antibodies against this protein in rabbits. The polyclonal antibodies will be used to quantitate receptor protein in cells transfected with chimeric receptor and also to characterize the TSH receptor protein. The glycoprotein hormone receptors are a unique subgroup of the G protein-linked receptor family, with a large extracellular amino terminal domain followed by a 7 membrane spanning domain. Evidence suggests that high affinity hormone binding to these receptors occurs somewhere in the amino terminal domain. The first chimeric receptor that we have constructed comprises the complete amino terminal domain of the TSH-R and the entire transmembrane spanning domain of the CG/LH-R. A retrovirus expression vector, pLJ, containing either the chimeric TSH/LH receptor or the wild type TSH-R was stably transfected into human 293 cells. When cells transfected with the chimeric receptor were stimulated with hTSH for 1 hour, a maximum 4-fold induction of cAMP was observed with $1x10^{-8}$ M TSH. In contrast, TSH stimulation of cells transfected with wild type TSH-R produced a 360-fold induction of cAMP with only $5\text{-}7x10^{-9}$ M TSH. This data suggest that there are specific regions in the transmembrane domain of the TSH-R that are necessary for activation of this receptor.

REGULATION OF PTPASE 1B IN HUMAN CELLS, Benjamin G. Neel, Victor Shifrin, Christine A. Jost and Pamela H. Beahm, Molecular Medicine Unit, Beth Israel Hospital, Boston, MA. 02215

We have previously cloned a cDNA for the major transcript (3.5 kb) of the human tyrosine phosphatase, PTPase 1B and are now studying its transcriptional and post-transcriptional regulation. We have identified a new, 4.5 kb transcript that is induced by treatment of quiescent human fibroblasts with serum or EGF. Induction requires protein synthesis, implying a requirement for early response gene products. We are currently cloning the cDNA for this transcript to determine whether it is an alternative splice product of PTPase 1B or the product of a new gene. We have also raised and affinity-purified antibodies to PTPase 1B. Interestingly, immunoprecipitations under stringent conditions suggest that PTPase 1B may be part of a complex with at least three higher molecular weight proteins. The possible implications of these finding for PTPase control and signalling will be discussed.

GLUCOCORTICOID RECEPTOR DIMERIZATION IS REQUIRED FOR EFFICIENT DNA-BINDING. **Sam Okret**, William Cairns, Carol Cairns, Ingemar Pongratz, Jan-Åke Gustafsson and Lorenz Poellinger
Dept. of Medical Nutrition, Karolinska Institute, Huddinge University Hospital, Novum F60, S-141 86 Huddinge, Sweden

Glucocorticoid hormones regulate specific gene activation through interaction with and activation of an intracellular glucocorticoid receptor (GR). The hormone-receptor complex recognises specific DNA sequences (GREs) in target genes and modulates the transcriptional activity of these genes. Functional GREs are palindromic, each half of the palindrome recognizing one receptor molecule. We were interested to see whether receptor dimerization occured in cytosol (crude receptor preparations) prior to DNA-binding and if this is a requirement for efficient DNA-binding. Centrifugation of cytosolic GR by density gradient centrifugation into a 4S monomeric and 6S multimeric complex showed that the 6S peak bound specific DNA sequences much more efficiently than the 4S peak. Gel retardation assays with full and half palindromic GREs resulted in GR-GRE complexes of identical mobilities. Taken together our results suggests that GR dimerization occurs prior to DNA binding and is required for efficient interaction of the GR with its GRE.

STRUCTURE OF THE RAT PEPTIDYLGLYCINE α-AMIDATING MONOOXYGENASE (PAM) GENE, Ouafik,L'H, Stoffers,DA, Campbell,TA, Bloomquist,BT and Eipper,BA, Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD, USA, 21205

