MEMBRANE RECEPTORS AND CELLULAR REGULATION

M. P. Czech and C. R. Kahn, Organizers

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Plenary Sessions

Poster Sessions

Receptor Turnover and Life Cycles

0626 ENDOCYTIC VESICLE ACIDIFICATION AND THE INTRACELLULAR PATHWAYS OF LIGANDS AND RECEPTORS. Frederick R. Maxfield, Darrell J. Yamashiro, Mario DiPaola, Benjamin Tycko, and Sharon R. Fluss. New York University School of Medicine, Department of Pharmacology, New York, New York 10016.

It has now been shown for several cell types that ligands and receptors which enter cells by receptor-mediated endocytosis are exposed to an acidic environment (pH 5.0 - 5.5) within the first few minutes after internalization. This exposure to an acidic pH can have several consequences, including dissociation of certain ligands from their receptors and the penetration of toxin chains or viral nucleocapsids into the cytoplasm. Experiments from several laboratories are consistent with the hypothesis that these pH-mediated events occur in a prelysosomal endocytic compartment. Acidification of endocytic vesicles occurs by an ATP-dependent mechanism which is similiar to, but not identical with, the acidification mechamism for lysosomes. Experiments with photolabelling reagents, lactoperoxidase iodination, and proteases suggest that the epidermal growth factor receptor and the asialoglycoprotein receptor undergo a large conformational change between pH 7.0 and pH 5.6, resulting in a decrease in the surface area exposed to solvent. This conformational change may facilitate ligand dissociation while protecting the receptors from any hydrolytic enzymes in the endocytic compartments. Similiar changes could facilitate the penetration of virus coats and toxin chains into the membrane.

0627 PROCESSING AND MATURATION OF FUNCTIONAL INSULIN RECEPTOR IN 3T3-L1 ADIPOCYTES. M. Daniel Lane, Gabriele V. Ronnett, Tracy L. Simpson and Ronald Kohanski. Department of Physiological Chemistry, The Johns Hopkins University Medical School, Baltimore, MD 21205

Glycosylation is required for the formation of functional insulin receptor in 3T3-L1 adipocytes (1,2). Following translation of the proreceptor polypeptide, about 1.5 h are required to form active receptor (3). An additional 1.5 h are needed for the active receptor to be inserted into the plasma membrane (4).

Tunicamycin (TM) causes the depletion of cell-surface and total cellular insulin binding activity, but does not affect the rate of degradation of existing receptors. Rather, no functional receptor is synthesized in the presence of TM. Inactive aglyco-proreceptor is synthesized in the presence of TM, since previously synthesized active light receptor can be rescued from these cells when TM is removed concomitant with addition of heavy amino acids.

To study the post-translational processing of the receptor, metabolic labeling of 3T3-L1 adipocytes was carried out. The first translation product of the receptor is a 190 Kd species which is rapidly ($t_{\frac{1}{2}}$ = 15 min) processed to a 210 Kd species. The latter precursor is converted slowly ($t_{\frac{1}{2}}$ = 2 h) by proteolytic processing to a 125 Kd (α') and 83 Kd (β') species. Immediately prior to its insertion into the plasma membrane, 3 h after synthesis, mature receptor containing the α(135 Kd) and β-(95 Kd) subunits is formed. When applied to wheat germ lectin-Sepharose, the 210 Kd precursor, the 125 Kd (α'-subunit) and 83 Kd (β'-subunit) species are specifically bound; the 190 Kd species is not. Upon incubation with Endo glycosidase H, the 210 Kd and 190 Kd species are reduced to 180 Kd (identical to aglyco -receptor synthesized in the presence of TM). The 125 Kd and 83 Kd species are Endo H-sensitive. The receptor precursors do not contain terminal sialic acid (or other capping sugars) as they are Endo H-sensitive. Just prior to insertion into the plasma membrane the mature 135 Kd and 95 Kd receptor subunits become Endo H-resistant and neuraminidase-sensitive.

In the presence of TM, a 180 Kd aglyco-receptor is synthesized which is incapable of further processing and does not reach the cell surface as is indicated by trypsin-insensitivity. Thus, oligosaccharide chains are either required for intracellular translocation to the clevage site or the glycosyl moieties confer the recognition necessary for cleavage. Thus, the oligosaccharide moieties of the insulin receptor are essential for proper processing and formation of functional receptor.

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0628 REGULATION OF H-2 AND IA ANTIGEN EXPRESSION. Patricia P. Jones, Donna King-Paulnock, Kathelyn Steimer, Samuel Strober, and Jane R. Parnes, Departments of Biological Sciences and Medicine (Immunology), Stanford University, Stanford, CA 94305

H-2 and Ia antigens, products of the H-2 major histocompatibility complex, serve as self-recognition structures in the induction of immune responses. Despite the essential role of macrophage Ia antigens in the presentation of macrophage-processed foreign antigen to T lymphocytes, macrophages do not express Ia antigens constitutively. Instead, the expression of Ia antigens appears to be regulated by the immune system itself. Products of activated T cells induce the expression of Ia antigens on macrophages (1-3) and a macrophage cell line (4). We have shown that the appearance of Ia antigens and the concomitant increase in H-2 antigen expression is due to enhanced synthesis of Ia α, β, and I_1 chains and of the heavy and β_2 microglobulin chains of the H-2 antigens (4). The increased synthesis of these polypeptide chains is paralleled by elevated levels of the corresponding mRNAs. Recent studies have shown that the active factor in the activated T-cell supernates is immune interferon (IFN-γ) (5). The stimulatory effect of IFN-γ on H-2 and Ia antigen expression is antagonized by a number of substances, including prostaglandins (6), glucocorticoids (7), and a protein factor made by spleen cells from mice treated with total lymphoid irradiation (our unpublished results). Current experiments are directed towards determining whether these agents block the IFN-γ-mediated stimulation of H-2 and Ia expression at the transcriptional, post-transcriptional, or translational levels, or alternatively, by affecting the sensitivity of the cells to IFN-γ action.

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Regulation of Cell Motility and Chemotaxis

0629 INTRODUCTION TO REGULATION OF CELL MOTILITY AND CHEMOTAXIS. Elmer L. Becker, Richard Freer, Claudio Toniolo, Peter Balaram, Department of Pathology, University of Connecticut Health Center, Farmington, CT 06032. Department of Pharmacology Medical College of Virginia, Richmond VA 23298. Biopolymer Research Center, University of Padova, 35100 Padova Italy. Indian Institute of Science, Bangalore 560012 India.

There are various means of regulating both bacterial and neutrophil chemotaxis, e.g. adaptation. As Dr. Koshland will demonstrate, in bacterial chemotaxis these can be described, in large part, in molecular terms. In contrast, as Dr. Zigmond will illustrate, knowledge of neutrophil regulation is still largely, although not wholly, descriptive. Chemotaxis is initially regulated by the selectivity of the given chemotactic receptor for its ligands. This is exemplified in the work that Dr. Wilmer will describe on the specificity of the α thrombin chemotactic receptor of the neutrophil and also in that of my colleagues and myself on the formyl peptide receptor of the neutrophil.

The chemotactic formylpeptides are synthetic oligopeptides that induce chemotaxis, chemokinesis and other functions in neutrophils and macrophages by acting at a specific receptor, the formylpeptide receptor. Recently, Marasco et al., have shown that formylmethionyl-leucinyl-phenylalanine (CHO-Met-Leu-Phe-OH) the prototypical, chemotactic synthetic peptide is the most chemotactically active constituent of butanol extracts of E. coli culture filtrates. On the basis of extensive structure-activity studies in which the various elements of f Met-Leu-Phe-OH were systematically varied, we have proposed a working model of the topology of the rabbit neutrophil receptor. According to this model, the receptor has sufficient "room" to accommodate tetra or pentapeptides. The receptor has at least five sites of interaction with the chemotactic peptide: an hydrogen bond acceptor which binds to the proton of the formyl group, a hydrophobic pocket accomodating the methionyl group; within the latter pocket, a positively charged region complementary to the electronegative sulfur of the methionine; a hydrophobic pocket accomodating the leucyl residue and another hydrophobic pocket into which the phenylalanine fits. The last is specifically adjusted to the aromatic ring of the phenylalanine. Within the pocket is an hydrogen bond donor specifically able to interact with the carbonyl oxygen of the phenylalanine. Various kinds of indirect evidence suggest that the chemotactic peptides exist on the receptor in an anti-parallel β-pleated sheet arrangement. Other work has shown that the biological activity of the stereochemically hindered formylmethionyl-α-aminoisobutyryl -phenylalanine and formyl-methionyl-cycloleucinyl-phenylalanine is high but less than that of CHO-Met-Leu-Phe-OH, suggesting that folded structures may be compatible with high although not optimal activity.
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0630 ABILITY OF LEUKOYCTES TO ORIENT THEIR LOCOMOTION IN A CHEMOTACTIC GRADIENT,

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Chemotaxis by polymorphonuclear leukocytes (PMNs) plays an important role in the inflammatory response and provides a model system for studies on eukaryote chemotaxis. The accuracy with which PMNs orient their locomotion along a gradient of a chemotactic peptide is proportional to the difference in the number of receptors occupied on the two sides of a cell in that gradient (1, 2). The mechanism by which such differences in receptor occupancy result in directed locomotion is unknown. Using low concentrations of chemotactic peptides we have evidence that PMNs can perceive concentration gradients so shallow that the statistical fluctuations in the number of molecules bound at each end of the cell is large relative to the difference in the number bound between the two ends of the cell. PMNs orient in these noisy gradients more accurately than would be possible by comparing instantaneous measurements of the receptor occupancies. We calculate the cells must be able to accumulate spatial information (have a memory) over time periods up to 5 minutes in order to respond as they do (3). Since PMNs extend lamellipodia within seconds after addition of chemotactic peptides and can move at rates greater than 10 um per minute, this accumulation of information does not retard rapid cell responses. We believe the information storage is linked to the polarity exhibited by locomoting cells. While most of the molecular changes known to occur upon peptide stimulation peak within one minute (4), the polar properties of PMN behavior and morphology have persistence times of several minutes and thus are capable of contributing to the time averaging (5).

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0631 STRUCTURE-FUNCTION RELATIONSHIPS IN THE ALPHA-THROMBIN CHEMOTACTIC RECEPTOR, Rachel Bar-Shavit, Arnold Kahn, and George D. Wilner, The Jewish Hospital and Washington University, St. Louis, MO 63110.

One of the early consequences of physical trauma in man and other mammals is fibrin clot formation. Such clots serve to staunch the flow of extravasated blood and to provide a physical matrix for the migration of cells involved in anti-infective and wound healing activities. In the latter regard, it is of interest that fibrin clots contain significant amounts of thrombin, a serine protease with a wide range of biological functions including the ability to stimulate mitosis.

To date, virtually all functions of thrombin have been linked to its esterolytic activity, requiring an intact active center. Recently, we found that thrombin's effectiveness as a monocyte and macrophage chemotaxin and mitogen remains unimpaired even if modified proteolytically or by blocking agents which sharply curtail enzymatic activity (1). These observations suggest the existence in thrombin of a unique region which is independent of the active center but which is involved in cell membrane recognition and the triggering of specific biological responses.

To further identify and characterize the cell recognition region, cyanogen bromide digests were prepared from human prethrombin 1, a single chain precursor of α-thrombin and the isolated fragments tested for their ability to stimulate chemotaxis and mitogenesis (2). One fragment, CB67-129 (representing residues 54-116 of the thrombin B chain) was found to elicit both stimulatory activities of the parent molecule. Moreover, this peptide competed with α-thrombin in a chemotaxis assay suggesting that the fragment and α-thrombin occupy the same receptor site.

The CB67-129 region of thrombin contains several unique features including a carbohydrate containing sequence termed loop B. Loop B is absent in homologous regions of several closely related serine proteases, including chymotrypsin and trypsin, suggesting a relationship between this insertion sequence and biological activity. Interestingly, data derived from computer-generated three dimensional models of α-thrombin indicate that the CB67-129 region of the molecule, including loop B, forms a discrete topographical feature on the intact enzyme which is, in fact, absent in closely related proteases. Collectively, these observations document a heretofore unsuspected role for thrombin in regulating monocyte function and suggest that the latter activity is affected by a phylogenitically unique sequence within the parent molecule.

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Tyrosine Kinases and Cell Regulation

0632 **ENDOGENOUS SUBSTRATES FOR TYROSINE PROTEIN KINASES IN GROWTH FACTOR-TREATED AND TRANSFORMED CELLS.** **Jonathan A. Cooper and Tony Hunter, Molecular Biology and Virology Laboratory, The Salk Institute, P. O. Box 85800, San Diego, CA 92138**

Proteins interacting with growth factor receptors and viral transforming proteins are implicated in the processes whereby the cell regulates its growth and metabolism. The cell surface receptors for insulin, insulin-like growth factor type I, epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) reportedly have associated tyrosine protein kinase activities, regulated by their respective ligands. Several retroviruses encode transforming proteins having tyrosine protein kinase activity. In these cases, therefore, cell proteins potentially involved in cell growth or transformation may be recognized by their content of phosphotyrosine.

In intact cells treated with growth factors, the growth factor receptors themselves contain increased amounts of phosphotyrosine. In addition, EGF and PDGF induce transient phosphorylation of a pair of related 42K proteins (p42) at tyrosine. Rather surprisingly, p42 is also phosphorylated at tyrosine in response to agents which most probably do not interact directly with tyrosine protein kinases, such as the mitogenic protease trypsin and tumor promoting agents. The tumor promoters are known to activate a serine/threonine protein kinase, C-kinase. One substrate for C-kinase is the EGF receptor. However, phosphorylation of the EGF receptor by C-kinase appears to reduce its EGF-stimulated tyrosine protein kinase activity, so the mechanism of p42 phosphorylation in cells exposed to tumor promoters remains obscure. The common phosphorylation of these proteins in response to mitogenic stimuli leads to the expectation that they may be important for at least one of the pleiotypic responses of cells to mitogens.

Many more phosphotyrosine-containing proteins can be detected in cells transformed by retroviruses encoding tyrosine protein kinases than in cells responding to growth factors. Since it is possible that some proteins may be phosphorylated simply because they are accessible to the viral transforming protein and their phosphorylation is not detrimental to the cell, it will be difficult to determine which of the observed phosphorylations are important for the transformed phenotype. Substrate proteins for retroviral tyrosine protein kinases include the cytoskeletal protein vinculin, the peripheral membrane protein p36, an 81K protein, and the enzymes enolase, phosphoglycerate mutase, and lactate dehydrogenase. Phosphorylation of the first three proteins could be involved in some aspect(s) of the transformed phenotype such as altered morphology. It seems unlikely, however, that phosphorylation of the three glycolytic enzymes accounts for the increased rate of glycolysis in all transformed cells, since many retroviruses do not encode tyrosine protein kinases.

0633 THE INSULIN RECEPTOR AS A PROTEIN KINASE. C.R. Kahn, H.U. Haring, S. Takayama, M. White, P. Roach, A. Roach and J. Niedel. Joslin Diabetes Center, Boston, MA Indiana University, Indianapolis, IN; Duke University, Durham, N.C.

Insulin stimulates a rapid phosphorylation of the β-subunit of its own receptor in both intact and broken cell preparations. In vitro this autophosphorylation reaction occurs with a $t_{\frac{1}{2}}$ of 30 sec and involves primarily tyrosine residues. In intact cells, the autophosphorylation reaction is slower ($t_{\frac{1}{2}}$=5-10 min) and involves serine, threonine and tyrosine residues. Studies have shown that the purified insulin receptor retains tyrosine kinase activity, and in vitro the insulin receptor kinase phosphorylates a number of substrates such as histone 2B, casein, synthetic peptides resembling the phosphorylation site of $pp60^{src}$ and antibodies to $pp60^{src}$. In an attempt to find a cellular protein which might function as a physiological substrate of the receptor kinase and also account for the serine and threonine phosphorylation in the intact cell, a number of serine/threonine kinases have been screened in in vitro reconstitution assays. The insulin receptor neither phosphorylates nor is phosphorylated by casein kinases I and II, the catalytic subunit of cAMP-dependent protein kinase, phosphorylase kinase, or glycogen synthase kinase 3. By contrast, purified protein kinase C will phosphorylate the insulin receptor in vitro. This occurs in a concentration-dependent manner and occurs on serine and threonine residues. In intact hepatoma cells, phorbol ester treatment also results in a variable increase in insulin receptor phosphorylation. In both the intact and broken cell experiments, the C-kinase effect is additive with the insulin effect. These data indicate that the insulin receptor may be phosphorylated both in vivo and in vitro by C-kinase and suggests an intimate relationship between these two protein kinases which may be important in cell growth and metabolism.

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0634 IN VITRO MUTAGENESIS TO PROBE THE FUNCTION OF TRANSFORMING PROTEINS, Alan E. Smith, Ben A. Oostra, Bill Markland and Sara A. Courtneidge, Biochemistry Division, National Institute for Medical Research, Mill Hill, London NW7 1AA, United Kingdom.

The transforming proteins of some retroviruses are membrane bound tyrosine kinases, for example Rous sarcoma virus $pp60^{v\text{-}src}$. The kinase activity is an intrinsic property of $pp60^{v\text{-}src}$ and is essential for the transformation process. Normal cells contain proteins related to retrovirus transforming proteins, for example the cellular homologue of $pp60^{v\text{-}src}$ is called $pp60^{c\text{-}src}$. $pp60^{c\text{-}src}$ is a tyrosine kinase and because it is conserved from Drosophila to man it is assumed to play a role in cellular regulation.

The transforming protein of polyoma virus is called middle-T antigen. It is necessary and sufficient to transform established cell lines and it too is membrane bound and has an associated tyrosine kinase activity. We have proposed however that middle-T itself is not a tyrosine kinase, but that it forms a stable complex with a cellular enzyme, namely $pp60^{c\text{-}src}$. This conclusion is based on the finding that (i) $pp60^{c\text{-}src}$ may be immunoprecipitated with a variety of antisera which recognise middle-T, including antipeptide sera and monoclonal antibodies; (ii) middle-T may be immunoprecipitated by antisera against $pp60^{c\text{-}src}$, and (iii) $pp60^{c\text{-}src}$ from polyoma virus transformed cells but not normal cells sediments rapidly on sucrose gradients.

Middle-T has been purified approximately 5,000 fold from tumour cells by immunoaffinity chromatography using immobilised purified antipeptide IgG. The enriched protein retains kinase activity and has associated $pp60^{c\text{-}src}$.

In vitro mutagenesis has been used to probe the sequence requirements for transformation by polyoma virus and to map the $pp60^{c\text{-}src}$ binding site. Targets for mutagenesis include tyrosine phosphorylation sites at residues 250 and 315, a glutamic acid tract, the hydrophobic carboxy terminal domain, and the region around the lesion in the non-transforming mutant NG59. The results show that all transformation-competent mutants tested have bound $pp60^{c\text{-}src}$, whereas some that are defective for transformation lack the complex.

We propose middle-T transforms cells by altering the activity or specificity of a cellular tyrosine kinase.

Coordinated Receptor Signalling and Cell Growth

0635 RECEPTORS AS TARGETS OF CELLULAR SIGNALLING MECHANISMS, Michael P. Czech, University of Massachusetts Medical Center, Worcester, MA 01605

Although receptors for hormones, neurotransmitters, and other agonists have long been recognized as components that initiate cellular signalling pathways, it is also apparent that they serve as targets for regulatory mechanisms. We have investigated in detail types of receptor regulations whereby one receptor system is modulated by a signalling mechanism initiated by a second, distinct receptor system. We have studied in parallel three model systems of such regulation in order to gain insight to underlying mechanisms: I. Insulin action rapidly stimulates ^{125}I-IGF-II binding to intact adipocytes and H-35 hepatoma cells (1). In order to distinguish between an increase in IGF-II receptor number versus affinity, we raised potent anti-IGF-II receptor antiserum in a rabbit immunized with IGF-II receptor we purified (2). The anti-receptor Ig bound the receptor in the presence or absence of saturating concentrations of IGF-II. Insulin action increased ^{125}I-IGF-II binding as well as anti-receptor Ig binding to intact fat cells to the same extent, indicating an increase in receptor number. II. Tumor promoting phorbol diesters (PD) appear to decrease the number of high affinity EGF receptors on the surface of cultured cells. Because the PD receptor appears to be associated with C-kinase, we investigated PD action in ^{32}P-labeled A431 cells. Specific phosphorylation of the EGF receptor on serine residues localized to a unique peptide fragment of the EGF receptor upon tryptic hydrolysis was found in response to PD. III. ß-adrenergic inhibition of ^{125}I-insulin binding to adipocytes appears to be mediated by cyclic AMP (3). Using anti-insulin receptor Ig, we find that the number of insulin receptors on the cell surface is not changed, but that a decrease in receptor affinity is responsible for the catecholamine-mediated ^{125}I-insulin binding inhibition. Furthermore, we find that the tyrosine kinase activity associated with insulin receptor purified from catecholamine-treated cells is markedly impaired in its ability to respond to saturating concentrations of insulin. Autophosphorylation of insulin receptor in response to insulin in intact cells is also inhibited by catecholamines.

These studies demonstrate at least three modes of receptor modulation. In I, IGF-II receptors are expressed on the cell surface in response to insulin, while in II, EGF receptors appear to disappear from the cell surface in response to PD. In contrast, ß-adrenergic action on the insulin receptor appears to involve no receptor number change, but rather structural alterations which lead to decreased affinity for ^{125}I-insulin and an impaired tyrosine kinase activation. These receptor regulatory mechanisms appear to be directly involved in modulating and coordinating receptor signalling.