Bioactive α-amidated peptides are synthesized from Gly-extended precursors by the sequential action of two enzymatic activities derived from the bifunctional PAM precursor. Peptidylglycine α-hydroxylating monooxygenase (PHM) catalyzes the Cu, ascorbate and O_2 dependent formation of a peptidyl-α-hydroxyglycine intermediate that is stable at physiological pH; its conversion into an active product is catalyzed by peptidyl-α-hydroxyglycine α-amidating lyase (PAL). The PAM precursor consists of an NH_2-terminal signal peptide followed by PHM, PAL, and putative transmembrane and cytoplasmic domains. Since tissue-specific and developmentally regulated alternative splicing generate a number of soluble and membrane associated forms of PAM from what appears to be a single gene, elucidation of the structure of the gene was undertaken. A rat genomic library constructed in Lambda FIX was screened with cDNA fragments spanning the sequence of PAM. Eight partially overlapping phage encompassing over 100 kb of genomic DNA were mapped and exon-containing fragments were subcloned and sequenced. Thus far, 13 exons have been identified, several corresponding to predicted functional domains of the protein. For example, Optional Exon A (expressed primarily in heart and CNS), Optional Exon B (which includes the putative transmembrane domain), and the 3'-untranslated regions are all contained within individual exons. In addition, genomic sequencing has confirmed the presence of at least one intron within the 5'-untranslated region, while primer extension utilizing RNA from adult rat atrium indicates that most transcriptional initiation begins approximately 550 bp upstream of the translational start.

THE PROMOTER FOR A BRAIN-SPECIFIC SUBUNIT OF cAMP-DEPENDENT PROTEIN KINASE DRIVES TRANSGENE EXPRESSION IN THE CNS. Kimberly V. Rogers, G. Stanley McKnight, Christopher H. Clegg*. Department of Pharmacology SJ-30, University of Washington, Seattle, WA, 98195; *Department of Molecular Biology, Oncogen Inc., Seattle, WA, 98121.

The cAMP-dependent protein kinase (cA-PK) is important in signal transduction and mediates the activity of numerous hormones and neurotransmitters. RIβ is a cA-PK regulatory subunit that is unique to the CNS and is expressed in a number of regions including the cortex, hippocampus, and several hypothalamic nuclei. We have isolated genomic clones for RIβ that span 80 kilobases. S1 analysis of the 5' region of the gene indicates that multiple transcription start sites are used within a 60 bp region. A fusion gene consisting of a 3.5 kb RIβ promoter fragment linked to the E. coli *lacZ* gene was used to generate transgenic mice. Expression of the transgene in these animals is specific to the CNS and the localization of transgene expression parallels that of the endogenous gene. Two of the three transgenic lines had *lacZ* mRNA levels similar to those of the endogenous gene whereas the third had undetectable levels of message despite the presence of β-galactosidase activity. The level of transgene expression did not correllate with the transgene copy number. These experiments demonstrate that some of the elements necessary for the tissue specific expression of the RIβ gene reside within this 3.5 kb fragment. Further analysis of this promoter should yield information regarding the transcription factors and the corresponding genetic elements that are important in the expression of neural specific genes. In addition, this promoter fragment will be useful for targeting genes to a specific subset of neurons within the CNS.

RAPID STIMULATION BY GROWTH HORMONE OF SOMATOSTATIN mRNA IN THE PERIVENTRICULAR NUCLEUS OF THE ADULT MALE RAT, Robert A. Steiner, Elizabeth Redmond, Philip Zeitler and Donald K Clifton, Departments of Physiology & Biophysics and Obstetrics & Gynecology, University of Washington, Seattle, WA 98195.

We have recently demonstrated that both SS and GHRH mRNA content in the hypothalamus of adult male rats vary in a manner consistent with the existence of an ultradian rhythm. However, the mechanism underlying this rhythm is unknown. Since previous investigations have demonstrated that GH can stimulate SS mRNA content, at least over a period of days, we tested the hypothesis that GH can stimulate SS mRNA content with a time course sufficiently rapid to account for the ultradian rhythm. Hypophysectomized adult male rats were fitted with jugular catheters, injected with either GH (1 mg) or vehicle and sacrificed at 2, 4 and 8 h after injection (n=4 at each time). Using *in situ* hybridization, we measured SS mRNA levels in individual neurons of the PeN and compared these levels among experimental groups. We report results (based on ~50 cells per animal) that SS mRNA content is increased at 2 and 4 h after the GH injection and returns to control values by 8 h. Conclusion: Rapid feedback of GH on SS gene expression may underlie, at least in part, the ultradian rhythm in SS mRNA content in the hypothalamus of the adult male rat.

I-POU: A non-DNA binding POU-domain protein act as a negative regulator of transcription through specific protein/protein interactions. Maurice N. Treacy and Michael G. Rosenfeld, University of California, San Diego, School of Medicine,M-068, La Jolla, CA 92093-0648.