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0636 REGULATION OF GENE EXPRESSION AND GROWTH CONTROL BY PLATELET-DERIVED GROWTH FACTOR, Charles D. Stiles*, Brent H. Cochran*, Kathleen Kelly+, and Philip Leder+, Department of Microbiology and Molecular Genetics and Dana-Farber Cancer Institute* and Department of Genetics+, Harvard Medical School, Boston, MA 02115

Platelet-derived growth factor (PDGF) initiates the first phase of the mitogenic response in quiescent fibroblast cells - a phase we have termed "competence"[1]. We have characterized a family of cell cycle genes whose expression is regulated by PDGF in BALB/c-3T3 cells[2]. These genes are rare, comprising perhaps 0.1% to 0.3% of the total genetic information expressed by 3T3 cells. The abundance level of the cognate mRNAs can be increased 60-fold within three hours following the addition of pure PDGF to the culture medium of quiescent 3T3 cell monolayers. Epidermal growth factor, insulin, and platelet-poor plasma have either a weak or undetectable effect on expression of these genes.

Inhibitors of protein synthesis block the progression of quiescent 3T3 cells through G_1 into S phase; however these drugs do not block the induction of cell cycle genes by PDGF. In fact these genes are super-induced by the combination of PDGF and protein synthesis inhibitors. The inhibitor data indicate i. that induction of these genes is not a secondary consequence of the mitogenic response and ii. that a labile protein may regulate transcription of the genes[2].

Overshadowing all of these observations is the finding that an oncogene (c-myc) is contained within this cell cycle gene family[3]. The cellular content of c-myc increases up to 40-fold within 3 hours following the addition of PDGF to quiescent BALB/c-3T3 cells. Moreover agents which initiate competence in lymphocytes (lipopolysaccharide or concanavalin A) stimulate expression of myc in these cells. Together, these observations suggest that some oncogenes encode products which function as intracellular mediators of the growth response to mitogens. Further, the work illustrates how oncogenes may be linked in functional hierarchies since the product of the c-sis gene (PDGF) stimulates expression of c-myc in fibroblasts.

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0637 ROLE OF THE PHORBOL DIESTER RECEPTOR AND THE Ca^{2+}-PHOSPHOLIPID-DEPENDENT PROTEIN KINASE C IN CELL DIFFERENTIATION, James E. Niedel and G.R. Vandenbark, Department of Medicine, Duke University Medical Center, Durham, N.C. 27710.

The phorbol diester tumor promoters are plant products known to enhance tumor formation in two-stage models of carcinogenesis. Paradoxically, these same compounds will induce most myeloid leukemia cells (cell-lines and fresh explants from patients) to cease proliferation and differentiate into cells with the morphologic, antigenic, biochemical and functional characteristics of mature macrophages. For a large series of synthetic analogs, structure-function studies suggest that tumor promotion and induction of cell differentiation are mediated via a common phorbol diester binding protein. This phorbol diester receptor is present in all cells and tissues, except erythrocytes; brain contains the highest concentration and several laboratories have suggested that the receptor is the Ca^{2+}-phospholipid-dependent protein kinase C.[1-3] The activity of this kinase is dependent upon Ca^{2+} and phospholipids and is enhanced by unsaturated diacylglycerols which act by lowering the K_m for Ca^{2+}. Diacylglycerol is a product of the phosphatidylinositol cycle and hence protein kinase C may be regulated by a variety of ligand-receptor interactions at the membrane that lead to PI turnover. The phorbol diesters can substitute for diacylglycerol, thereby activating the kinase and bypassing the normal membrane regulatory pathway. Operationally, three pools of the phorbol diester receptor and protein kinase C can be defined in myeloid leukemia cells; a cytosolic pool that remains soluble in the presence of Ca^{2+} or chelators; a cytosolic pool that becomes membrane-associated in the presence of Ca^{2+} and a membrane pool that requires detergent-extraction for solubilization. The soluble receptor and C kinase co-purify from each of these pools.[4] The soluble aporeceptor and apokinase share common phospholipid and Ca^{2+} requirements for reconstitution. Identical potencies are seen for several phorbol diesters as inducers of cell differentiation, inhibitors of receptor binding and activators of protein kinase C. In myeloid leukemia cells loaded with $^{32}P_i$, treatment with nanomolar concentrations of active phorbol diesters leads to rapid and discrete changes in cellular phosphoproteins. Within one minute of treatment, very basic proteins with molecular weights of 13k - 15k are phosphorylated, as are neutral and acidic proteins of molecular weights 20k(pI-6.1), 52k(pI-5.4), 60k and 80k(pI-5.8). In addition, a 20k protein (pI-6.9) shows decreased phosphorylation. The phosphoprotein changes are greatest at 1h of treatment, decline by 6h and return to control levels at 14h. Dose-response experiments with several phorbol analogs indicate that these changes are mediated via the phorbol diester receptor/C kinase. The phorbol sensitive phosphoproteins are stable to alkali treatment.

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0638 DICTYOSTELIUM DIFFERENTIATION: SEQUENTIAL INDUCTION OF CELL-TYPE SPECIFIC mRNAS IS MEDIATED BY DEVELOPMENTALLY REGULATED CELL SURFACE PROTEINS, Harvey F. Lodish, Rex L. Chisholm, Eric Barklis and Stephen M. Cohen, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

The developmental program of the cellular slime mold Dictyostelium discoideum exhibits many features of differentiation in higher organisms: a single type of cell differentiates into two very different ones; specific cell-cell contacts are formed; and cell-cell signalling by small extracellular molecules plays a critical role in the differentiation process. We have characterized the induction of a large number of developmentally regulated mRNAs, most of which are expressed in a specific cell type (prestalk or prespore cells) and the induction of developmentally regulated cell surface components. We have shown that the Dictyostelium developmental program can be explained by a small number of dependent sequences, each presumed to be mediated by specific surface molecules. Progression through at least four dependent stages is required for Dictyostelium amoebae to complete the aggregation stage of their developmental cycle. Discrete classes of cell type-specific mRNAs are induced at each stage, as are cell surface molecules that enable the cell to sense the signal responsible for triggering the next stage of the program.

Two factors are involved in initiating the developmental program: starvation for amino acids and placing the cells at a high cell density. Induction of actin mRNA and a set of mRNAs encoded by a family of heat shock genes is induced solely by high cell density. Starvation induces one class of genes specific for prestalk cells (prestalk I) and also the cell surface cAMP receptor. The appearance of the cAMP receptor enables the cells to undergo chemotaxis, a process mediated by cell-cell signalling of extracellular cAMP. Binding of cAMP to cell surface receptors induces synthesis of the second class of prestalk cell-specific mRNA (prestalk II) and one class of prespore cell-specific mRNA (prespore I). Cyclic AMP also induces the expression of cell surface molecules necessary for the formation of cohesive cell contacts. The step in the developmental cascade following chemotaxis is the formation of multicellular aggregates. Cell-cell contact induces the expression of a large class of genes, the second class of prspore mRNAs (prespore II). Cells at this stage of development appear poised to proceed through the directed cell movement responsible for morphogenesis, eventually culminating in the formation of the fruiting body containing mature spore and stalk cells.

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Receptor Aggregation, Adaptation and Cytoskeletal Linkages

0639 GnRH STIMULATION OF GONADOTROPIN RELEASE FROM THE PITUITARY: REGULATION OF CELL RESPONSES AND GnRH RECEPTORS BY GnRH-RECEPTOR MICROAGGREGATION, P. Michael Conn, Deloris Rogers, Sallie Seay, Dept. Pharmacology, Duke Univ. Medical Center, Durham NC 27710

Gonadotropin releasing hormone (GnRH) binds to a plasma membrane receptor (1) and stimulates luteinizing hormone (LH) release by a Ca^{2+}-mediated mechanism (2). Indeed, Ca^{2+} fulfills the requirements of a second messenger in this system (3) and it can be shown that GnRH-stimulated LH release can be blocked by depletion of extracellular Ca^{2+} or by addition of Ca^{2+} ion channel blockers such as methoxyverapamil (2). These observations suggested that GnRH stimulated LH release is mediated by receptor regulated Ca^{2+} ion channels in the plasma membrane. The precise mechanism by which receptor occupancy is coupled to these channels remains unclear. It has been shown that treatments which provoke GnRH receptor-receptor interactions (microaggregation) are sufficient to stimulate release of LH (4-6). In these studies a GnRH antagonist was used. This analog inhibits GnRH stimulated LH release and stimulates no release when it is added to pituitary cultures alone. When the antagonist is first dimerized then incubated with a cross-reactive antibody the product appears to contain four molecules of antagonist. Two of these are bound to the antibody and two others are extended in space and separated by about 150 A. This conjugate behaves as an agonist, stimulating LH release from pituitary cultures (4). The observed behavior has been described by a mathematical model supporting the simultaneous interaction with more than one receptor (6); monomeric antibody fragments (i.e. reduced pepsin cleavage products) do not stimulate LH release. Like GnRH stimulated LH release, release in response to the conjugate requires extracellular Ca^{2+} and is antagonized by calmodulin inhibitors. Gonadotrope desensitization and receptor regulation in response to GnRH can be mimicked by the antagonist conjugate but not by antagonist alone. Thus, four physiological actions of the releasing hormone (LH release, desensitization, up- and down-regulation of the GnRH receptor) occur in response to the conjugate. The data are consistent with a model of GnRH action in which microaggregation of the GnRH receptor in the final step in common to a branched pathway consisting of Ca^{2+} dependent actions (LH release, up-regulation) and Ca^{2+} independent actions (desensitization, down-regulation) of the hormone. (Supported by NIH HD13220, HD00337, and the Mellon Foundation)

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0640 MOUSE MACROPHAGE Fc RECEPTOR: STRUCTURE AND SIGNAL TRANSMISSION. J.C. Unkeless, D.-E. Young, and I.S. Mellman*, Department of Cellular Physiology and Immunology, Rockefeller University, New York, NY 10021 and *Section of Cell Biology, Yale University School of Medicine, New Haven, CT 06510

A mouse Fc receptor with specificity for murine IgG2b and IgG1 immune complexes ($Fc_{\gamma 2b/\gamma 1}R$) has been purified from detergent lysates of the J774 macrophage cell line by affinity chromatography using the Fab fragment of the monoclonal antibody, 2.4G2 (1,2). The purified FcR has been used as immunogen to generate a second set of monoclonal antibodies. Crude lysates of the J774 macrophage cell line and the Fc receptor-positive T cell line S49.1 were analyzed by immunoblotting using a series of monoclonal antibodies directed against different epitopes. The FcR from S49.1 is clearly smaller than that on J774. The molecular basis of the difference in size between the two receptors will be discussed.

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0641 REGULATION OF THE PHAGOCYTOSIS-PROMOTING CAPACITY OF THE C3 RECEPTORS ON HUMAN MACROPHAGES, Samuel D. Wright, Laboratory of Cellular Physiology and Immunology, Rockefeller University, New York, NY 10021

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Monoclonal antibodies directed against CR3 have been used to study the biochemical basis for activation of complement receptors by FN and PMA (3). Monoclonal antibodies OKM1 and OKM9 bind to distinct epitopes on CR3 without inhibiting binding of the ligand, C3bi. OKM10, on the other hand, binds and masks the ligand-binding site of CR3. CR3 is composed of two polypeptide chains, an α of M_r 185,000 and a β of 105,000 (3), and appears to be a member of a closely related family of two-chain receptors found on leukocytes. Proteins that are members of this family are composed of an α chain that is unique in association with a β chain that is biochemically indistinguishable from member to member (4).

Experiments with labelled monoclonal antibodies against CR3 suggest that activation of CR3 is not accompanied by changes in the number or cellular distribution of CR3. Further, activation is not accompanied by changes in the molecular weight or polypeptide composition of CR3. Activation of C3 receptors caused by either phorbol esters or FN is readily reversible since activity rapidly declines after removal of the stimulant and can be restored by fresh stimulant (manuscripts in preparation). These observations suggest that activation of CR3 may occur through reversible posttranslational modification of the receptor protein.

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0638 DICTYOSTELIUM DIFFERENTIATION: SEQUENTIAL INDUCTION OF CELL-TYPE SPECIFIC mRNAS IS MEDIATED BY DEVELOPMENTALLY REGULATED CELL SURFACE PROTEINS, Harvey F. Lodish, Rex L. Chisholm, Eric Barklis and Stephen M. Cohen, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

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0642 INTERACTIONS BETWEEN SURFACE RECEPTORS AND THE CYTOSKELETON. Richard G. Painter, Rodger A. Allen, Mark H. Ginsberg and Algirdas J. Jesaitis. Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037

The interaction of various ligands with their specific receptors can induce apparent associations between the receptor-ligand complex and the cytoskeleton. In the human blood platelet, Concanavalin A or F(ab')2 fragments (but not Fab fragments) of anti membrane surface glycoproteins cause the isolation of their "receptors" with the Triton X100-insoluble cytoskeletons derived from whole cells. These interactions were disrupted by treatment of the Triton lysate with actin depolymerizing agent, DNAse I. Similar interactions were demonstrated in Triton lysates of plasma membranes isolated from ConA pretreated platelets. Evidence will be presented showing that, in the platelet system, a small fraction (<10 percent) of lectin binding surface glycoproteins are preassociated with the cytoskeleton. Binding of multivalent ligands like ConA or F(ab')2 fragments results in a passive clustering of unanchored molecules around that fraction already associated with the cytoskeleton. ConA treatment of isolated platelet membranes caused platelet surface membrane proteins to coisolate with the Triton-insoluble-actin rich membrane matrix.

In the human neutrophil, we have shown that the receptor for chemotactic N-formyl peptides becomes rapidly (<5 seconds) associated at the cell surface with the Triton cytoskeleton of such cells at 37°C more slowly at 15°C, but not at 4°C. The receptor-ligand complex can also be isolated from membrane preparations in a Triton-insoluble, high molecular weight form from which the ligand dissociates very slowly ($t_{1/2}$>3h). In contrast, surface receptor can be extracted quantitatively from isolated plasma membrane obtained from unstimulated neutrophils in a low molecular weight form. Physiochemical studies indicate that the receptor isolated in this manner was monomeric in Triton X100 with a molecular weight of 62,000 as compared to 50-60,000 as determined by SDS-PAGE. The Triton-solubilized receptor, which bound to wheat germ agglutinin, had a partial specific volume of 0.88 cc/g, pIs of 6.0 and 6.5 and a Stoke's Radius of 38Å, indicating a globular protein with properties characteristic of an intrinsic membrane glycoprotein. Pulse chase studies at 37°C indicate that the ligand-induced conversion of receptor to a high affinity, cytoskeletally associated form was transient and formed at the cell surface prior to internalization and transport to the Golgi and lysosomal compartments. Pretreatment of cells with dihydrocytochalasin B inhibited the cytoskeletal association, high affinity conversion and subsequent internalization of ligand, suggesting that cytoskeletal-receptor interactions may participate in these cellular responses.

0643 DELETIONS IN THE N-TERMINUS OF ROUS SARCOMA VIRUS SRC PROTEIN CAN PREVENT SRC MEMBRANE ASSOCIATION AND CELL TRANSFORMATION. Frederick R. Cross, Ellen A. Garber, David Pellman, Marius Sudol, and Hidesaburo Hanafusa. The Rockefeller University, N.Y.C., N.Y. 10021.

Using a DNA clone of Rous sarcoma virus, deletions including varying portions of the DNA coding for the N-terminal 10 kd (80 amino acids) of src protein were constructed in vitro, and infectious mutant virus recovered. Membrane association of mutant src proteins was measured, as well as attachment of myristic acid to the N-termini. We measured tyrosine kinase activity of the mutant proteins, and determined the state of transformation of infected cells. Deletion of amino acids 15 to 81 had little effect on membrane association, myristic acid attachment, or kinase activity, and infected cells were fully transformed. However, if the deletion extended from amino acids 2 to 81, membrane association and myristic acid attachment were abolished. Kinase activity was unaffected, but infected cells were not transformed. RSV transformation causes an induction of plasminogen activator production. Plasminogen activator induction correlates partially with tumorigenicity in chickens among the N-terminal deletion mutants we have constructed.

Receptor-Mediated Regulation of Gene Expression

0644 STRUCTURE AND REGULATION OF PEPTIDE HORMONE RESPONSIVE GENES, Jeffrey M. Rosen, William K. Jones, Sheldon M. Campbell and Li-Yuan Yu-Lee, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030

The multihormonal regulation of milk protein gene expression in the mammary gland provides a model system for elucidating the mechanism of action of both peptide and steroid hormones. Both glucocorticoids and prolactin are required for maximal expression of the milk protein genes, while progesterone antagonizes the inductive effects of these lactogenic hormones. As a necessary prerequisite for elucidating the mechanism of action of the hormones, we have isolated a series of recombinant cDNA and genomic DNA clones encoding three members of the casein multigene family and a fourth, abundant rat milk protein, the whey acidic protein. Complete nucleic acid sequences have been determined for all four of these milk protein mRNAs and for portions of the large and complex, split genes encoding these mRNAs. Comparison of these sequences has revealed three regions of unusual conservation among the rapidly diverging members of the casein gene family: the 5' noncoding region of these mRNAs, the signal peptide sequences, and the major sites of casein phosphorylation and calcium binding. The first two regions are each encoded by a separate exon, while the site of phosphorylation is generated by an RNA splicing event. Analysis of the 5' flanking regions of the casein genes have revealed an unexpected degree of homology with a sequence found at the 5' end of the chicken ovalbumin gene shown to be involved in progesterone receptor binding and the induction of ovalbumin gene expression (Compton et al., Proc. Natl. Acad. Sci. USA 80, 16, 1983; Dean et al., Nature 305, 551, 1983). Analysis of chicken progesterone receptor binding to the rat γ-casein gene have revealed sites of preferential binding. Additional sequence comparisons of te 5' flanking regions of the milk protein genes have revealed homologies with a putative glucocorticoid receptor binding site observed in the mouse mammary tumor virus long terminal repeat (Scheidereit et al., Nature 304, 749, 1983) and DNA sequences similar to the core sequence 5'GTGG$^{A}_{T}$T$^{A}_{T}$TG3' present in several viral enhancers (Khoury and Gruss, Cell 33, 313, 1983). The functional importance of these sequences is currently under investigation by DNA mediated gene transfection using intact genes, minigenes and fusion genes in mammary tumor cells containing both steroid and peptide hormone receptors. Supported by NIH grant CA16303.

0645 IDENTIFICATION OF A cAMP REGULATORY REGION OF THE P-ENOLPYRUVATE CARBOXYKINASE GENE: USE OF FUSION GENES TRANSFECTED INTO RAT HEPATOMA CELLS, Anthony Wynshaw-Boris, Tracy Gross Lugo, R.E.K. Fournier and R.W. Hanson, Department of Biochemistry, Case Western Reserve University School of Medicine, Cleveland, OH 44106 and the Comprehensive Cancer Center, University of Southern California, Los Angeles, CA. 90033.

P-enolpyruvate carboxykinase (GTP) (EC 4.1.1.32) (PEPCK) from the cytosol of rat liver is markedly induced by cAMP. The administration of Bt_2cAMP to a starved-refed rat causes an 8-fold increase in the concentration of enzyme mRNA (1) and in the transcription rate of the PEPCK gene (2). Recently, the gene for the enzyme, as well as its 5'-flanking sequence, has been isolated in a 7.0 kb Bam Hl fragment and subcloned into pBR322 (3). The 5'-regulatory region contains the promoter for the PEPCK gene, in a 600 bp Bam Hl-Bgl II fragment. A variety of vectors have been constructed using this fragment in order to determine the sequences required for both promoter activity and cAMP regulation. All vectors employ the pBR327-based vector, pOPF, which contains the Herpes simplex virus thymidine kinase (TK) gene (pOPF was a gift of Dr. Richard Flavell). A DNA fragment containing the PEPCK promoter was ligated to the TK structural gene which lacked its promoter, either in the proper orientation for transcription or in an orientation opposite of that required for transcription. These fusion genes, as well as the parent vector containing the intact TK gene, were then transfected into TK-deficient rat hepatoma cells and transformants selected in HAT medium. Cells selected by this procedure were isolated as individual clones and tested further for their ability to respond to cAMP. Cells were treated with Bt_2cAMP (0.1 mM) and theophylline (1 mM) for 16 hrs, and TK activity measured. We noted a 2 to 4-fold induction of TK activity by Bt_2cAMP and theophylline in several of the cell lines transfected with the PEPCK-TK fusion gene, with the promoter in the correct orientation. However, the intact TK gene was not induced by hormonal treatment, nor was there any expression of the PEPCK-TK fusion gene in which the promoter from PEPCK was present in the opposite orientation. Analysis of the transfected cells by Southern blotting indicated that fragments of the predicted size were integrated into the host cell genome, and were present in varying copy number. These findings indicate that the sequences required for the cAMP-induced regulation of PEPCK gene expression are contained within a 500 bp segment of 5'-flanking sequence. We are currently determining the sequences required for cAMP regulation of the fusion gene by deletions within this 5'-regulatory region.

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0646 REGULATION OF THE GENES FOR GROWTH HORMONE AND PLACENTAL LACTOGEN by John D. Baxter, N.L. Eberhardt, M. Birnbaum, E. Slater, N. Lan and D. Gardner.