We have isolated two POU-domain proteins from *Drosophila*; Inhibitor POU(I-POU) and *Drosophila* POU-1(DP-1). I-POU and DP-1 are co-expressed temporally in a subset of neurons in the CNS of *Dropophila*. I-POU lacks two basic residues in the POU-homeodomain and has no intrincic DNA binding activity. I-POU specifically forms a heterodimeric complex with DP-1 and specifically inhibits DP-1's ability to transactivate the *Drosophila* Dopa-decarboxylase gene. We propose that I-POU is a dominant negative regulator of DP-1 and constitutes a new class of POU-domain protein genes.

THE REGULATION OF PDGF AND PDGF RECEPTOR EXPRESSION IN F9 TERATOCARCINOMA CELLS. C. W. Wang, M. Mercola, and C. D. Stiles, Division of Cellular and Molecular Biology, Dana-Farber Cancer Institute, Boston, MA 01115.

The PDGF A gene (but not the PDGF B gene) and the PDGF α-receptor gene (but not the β-receptor) are expressed at early times in embryonic development of mice (1). Interaction of PDGF A:A homodimers with PDGF α:α-receptors may be essential for correct development of the mouse embryo. An embryonic lethal mutation in mice termed "patch" incorporates a deletion of coding sequences for the PDGF α-receptor (2). The apparent role of PDGF A:A and its receptor in early embryonic development prompted us to study regulation of the PDGF A and α-receptor genes in an experimentally more pliable system - mouse F9 embryonic carcinoma cells.

We show that expression of PDGF A and PDGF α-receptor is regulated by the morphogen retinoic acid. Retinoids regulate PDGF A gene expression at the translational level. F9 stem cells and retinoid treated differentiated F9 cells express PDGF A mRNA but only the stem cells produce PDGF A:A protein dimers. In contrast to PDGF A, which is produced only in undifferentiated F9 stem cells, the PDGF α:α-receptor protein is expressed only in retinoid-treated, differentiated F9 cells. The F9 stem cells do not transcribe the PDGF α-receptor gene until they are exposed to retinoic acid.

MAPPING THE CRITICAL IL-3 BINDING STRUCTURE IN CLONED MURINE IL-3 RECEPTOR. Huey-Mei Wang, Toshiya Ogorochi, Ken-ichi Arai, and Atsushi Miyajima, Department of Molecular Biology, DNAX Research Institute, Palo Alto, CA 94304

We recently isolated two highly homologous cDNAs (AIC2A and AIC2B) from a mouse mast cell line by expression cloning using an anti-IL-3 receptor antibody (anti-Aic2). The extracellular regions of the proteins encoded by these two cDNAs can be devided into two homologous domains, and each contains common motifs that are shared by the cytokine receptor gene family. Fibroblasts transfected with the AIC2A cDNA bound IL-3 with low affinity (*kd* of 18 nM). The soluble IL-3 receptor produced by transfected COS7 cells bound IL-3 with a similar affinity, indicating that IL-3 binding is mediated only by the external domain. Despite its 91% identity to AIC2A protein in amino acid sequence, the AIC2B protein did not bind IL-3 at all. Chimeric receptors made by exchanging regions between these two AIC2 cDNAs demonstrated that the second domain, located close to the membrane, determined the IL-3 binding capacity of the receptor. Detailed site-directed mutagenesis of the AIC2A cDNA revealed that IL-3 recognized the structure formed by both domains and bound to a critical region in the second domain.

PHOSPHORYLATION REGULATES CHICKEN PROGESTERONE RECEPTOR STRUCTURE AND FUNCTION. Nancy L. Weigel, Larry A. Denner, William T. Schrader and Bert W. O'Malley, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030

We have identified 5 phosphorylation sites in the chicken progesterone receptor. These sites can be divided into three classes: 1. those which are partially phosphorylated, but whose phosphorylation increases in response to progesterone, 2. a site which is phosphorylated only in response to progesterone administration, 3. sites which are constitutively phosphorylated. The class 1 sites are in the amino terminal portion of the receptor. The class 2 site is in the hinge region between the hormone binding and DNA binding domains. The class 3 sites are in a transactivation domain on the amino terminal side of the DNA binding domain. Phosphorylation of the hinge site correlates with receptor hsp90 dissociation suggesting that this phosphorylation is important for dissociation of the complex and subsequent formation of receptor dimers. Using transient transfection of CV1 cells, we have found that stimulation of kinase activity or inhibition of phosphatase activity enhances hormone dependent receptor mediated transcription. Surprisingly, we found that these compounds were able to substitute for progesterone to produce receptor mediated hormone independent transcription. Studies are in progress using site directed mutagenesis to determine the roles of the individual phosphorylation sites in receptor function.