The genes for growth hormone (GH) and placental lactogen (chorionic somatomammotropin, CS) along with prolactin form a multigene family that evolved from a common precursor gene. An analysis of their structures suggests that the precursor gene formed from segment duplication, insertion of coding domains and intron removal, whereas diversification of the family involved nucleotide substitutions, insertion of additional intron DNA, the separate insertion of promoter elements, and concerted evolution. Of interest is that the human GH and PL genes show over 90% nucleic acid homology (also in the flanking DNA) and yet they are expressed in different tissues. In the male rat pituitary the major expressed gene is that for GH. This gene is expressed at a much lower level in cultured rat pituitary tumor cells, not at all in certain other tissues and to a very low level in a number of cell types into which the cloned rat and/or human growth hormone genes have been transferred. In some, but not all of the latter circumstances the transferred genes have been found to be regulated by glucocorticoids, but not thyroid hormone. In some cases, regulation of expression of the transferred GH gene does not occur even when the co-transferred human metallothionein-II_A gene does respond to glucocorticoids. In the latter case, the steroid regulatory site can be shown to stimulate transcription by heterologous promoters located several hundred nucleotides away. Although important regulatory information may reside in the 5'-flanking DNA of the GH gene, all of the information is not found in this portion, since the rGH gene deleted in its entire 5'-flanking DNA and transferred into mouse L cells can still be glucocorticoid responsive. These studies indicate that a number of different factors contribute to the high level tissue-specific expression of the GH gene. Indeed, using cultured pituitary cells it appears that regulation occurs by multiple mechanisms, including: direct control of transcription by methylation and thyroid and glucocorticoid hormones; indirect control of transcription by factors regulated by thyroid and glucocorticoid hormones; down regulation of hormone responsiveness by receptor-changes and receptor-independent mechanisms; control of mRNA stability by glucocorticoid and possibly thyroid hormones; and, possibly mRNA translational activity by glucocorticoid hormones.

Regulation of Complex Enzyme Systems

0647 EVIDENCE FOR TWO DISCRETE CATALYTIC SITES ON RAT LIVER 6-PHOSPHOFRUCTO 2-KINASE/FRUCTOSE 2,6-BISPHOSPHATASE. S.J. Pilkis, D.M. Regen, M.R. El-Maghrabi, Department of Physiology, Vanderbilt University, Nashville, TN 37209.

Two enzyme activities catalyzing the synthesis and degradation of fructose 2,6-P_2 have been detected in rat liver and subsequently purified to homogeneity. We have presented evidence that these two activites are present in a single enzyme protein, i.e. the enzyme is bifunctional. This protein, which we have designated 6-phosphofructo 2-kinase/fructose 2,6-bisphosphatase, is an important enzyme in the regulation of carbohydrate metabolism. Its activity determines the steady state concentration of fructose 2,6-P_2, an activator of 6-phosphofructo 1-kinase and an inhibitor of fructose 1,6-bisphosphatase. The enzyme is a substrate for cAMP-dependent protein kinase-catalyzed phosphorylation with concomitant inhibition of the kinase activity and activation of the bisphosphatase activity. The amino acid sequence around the phosphorylation site is Val-Leu-Gln-Arg-Arg-Arg-Gly-Ser(P)-Ser-Ileu-Pro-Gln. The enzyme's activities are also modulated by low molecular weight effectors such as P_i and α-glycerol-P. Regulation of this enzyme in intact cells is a complex function of both covalent modification and the influence of substrates and allosteric effectors.

Recent evidence suggests that the two reactions proceed by a two step transfer mechanism with a phosphoenzyme intermediate. The enzyme catalyzes exchange reactions between ADP and ATP and between fructose 6-P and fructose 2,6-P_2. The existence of such an intermediate for the bisphosphatase reaction was directly demonstrated by the isolation of a ^{32}P-labeled enzyme after incubation with [2-^{32}P]fructose 2,6-P_2 and the subsequent identification of the product as 3-phosphohistidine. However, it was not possible to demonstrate significant labeling of the enzyme directly from [γ-^{32}P]ATP. These results can be most readily explained in terms of two catalytic sites, a kinase site whose phosphorylation by ATP is negligible (or whose E-P is labile) and a fructose 2,6-bisphosphatase site which is readily phosphorylated by fructose 2,6-P_2. Additional evidence in support of two active sites include: 1) limited proteolysis with thermolysin results in loss of 6-phosphofructo 2-kinase activity and activation of fructose 2,6-bisphosphatase; 2) mixed function oxidation results in inactivation of the 6-phosphofructo 2-kinase but no affect on the fructose 2,6-bisphosphatase; 3) N-ethylmaleimide treatment also inactivates the kinase but does not affect the bisphosphatase; and 4) p-chloromercuribenzoate immediately inactivates the fructose 2,6-bisphosphatase but not the 6-phosphofructo 2-kinase. Our findings indicate the bifunctional enzyme is a rather complicated enzyme: a dimer, probably with two catalytic sites reacting with sugar phosphates, and with an unknown number of regulatory sites for most of its substrates and products. Work is in progress to characterize the catalytic and regulatory ligand action further.

0648 PROTEIN PHOSPHORYLATION AND HORMONE ACTION - AN OVERVIEW. Professor Philip Cohen, Department of Biochemistry, University of Dundee, DD1 4HN Scotland, Great Britain.

Over the last 5-10 years there has been a gradual realization that protein phosphorylation is the major general mechanism by which intracellular events are controlled by extracellular stimuli. About 40 enzymes as well as countless other proteins are now known to be regulated in this manner. An important principle to have emerged is that enzymes involved in biodegradative pathways are usually activated by phosphorylation whereas biosynthetic enzymes are normally inactivated by phosphorylation. This finding has been important conceptually since it has raised the possibility that different cellular functions may be regulated by the same protein kinases and phosphatases. Thus an interlocking network of "cascade" systems may exist, that allow co-ordinated and syncrhonous control of many biochemical functions. Recent evidence from this laboratory that supports these concepts will be reviewed.

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0649 ADENYLATE CYCLASE-COUPLED BETA-ADRENERGIC RECEPTORS: BIOCHEMICAL BASIS FOR PHYSIOLOGICAL REGULATION, Robert J. Lefkowitz and Marc G. Caron, Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC 27710

Adenylate cyclase-coupled beta-adrenergic receptors have been among the most thoroughly studied plasma membrane receptors. Several reasons account for this including the ubiquity of the receptors, their close coupling to a well-defined biochemical effector unit, the adenylate cyclase, and the important clinical consequences of stimulation or blockade of these receptors by drugs. Over the past decade there have been numerous advances in the study of the beta-adrenergic receptors including elucidation of the mechanisms by which the receptors are coupled to the adenylate cyclase by their interaction with guanine nucleotide regulatory proteins; photoaffinity labeling of the receptors; purification to homogeneity of the receptors; and reconstitution of the purified receptor proteins with other components of the adenylate cyclase system. The mammalian beta-adrenergic receptor binding site resides on a glycoprotein of M_r 62,000-65,000. The protein is readily subject to proteolysis in situ. This beta-adrenergic binding protein also contains the essential structural requirements for coupling to the other components of the adenylate cyclase system. When the pure receptor protein is reconstituted into lipid vesicles and fused with erythrocytes of Xenopus laevis which lack beta-adrenergic receptors, the Xenopus erythrocyte adenylate cyclase becomes sensitive to catecholamine stimulation. Thus, the single M_r 62,000-65,000 peptide contains both the ligand binding and coupling sites of the beta-adrenergic receptor.

When intact cells are exposed to beta-adrenergic agonist stimulation they rapidly become desensitized to further stimulation. Antagonists block this process. At least two distinct mechanisms account for such tachyphylaxis in different cell types. In one model, the frog erythrocyte, the receptors appear to be sequestered away from the cell surface. The internalized receptors can be quantitatively recovered in a light membrane fraction which sediments at only high centrifugal speeds. The receptors in these light membrane vesicles are structurally unaltered as assessed by photoaffinity labeling and functionally unaltered as assessed by reconstitution and fusion methodology. However, they are physically sequestered away from the nucleotide regulatory protein and the adenylate cyclase and are thereby physiologically uncoupled. These internalized receptors can recycle to the cell surface where they become recoupled to the adenylate cyclase system. By contrast, in turkey erythrocytes agonists do not promote internalization of the receptors. Rather they appear to promote the covalent modification of the receptors by phosphorylation by a cyclic AMP dependent mechanism. The phosphorylated receptors appear to be desensitized. Upon removal of agonist the phosphorylation is reversed as is the desensitization. The phosphorylated and desensitized receptors are functionally deficient as assessed by reconstitution methodologies.

Regulatory Actions of Oncogenes and Inserted Genes

0650 HUMAN LEUKEMIA VIRUSES, ONC GENES AND GROWTH FACTORS, Robert C. Gallo, Flossie Wong-Staal, Mikulas Popovic, Prem Sarin, Phillip Markham, Suresh Arya, S. Zaki Salahuddin, Marjorie Robert-Guroff, Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD 20205

HTLV represents a spectrum of closely and distantly related exogenous human T-lymphotropic retroviruses. About 35 isolates are now known. Most of these are very closely related, belonging to a subgroup we call HTLV-I, and have been obtained from a very aggressive adult T-cell leukemia or lymphoma (ATLL). Others have come from patients with AIDS. We also detected and isolated a different human T-lymphotropic retrovirus termed HTLV-II. There are two members of this subgroup, one a hairy cell leukemia and one from AIDS. Other very recent isolates (again from ATLL and AIDS) are envelope variants of HTLV-I. The genome of several HTLV isolates have been cloned and subcloned, and the complete nucleotide sequence of one is known. In addition to the usual genes (gag, pol, and env) of a chronic leukemia virus HTLV contains near its 3' end a region known as pX with open reading frames. HTLV does not carry a cell derived onc gene and produces monoclonal tumors. Although these are characteristics of a chronic leukemia virus, paradoxically HTLV can transform fresh human blood or bone marrow derived T-cells. The mechanism of this transformation is under study. One of the hallmarks of all HTLV transformed cells (in vitro or in vivo derived tumors) is the presence of TCGF (IL-2) receptors on the surface of these cells. We suggested this on the basis of the direct response of these cells to TCGF in contrast to normal T-cells which first must be activated by appropriate antigens or lectins. T. Waldmann and colleagues developed a monoclonal antibody (α-TAC) to TCGF receptors, enabling the purification and quantitation of the TCGF receptors by W. Greene et al. The number of receptors is about 10X greater in HTLV tumors than in normal PHA activated cells. However, in a collaborative study with S. Clark and colleagues of Genetics Institute, utilizing cloned human TCGF gene we do not find TCGF in mRNA expression in these cells, thereby ruling out an autostimulation model of cell proliferation involving TCGF and its receptor. Nonetheless, the consistency of receptor expression and the high number of receptors both in the in vitro transformed cells and in the tumors suggest that the receptors are involved in the abnormal growth of these cells. Some ideas on how this may occur and the possible role of another growth factor, the c-sis derived PDGF, will be discussed.

0651 REGULATION OF GLOBIN GENES AFTER DNA TRANSFER TO MOUSE ERYTHROLEUKEMIA CELLS, Pamela Mellon*, Patrick Charnay*, Moses Chao#, Richard Axel#, Tom Maniatis*. Harvard University, Cambridge, MA, 02138* and Columbia University, New York, NY, 10032#.

We have introduced cloned globin genes into the genome of Mouse Erythroleukemia cells (MEL) using DNA-mediated gene transfer. These cells are erythroid precursors thought to be arrested at the proerythroblast stage of adult red cell maturation. Treatment with one of a variety of chemical inducers will cause them to differentiate in culture into erythrocytes, during which the transcription of the adult globin genes is significantly increased. We have shown that transferred mouse and human adult-β globin genes are coordinately regulated with the endogenous globin genes during this differentiation. Nuclear transcription experiments demonstrate that this induction is due, at least in part, to an increase in the rate of transcription (1).

In contrast, the transcription of cloned human α-globin genes introduced into MEL cells by DNA transformation was not correctly regulated in 35 out of 36 cell lines examined. The α-globin genes studied were carried on DNA segments with as much as 40 kb of human flanking sequences. These results were unexpected, since human α-globin genes transferred on a chromosome to MEL cells by cell-cell fusion were accurately regulated (2).

In order to more precisely define the sequences involved in the induction of the mouse β major globin gene, we have analysed a series of truncated plasmids which contain from 1.2 to zero kb of 5' flanking sequence. We find that the level of transcription of these genes is reduced for genes containing less than 97 bp of 5' flanking sequence but that the inducibility of transcription is not affected for genes containing as little as 78 bp of 5' flanking sequence. In addition, a mutant globin gene that contains a single base mutation at 87 bp 5' to the gene(isolated from a patient with β thalassemia, a severe anemia) and is defective for transcription (3), was shown to be fully inducible after transfer to MEL cells. This data indicates that the DNA sequences required for constitutive transcription of the β-globin gene are overlapping but seperable from those involved in its regulation during erythroid differentiation.

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Regulation of Membrane Receptors and Transporters

0652 Metabolic Degradation of the Red Cell Anion Transporter Implanted into the Plasma Membrane of Dividing Hepatoma Cells, James F. Hare and Michael Huston, Oregon Health Sciences University, Portland, OR 97201

Since relatively few transport or receptor proteins have been purified well enough to allow their thorough characterization, regulation of transport or receptor function by metabolic turnover or covalent modification in growing cells has not been extensively investigated. We have attempted to study these regulatory aspects of membrane protein function by implanting isolated proteins into the cell membrane of cultured cells. The red cell anion transporter, a prototype transport protein used in our studies, was radiolabled in situ by an endogenous kinase with ^{32}P or by exogenous lactoperoxidase with ^{125}I. The protein was then isolated by a novel method and incorporated, along with Sendai envelope HA protein, into small (0.1 μM) lipid vesicles. The vesicles were absorbed onto the surface of cultured hepatoma cells and then fused into the plasma membrane with polyethylene glycol at a 10% efficiency. The implanted protein catalyzed the transport of SO_4^{-2} and was degraded at the same rate as were lactoperoxidase accessible, endogenous hepatoma membrane proteins ($T\frac{1}{2}$ = 12-16 h). Its degradation was 90% sensitive to lysosomotropic amines. This model system thus represents a new approach to study metabolic regulation of membrane protein function as well as the mechanism of membrane turnover. The intracellular sites of anion transporter peptide domain degradation will be probed by their covalent labeling with membrane impermeable radiolabels prior to implantation, chase, and cell fractionation.

0653 DOWN-REGULATION OF GONADOTROPIN AND β-ADRENERGIC RECEPTORS BY HORMONES AND CYCLIC AMP, Peter H. Fishman, R. Victor Rebois and Terrye Zaremba, Membrane Biochemistry Section, Developmental and Metabolic Neurology Branch, NINCDS, NIH, Bethesda, MD 20205

Loss of gonadotropin receptors in murine Leydig tumor cells (MLTC-1) and of β-adrenergic receptors in rat glioma C6 cells occurred following exposure of the cells to human chorionic gonadotropin (hCG) or isoproterenol (ISO), respectively. Loss of receptors (down-regulation) was time dependent, was not accompanied by loss of regulatory and catalytic components of adenylate cyclase, and was mimicked in part by agents that elevated cyclic AMP. Thus, cholera toxin, dibutyryl cyclic AMP and isobutylmethylxanthine all caused down-regulation in both cell lines but only after a delay of several hours in contrast to the rapid agonist-mediated receptor loss. In MLTC-1 cells, hCG-mediated down-regulation was biphasic; initially, only occuppied receptors were lost but after a lag of several hours, the unoccuppied receptors also were down-regulated. We believe that the second phase is mediated by cyclic AMP. The first phase required the presence of an agonist as deglycosylated hCG, which is an antagonist, did not induce down-regulation. In C6 cells, cyclic AMP-mediated loss of β-receptors occurred in the absence of desensitization of ISO-stimulated adenylate cyclase activity whereas ISO caused both down-regulation and desensitization. In both cell lines, down-regulation induced by either the agonist or cyclic AMP required protein synthesis as cycloheximide inhibited receptor loss. We conclude that down-regulation of receptors in these cells is a complex process involving both cyclic AMP-independent and -dependent events. As the receptors for several growth factors have protein kinase activity and become phosphorylated in the presence of their ligands, phosphorylation may be involved in both phases of receptor down-regulation.

0654 INSULIN RESISTANCE AND PLASMA MEMBRANE INSTABILITY IN MYOTONIC DYSTROPHY. Arthur J. Hudson, Margo E. Miller and Kenneth P. Strickland. Univ. of Western Ont., London, Ont. N6A 5C1 Canada.

Myotonic dystrophy (MyD) is a generalized metabolic disease accompanied by insulin resistant diabetes. It has been shown that insulin binding to MyD monocytes and cultured fibroblasts under optimal binding conditions is reduced. In apparent agreement with this finding insulin-stimulated 2-deoxyglucose transport and glucose uptake into freshly biopsied adipose tissue under optimal conditions of 37°C and pH 7.4 is also decreased. However, with cultured fibroblasts and the same optimal conditions, except for the 5% CO_2 incubation environment, both the basal and insulin-stimulated glucose uptake into the MyD cells is increased. Insulin stimulation is 35% greater in MyD than in control fibroblasts and the insulin-stimulated to basal (S/B) ratio is 1.71 compared with 1.57 in control cells. When the CO_2 in the incubation environment is elevated from 5% to 20% or the pH of the incubation medium is lowered by use of buffers to 6.5, the insulin-stimulated glucose uptake in MyD cells falls to a half of the uptake at pH 7.4 and, moreover, the glucose uptake becomes 15% to 20% less than in control fibroblasts. The S/B ratio for MyD and control cells at pH 6.5 is 1.36 and 1.28, respectively. The decrease in the insulin-stimulated glucose uptake in MyD fibroblasts at pH 6.5 and 37°C corresponds to the reduced insulin binding to these cells with optimal binding conditions of pH 8.0 and 15°C. These studies, in combination with other findings in MyD cells, suggest that the cell surface membrane in MyD may be unstable and markedly affected by the cellular milieu. The increased glucose uptake in fibroblasts under optimal culture conditions compared with the reduced glucose uptake in freshly biopsied adipose tissue may relate to the active growth of the fibroblasts in vitro versus the static state of cells in vivo.

0655 IMMUNOLOGICAL IDENTIFICATION AND REGULATION OF THE GLUCOSE TRANSPORTER FROM CHICKEN EMBRYO FIBROBLASTS, Sue A. Olson, Theodore F. McNair, and Michael J. Weber, Department of Microbiology, University of Illinois, Urbana, IL 61801.

Antiserum raised against the human erythrocyte glucose transporter immunoprecipitates two membrane proteins of average M_r 46,000 and 53,000 from chicken embryo fibroblasts (CEF) that appear to be components of the CEF D-glucose transport system. Immunoprecipitation could be blocked by the human glucose transporter or by an extract of human erythrocyte membrane proteins but not by an extract from bovine erythrocytes, which do not possess a glucose transporter of this type. Both of the proteins become tritium labeled when the glucose transporter-specific ligand [^{3}H]-cytochalasin B is photo-incorporated into CEF membrane proteins, and this incorporation is blocked in the presence of D-glucose, an inhibitor of cytochalasin B binding to the transporter. As is the case with the human erythrocyte transporter, the migration of the proteins as diffuse bands on SDS polyacrylamide gels appears to be due to glycosylation: when immunoprecipitated from tunicamycin-treated cells, the two protein bands appear considerably sharper, and migrate at molecular weights approximately 10 kdaltons lower than those of the proteins from untreated cells. Partial peptide maps of the two proteins are different, and the proteins also seem to be regulated differently. When CEF are transformed by Rous sarcoma virus, there is an increase in the glucose transport rate and in the amount of the M_r 53,000 protein, while the amount of the M_r 46,000 protein increases when the transport rate is elevated in response to glucose starvation of the cells.

0656 ISOLATION OF EGF AND INSULIN RECEPTOR FROM AN ORGANOMERCURIAL AGAROSE GEL, L.S. Aglio,[1] J.M. Maturo,[2] and M.D. Hollenberg,[3] [1]Albert Einstein College of Medicine Bronx, N.Y., [2]C.W. Post College, Department of Biology, Greenvale, N.Y., and [3]University of Calgary, Faculty of Medicine, Alberta, Canada

Placental membranes, prepared in the presence of EGTA and EDTA, were incubated in the presence of either labeled EGF or insulin in the presence or absence of saturating unlabeled ligand until equilibrium. After addition of the cross-linking agent, disuccinimidyl suberate, washed membranes were extracted with 1% Triton X-100. The high speed clarified supernatants were used in the following experiments without further purification. An organomercurial agarose support with selectivity for sulfhydryl groups (Affi-Gel 501, BioRad) was washed with 50 mM sodium acetate pH 5 followed by a 10 column volume wash with 4 mM mercuric acetate - 50 mM sodium acetate pH 5. A thorough washing with 10 mM Tris - 0.1% Triton pH 7.5 preceded sample additions. Following sample additions, the column was washed thoroughly with the sample buffer and elution was achieved with 10 mM dithiotreitol. Samples of non-adsorbed as well as adsorbed-eluted material were prepared for SDS-polyacrylamide gel electrophoresis on 5-15% linear gradients. The results indicated that 30% of the specifically cross-linked labeled EGF receptor and 20% for that of insulin could be absorbed and eluted from the organomercurial agarose gel. Preincubation with N-ethylmaleimide would completely abolish the association of specifically bound material. SDS-PAGE analysis indicated that the absorbed material was cross-linked labeled EGF and insulin receptors, respectively.

0657 TRANSFERRIN RECEPTOR EXPRESSION IS UNCOUPLED FROM CELL PROLIFERATION BY A DNA POLYMERASE INHIBITOR, W.K. Funkhouser, J.B. Trepel, and L.M. Neckers, NCI, NIH, Beth. MD

One of the most consistent elements of the proliferating cell phenotype, regardless of cell origin, is expression of the surface transferrin receptor (TFR). Although malignant cells may lose their surface receptors for specific growth factors, (i.e. IL-2 independent T-cell lymphomas), all rapidly proliferating cells express TFR. We have investigated the relationship of cell surface TFR expression to replicative DNA synthesis. Aphidicolin, an alpha DNA polymerase inhibitor, inhibits DNA replication in CEM cells. Numerical population growth and mean cellular ^{3}H-TdR uptake are subsequently decreased. Unexpectedly, there is an associated increase in surface transferrin receptor density using FACS analysis of OKT9 or FITC-transferrin fluorescence intensity, after incubation of cells with aphidicolin over a 1-2 day period. Trypsin stripping of surface marker antigens, with measurement of antigen reappearance rates, demonstrates that aphidicolin incubation does not increase the transferrin receptor reappearance rate. Inhibition of internalization of transferrin receptor with dansylcadaverine is mimicked, and possibly augmented, by aphidicolin pre-incubation, suggesting that steady-state surface transferrin receptor density is increased after aphidicolin by inhibiton of internalization of the receptor. These observations stimulate further interest in the membrane event/nuclear event interface when it is recognized that monoclonal antibody blocking of CEM transferrin receptor ligand sites yields inhibition of DNA replication and subsequent decrease in both average ^{3}H-TdR incorporation and numerical population growth. In summary, these observations suggest that regulation of transferrin receptor density at the cell surface may be one of the mechanisms by which the cell controls DNA synthetic rate during replication.