CLONING AND CHARACTERIZATION OF G PROTEIN-COUPLED RECEPTORS IN THE MOUSE-GUINEA PIG COCHLEA, Edward R. Wilcox, Kenneth M. Grundfast, Masayoshi Tachibana, and Jörgen Fex, Laboratory of Molecular Otology, Bldg. 36 Rm. 5D-08, NIDCD/NIH, Bethesda, MD 20892.

All known members of the family of G protein-coupled receptors show some amino acid homology. By using oligonucleotide sequences that are common to the genes of members of this family of proteins, it was possible to PCR amplify sequences of DNA that then also share some homology with these family members. PCR was performed on cDNA made from RNA isolated from total mouse cochlea as well as from micro-dissected guinea pig organ of Corti and stria/spiral ligament. Such amplified and isolated sequences are for putative G protein-coupled receptors and may lead to new members of the family. The putative G protein-coupled receptors possibly represent neuro-transmitter receptors in the organ of Corti as well as putative hormonal and ligand receptors in the vascular stria of the cochlea. Work is in progress in the cellular localization of these receptors. As these receptors are examined using the tools of molecular biology, their role in the function of the cochlea may become more apparent.

ANDROGEN RECEPTOR BINDING SEQUENCE ON THE ANDROGEN INDUCIBLE PSBP C3(1) GENE PROMOTOR, Yang-Lian Zhang[1], Malcolm G. Parker[2] & Chawnshang Chang[1] [1]Department of Human Oncology, University of Wiscosin, Madison, WI 53792. [2]Molecular Endocrinology Lab. Imperial Cancer Research Fund. London WC2A 3PX U.K.

Androgen responsive elements(ARE) is still remaining uncertain and being searched so far on some androgen inducible genes. By DNase I footprinting analysis, we have observed a print around -140 to -119 on the PSBP C3(1) gene promotor (-160 to +40) which has a TGTTCT core sequence conserved for GRE and followed a inperfect repeat of a TCTTCT modified sequence. The androgen receptor(AR) binding activity of this 31 b.p. (-149 to -119) oligos was tested *in vitro* by gel retardation analysis. Cytosol from androgen target tissues incubated with ^{32}P end labeled 31 b.p. probe gave a specific shifting band for the DNA-protein complex. The intensity of the shifting band was correlated well with the AR content in the cytosol. The shifting band can be abolished by incubating cytosol with monoclonal antibody against AR (amino acid 331 to 572 , 40% of the N-terminal domain and 25% of the DNA binding domain of the human AR) . Comparing with the MMTV(-188 to -163) fragment, a known HRE for PR, GR & AR, the AR binding activity of the C3(1) (-149 to -119) fragment is 10 times stronger than that of the MMTV. The prospects of applying this assay to clinical use are in progress. The binding characterization of different forms of AR with this 31 b.p. oligos was also being investigated.

IGF-II, IGF-II RECEPTOR AND WILMS' TUMOR GENE EXPRESSION IN THE PLACENTA.

Jian Zhou, Edward Chin and Carolyn Bondy, Developmental Endocrinology Branch, NICHD, NIH, Bethesda, MD.

The placenta's tumor-like growth and invasion of maternal tissue provides an opportune model for the study of factors involved in the regulation of cell proliferation and differentiation. We have used high resolution in situ hybridization to map the cellular pattern of IGF-II, IGF-II receptor and Wilms' tumor gene expression in the developing rat placenta. During the course of placental growth, IGF-II mRNA levels are very high in the proliferative cytotrophoblastic layer, but very diminished in the differentiated, post-mitotic syncytiotrophoblast layer, which arises from the cytotrophoblastic lineage. Very high levels of IGF-II receptor mRNA are co-localized with IGF-II, strongly suggesting an autocrine mode of action for IGF-II and a significant role for the controversial IGF-II receptor in this setting. Since the Wilms' tumor gene product has been implicated in the regulation of IGF-II gene expression, we examined its pattern of expression in these same placental tissues. Preliminary results show a negative relationship between IGF-II and Wilm's tumor gene expression. No Wilms' mRNA is detected in the cytotrophoblastic layers where IGF-II mRNA is highly abundant and, conversely, Wilm's tumor gene expression appears significant in the differentiated portion of the placenta where IGF-II mRNA is absent. These findings support the hypothesis that the Wilm's tumor gene product is a transcription factor normally involved in the suppression of growth factor gene expression during the course of cellular differentiation.