0658 ADENOSINE-SENSITIVE ADENYLATE CYCLASE IN HEART SARCOLEMMA, Madhu B. Anand-Srivastava and Marc Cantin, Clinical Research Institute of Montreal, Montreal, Canada H2W 1R7.

Adenosine-sensitive adenylate cyclase was characterized in rat heart sarcolemma. Heart sarcolemma was isolated by hypotonic shock-lithium bromide treatment method. This preparation contained negligible amounts (2-4%) of contamination by other subcellular organelles such as mitochondria, sarcoplasmic reticulum and myofibrils as verified by electron microscopic examination. N-Ethylcarboxamide adenosine (NECA), L-N -phenylisopropyl adenosine (PIA) and adenosine N'-oxide (Ado N'-oxide) all stimulated adenylate cyclase in a concentration dependent manner in an order of potency NECA >Ado N'-oxide >PIA. The activation of adenylate cyclase by NECA was dependent on the concentrations of Mg^{2+} or Mn^{2+}.The maximal stimulation was observed at lower concentration of the metal ions (0.2 -0.5 mM). At 5 mM Mg^{2+} or Mn^{2+}, the stimulation by NECA was completely abolished. The stimulatory effect of NECA on adenylate cyclase was blocked by 3-isobutyl-1-methylxanthine (IBMX). In addition 2'deoxy adenosine showed an inhibitory effect on adenylate cyclase. The stimulation of adenylate cyclase by NECA was additive with maximal stimulation obtained by epinephrine. These data suggest: 1. the presence of adenosine "Ra" receptors in heart sarcolemma and 2. adenosine and catecholamines either stimulate different cyclase populations or stimulate a common cyclase by distinct mechanisms. (Supported by grants from Quebec Heart Foundation, the Fonds de la recherche en santé du Québec and Medical Research Council of Canada to the Multidisciplinary Research Group on Hypertension).

0659 DEPHOSPHORYLATION OF (NA,K)ATPASE IN FRIEND CELLS DURING CELL DIFFERENTIATION, Li-An Yeh, Ian Macara and Lewis C. Cantley, Harvard University, Cambridge, MA 02138

A decrease in the activity of the plasma membrane (Na,K) pump is an early and essential step in commitment of Friend virus infected murine erythroleukemia cells to terminal erythroid differentiation. The mechanism by which dimethyl sulfoxide, an inducer of Friend cell differentiation, causes the decrease in pump activity has been investigated. Friend cells treated with 1.5% dimethyl sulfoxide for 20 hours exhibited approximately a 50% decrease in ouabain sensitive $^{86}Rb^{+}$ uptake. The cellular ATP concentration and K 1/2 for extracellular K^{+} were unaffected. The intracellular Na^{+} concentration increased approximately 30%. The number of copies of (Na,K)ATPase in the plasma membrane did not change as judged by the specific activity in purified plasma membranes or by active site phosphorylation with [^{32}P]-orthophosphate in the presence of ouabain plus Mg^{2+}. We previously showed that the α subunit of (Na,K)ATPase in Friend cells is phosphorylated by a cAMP independent and Ca^{2+} independent protein kinase (Yeh et al 1983 J. Biol. Chem. 258,6567). Here we show by labeling cells in culture with ^{32}P and [^{3}H] leucine that the phosphorylation stoichiometry per α subunit decrease by approximately 50% after treatment with dimethyl sulfoxide. These results suggest that the (Na,K) pump activity of Friend cells can be regulated by an endogenous kinase which may also be important for regulating cell differentiation.

0660 THE (NA,K)ATPASE OF FRIEND ERYTHROLEUKEMIA CELLS IS PHOSPHORYLATED NEAR THE ATP HYDROLYSIS SITE BY AN ENDOGENOUS, MEMBRANE BOUND KINASE, Leona Ling and Lewis Cantley, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA

Friend murine erythroleukemia cells(MEL cells) contain a cAMP independent protein kinase which phosphorylates the 100K catalytic subunit of the (Na,K)ATPase both in living cells and purified plasma membrane (Yeh et al., 1983, J. Biol. Chem., 258, 6567-6574). The region of phosphorylation was mapped to the central portion of the 100K polypeptide chain. The sites of chymotryptic cleavage on the MEL cell 100K polypeptide were located by comparing the active site phosphoaspartate labeled proteolytic peptides from the MEL cell and dog kidney 100K peptides. These proteolytic cleavage sites have previously been mapped on the dog kidney enzyme (Castro and Farley, 1979, J. Biol. Chem., 254, 2221-2228). The comparison of proteolytic products from chymotryptic digestion of native MEL cell and dog kidney 100K polypeptide suggested the sites of chymotryptic cleavage are similar for the two enzymes. The MEL cell 100K polypeptide was also labeled by the endogenous kinase by incubating purified plasma membrane with [γ-^{32}P]ATP. Chymotryptic digestion of these membranes produced a 35K phosphorylated peptide which contained almost all of the label present in the phosphorylated, but undigested 100K polypeptide. This 35K peptide also contained the active site aspartate which identifies it as originating from the central portion of the 100K polypeptide. These results indicate that the majority of the in vitro kinase phosphorylation occurs within this central portion of the 100K polypeptide. This region also contains the ATP hydrolysis site(active site aspartate) and an ATP binding site(Carilli et al., 1982, J. Biol. Chem., 257, 5601-5606) which suggests that phosphorylation may serve to regulate the activity of this enzyme.

0661 ESSENTIAL ROLE OF DISULFIDE BONDS IN THE HORMONE INDUCED CONVERSION BETWEEN CONFORMATIONAL STATES OF THE INSULIN RECEPTOR, Kenneth E. Lipson, Kazuyo Yamada and David B. Donner, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

^{125}I-insulin was incubated with receptors on control or dithiothreitol treated rat liver plasma membranes. The release of ^{125}I-insulin from control membranes was biphasic and resolved into rapid and slow components. All hormone dissociated from treated membranes at a rapid rate. As the incubation time between control membranes and ^{125}I-insulin was lengthened the fraction of hormone which dissociated at a slow rate increased; this was not true of dithiothreitol treated membranes. Thus, reduction of disulfide bonds impaired the ability of binding sites to undergo an occupancy-mediated increase of affinity. In affinity labeling experiments, unoccupied receptors were cleaved by trypsin to produce fragments of 94,000 and 37,000 Da. Trypsinizing membranes after they were incubated with hormone gave an additional fragment of M_r 64,000. Membranes treated with dithiothreitol were unable to undergo this hormone-induced alteration of sensitivity to trypsin (conformational change). As the incubation time between insulin and control membranes was lengthened, the fraction of receptors in a high affinity, trypsin insensitive state increased due to a second, slow conformational change. After brief or extended incubations with ^{125}I-insulin, receptors on treated membranes remained labile to trypsin. Thus, disulfide bonds play an obligatory role in facilitating the conversion between affinity and conformational states of the insulin receptor. Supported by NIH and the Juvenile Diabetes Foundation.

0662 STAGE-SPECIFIC EXPRESSION AND LOCALIZATION OF MEMBRANE ANTIGENS DURING MUSCLE DEVELOPMENT. Stephen J. Kaufman, Thomas I. Doran, and Rachel F. Foster, Department of Microbiology, University of Illinois, Urbana, IL 61801.

We have used monoclonal antibodies to define quantitative and topographic changes of antigens on the surface of L8E63 rat myoblasts that accompany differentiation. The determinants on the myoblast reactive with these monoclonal antibodies have been studied by immunofluorescence and photometry, and by radioimmunoassay. Four levels of complexity on the membrane have been discerned during myoblast development: 1) Distinct patterns of quantitative changes of membrane antigens parallel the morphologic differentiation of myoblasts in vitro; 2) Changes in the distribution of membrane antigens accompany myogenesis. These include: general aggregation and dispersal of antigens, concentration of antigens on cell projections and between aligned myoblasts, and specific alignments of select antigens into linear arrays; 3) The prevalence and distribution of the same determinant on the attached and upper surfaces of myoblasts differ for many antigens; 4) Many antigens on myoblasts are not extracted with Triton X-100 and are therefore believed to be associated with the cell cytoskeleton. For some antigens this linkage to the cytoskeleton exists de novo; crosslinking of other determinants with antibody promotes this association. The association of specific membrane antigens with the cytoskeleton changes with myoblast development. These experiments demonstrate that the myoblast membrane is in a dynamic state and undergoes a dramatic remodeling that accompanies differentiation. Supported by the Muscular Dystrophy Association and USPHS-GM28842.

0663 REVERSIBLE ALTERATION OF ASIALOGLYCOPROTEIN RECEPTORS IN DIABETIC RATS. Hak-Joong Kim, and Indira V. Kurup. Medical College of Wisconsin, Milwaukee, Wis. 53226

Previously we reported that the catabolism of galactose- or glucose-conjugated plasma albumins or low density lipoproteins (neo-glycoproteins) were reduced in diabetic rats, and the reduction was due to decreased uptake by the asialoglycoprotein receptors (ASGR) of diabetic liver (J. Cell Biochem. Suppl #6:135, 1982). To assess the ASGR activity, chromatographically purified bovine asialofetuin (ASGR) was labeled with I^{125} using ICl method of McFarlane, and I^{125}-ASF turnover in vivo and I^{125}-ASF binding to isolated liver cells in vitro were compared among groups of normal (N), diabetic (D) and diabetic rats treated with insulin (D+I). I^{125}-ASF catabolism was not different between N and D+I ($T_{\frac{1}{2}} < 10$ min); however, the turnover was reduced in D ($T_{\frac{1}{2}} = 20$ min). The reduced turnover in D was due to a significant reduction of hepatic uptake of the I^{125}-ASF. Liver cells isolated by a collagenase digestion showed sturable binding of I^{125}-ASF, but maximum binding to D cells were less than 50% of the N or the D+I cells. Scatchard analysis of binding data revealed that the D cells had significantly reduced binding sites per cell ($\times 10^{-3}$) at both 4°C and 37°C (12±2; 42±8.5, mean ± SEM n = 7) compared to the N and the D+I cells (45±13, 59±8 at 4°C; 221±32, 219±35 at 37°C, respectively). The D cells also showed significantly low affinity at 4°C (Kd: 0.59±0.14 at 0.52 ±0.02 nM) compared to the N cells (Kd:0.11±0.025 nM at 4°C 0.1±0.013 nM at 37°C). The affinity of the D+I cells were not significantly different from that of the N cells. The results suggest that the ASGR activity was reduced in the D cells due to reduced receptor sites and a reduced affinity. The alterations were reversible by an insulin treatment.

0664 COATED VESICLE ASSEMBLY POLYPEPTIDES: ROLE IN COAT REASSEMBLY. James H. Keen and Sam Zaremba, Fels Research Inst., Temple Univ. Schl. Med., Phila., PA 19140

Factors governing the reassembly of clathrin coat structures, the cellular organelle involved in receptor-mediated endocytosis and other intracellular membrane activities, have been studied. Coated vesicles treated with 0.5 M Tris Cl at neutral pH release clathrin, the structural component of the coat, and a number of other polypeptides in soluble form. Gel filtration of this extract resolves a protein complex, containing polypeptides of $M_r \sim$ 110K, 100K, 52K and 16.5K, that has been shown to promote clathrin reassembly into unique coat structures of sharply defined size (80 nm $\pm$ 5 nm). In contrast, reassembly of clathrin triskelions alone generates larger and more heterogeneous coats (100 nm $\pm$ 15 nm). These assembly polypeptides (AP) are specifically and stoichiometrically incorporated into reassembled coats and their presence permits reassembly under conditions of pH (6.8-7.4) and salt concentration ($\sim$0.1 M) under which clathrin triskelions alone remain dissociated. We have found the 52K M_r assembly polypeptide to be a major substrate of a coated vesicle kinase and this phosphopolypeptide has also been shown to be immunologically related to the microtubule associated τ proteins (Pfeffer et al., J. Cell Biol. 97:40, 1983). Thus the AP, which have been purified by affinity chromatography, promote reassembly under physiological conditions of coat structures whose sizes strikingly parallel those seen in the Golgi region of cells. The effects of phosphorylation and the intracellular action of the AP are being studied.

0665 IDENTIFICATION AND CHARACTERIZATION OF THE RAT ADIPOCYTE GLUCOSE TRANSPORTER BY PHOTOAFFINITY CROSSLINKING, Richard Horuk, Martin Rodbell, Samuel W. Cushman and Ian A. Simpson, NIH, Bethesda, MD 20205

The photoaffinity crosslinking agent hydroxysuccinimidyl-4-azidobenzoate has been used to attach [^{3}H]-cytochalasin B to a rat adipocyte low-density microsomal membrane protein of molecular weight 45-50K. The characteristics of the [^{3}H]-cytochalasin B-labeled protein are consistent with those of the adipocyte glucose transporter. The low-density microsomes from cells incubated without insulin incorporate twice the amount of radioactivity per mg of membrane protein than the low-density microsomes derived from insulin-stimulated cells. This value agrees with the distribution of glucose transporters measured in this intracellular membrane fraction prepared from basal and insulin-treated cells by [^{3}H]-cytochalasin B binding. Preincubation of membranes with 500 mM D-glucose reduces the photoaffinity crosslinking by 48% relative to that observed with 500 mM L-glucose. Isoelectric focusing of low-density microsomes containing the photoaffinity crosslinked transporter yields three bands of radioactivity focusing at pH's of 5.5, 4.5, and 4.2, respectively. Following isolation from the isoelectric focusing gel and SDS polyacrylamide gel electrophoresis, all three peaks can be shown to contain a band of molecular weight 45-50K which crossreacts with an antiserum raised against the purified human erythrocyte glucose transporter. These results suggest that identification, isolation, and purification of the adipocyte glucose transporter is now possible using the techniques described above.

0666 THE HUMAN ERYTHROCYTE Rh D ANTIGEN IS A PROTEOLIPID, Lyle T. Sinor, Jane M. Rachel, Ralph J. Butkowski, and Fred V. Plapp, Community Blood Center, Kansas City, MO 64111, and University of Kansas Medical Center, Kansas City, KS 64102

The Rh D antigen is a small integral membrane protein which may be involved in membrane cation transport and ATPase activity. Since the molecular weight of the D antigen closely resembled the molecular weight of proteolipids, which are also integral proteins associated with ATPase, we investigated to see if the D antigen was a proteolipid. These investigations have revealed that the D antigen is a proteolipid because it partitioned into chloroform-menthanol and specifically reacted with dicyclohexylcarbodiimide (DCCD), an ATPase inhibitor. DCCD was also shown by ELISA to inhibit anti-D binding. The Rh D receptor closely resembles proteolipids in other aspects including size and amino acid composition. HPLC and SDS-PAGE revealed a MW of 35,000 and an acidic pI of 6.1. The amino terminus of the purified Rh D receptor is histidine. The amino acid composition of the Rh D receptor was almost identical to the proteolipids from canine cardiac sarcoplasmic reticulum (phospholamban) and canine cardiac sarcolemma (calciductin) (1). Thus the Rh D receptor is a proteolipid and may be an important regulator of human erythrocyte cation transport. (1) Biochimica et Biophysica Acta, 728:83-91 (1983).

AMINO ACID COMPOSITION OF Rh D PROTEOLIPID
(Residues/1000 amino acid residues, N=6)

A.A.	MEAN	± S.D.	A.A.	MEAN	± S.D.
Asp	82.8	2.1	Cys	6.2	1.9
Thr	54.9	1.9	Met	17.1	4.8
Ser	80.2	3.2	Ile	40.4	2.4
Glu	129.0	3.1	Leu	112.5	3.4
Pro	50.4	5.6	Tyr	25.7	1.4
Gly	94.5	5.2	Phe	44.1	2.4
Ala	79.7	4.3	His	30.0	8.0
Val	56.9	3.0	Lys	48.0	2.5
Arg	47.4	4.4			

0667 LYMPHOCYTE BINDING SITES FOR BACTERIAL LIPOPOLYSACCHARIDE (LPS), Diane M. Jacobs and Frank Swartzwelder, SUNY at Buffalo, Buffalo, NY 14214.

LPS stimulates division (DNA synthesis) and differentiation (immunoglobulin synthesis and secretion) in murine B lymphocytes. Although preferential binding of the ligand to target cells has been demonstrated, the binding site responsible has not been identified. Using immunofluorescence microscopy, we have found that LPS caps on lymphocytes 1) preincubated with LPS in the cold and then exposed to either intact anti-LPS or Fab-anti-LPS at 20°C, or 2) incubated with LPS only at 20°C. No capping was observed at 0°C. Capping was inhibited by cytochalasins b and d, fluoride, iodoacetamide, azide and oligomycin. Thus binding of LPS to sites on lymphocytes, like the binding of antigen or anti-Ig to sIg, is followed by lateral mobility of the ligand-binding site complex which is dependent on temperature, aerobic and anaerobic glycolysis, and an intact cytoskeleton. sIgM, sIgD and Ia did not co-cap with LPS, indicating that the LPS binding site on B lymphocytes is distinct from these surface antigens. LPS binding to lymphocytes at 0°C was inhibited when the incubation mixture included polymyxin B, a cyclic cationic peptide antibiotic that interacts with LPS and inhibits its activity, but such inhibition was ineffective when addition was delayed 30 min or the incubation was at 20° or 37°C. The positively charged proteins egg white lysozyme and protamine chloride, and the polyanion dextran sulfate also inhibit binding. Lymphocytes pretreated with pronase fail to bind detectable LPS. These results suggest that negatively charged LPS interacts with lymphocytes in two stages: an initial step dependent on a positively charged membrane protein followed by irreversible hydrophobic interaction with membrane lipids. (Supported by NIH Research Grant AI 16915).

0668 CELLULAR METABOLISM OF BETA-ADRENERGIC RECEPTORS (BAR) ON S49 LYMPHOMA CELLS Lawrence C. Mahan and Paul A. Insel, U.C.S.D., La Jolla, CA. 92093

We have developed a receptor-specific method, which involves recovery of radioligand binding sites after irreversible receptor blockade, to assess BAR metabolism in S49 lymphoma cells. Rates of receptor appearance and disappearance were obtained in intact cells under physiological growth conditions after alkylating BAR with the antagonist bromoacetylalprenololmenthane (BAAM). Receptor-specific inactivation was time and dose dependent; concentrations of BAAM (0.1 - 2.0μM) could be used to achieve up to 85-90% BAR blockade as measured by the inhibition of binding of the radiolabeled antagonist [^{125}I]iodocyanopindolol (ICYP). Blockade was inhibited stereoselectively: (-)-propranolol was more effective in the inhibition of BAAM alkylation than (+)-propranolol. Scatchard analysis of sites remaining on extensively washed cells revealed a decrease in receptor number with essentially no change in the affinity of the receptors for ICYP, indicating lack of residual free BAAM or nonspecific effects of BAAM. Cells treated in growth medium containing 10% horse serum have unaltered viability, growth characteristics (cell doubling time=16-17hrs) and cell cycle phase distribution. Return of ICYP binding sites in cells growing over 100 hrs was analyzed by Marquardt non-linear least squares fit of data according to $R_t=k_a/k_d(1-\exp-k_dt)+R_o\exp^{-k_dt}$ where k_a, k_d and R_o represent rates of receptor appearance, disappearance and initial receptor levels respectively. Observed values of k_a=38 sites/cell/hr(2% control sites/cell/hr) and k_d=0.023 hr^{-1} yield a turnover rate of 30 hrs for BAR on S49 cells. Because BAR turn over more slowly than S49 cells divide, receptor protein appears to be conserved through successive cell generations.

0669 REGULATION OF INSULIN RECEPTOR FUNCTION BY ACIDIC INSULIN-LIKE GROWTH FACTOR, T.J. Brown, M.N. Lioubin, and B.H. Ginsberg, University of Iowa, Iowa City, IA 52242.

An acidic insulin-like growth factor (IGF) has been purified from human plasma and found to exert effects on both insulin action and insulin receptor properties. The effects of IGF must be demonstrated in the absence of serum binding proteins. IGF is able to antagonize the growth stimulating action of insulin by 75% in thymidine pulse experiments. Friend erythroleukemia cells (FLC) pretreated with IGF in vitro are "desensitized" to insulin action. Growth studies reveal that in the presence of IGF these cells undergo premature growth arrest. IGF treatment results in a decrease in the high affinity form of the insulin receptor. IGF does not induce/block insulin receptor down-regulation. Immunoprecipitation experiments using anti-insulin receptor antibody indicate that IGF does not bind to the insulin receptor per se. The IGF receptor demonstrates a 35 fold greater affinity over the insulin receptor for homologous hormone. The IGF receptor demonstrates linear Scatchard binding whereas the insulin receptor demonstrates curvilinear binding. We conclude from the above data that: (a) distinct receptors for IGF and insulin exist on FLC; (b) IGF antagonizes the insulin response of FLC; (c) IGF induced "desensitization to insulin" is associated with decreased insulin receptor affinity; (d) IGF interacts indirectly with the insulin receptor. The IGF effect probably mediated through a distinct IGF receptor appears to involve interference with the growth regulatory mechanisms of erythroleukemia cells.

0670 INCREASED RATE OF TURNOVER OF SURFACE INSULIN RECEPTORS IN FIBROBLASTIC CULTURES FROM GENETICALLY DIABETIC MICE, R.E. Fellows, R. Kadle, The University of Iowa, Iowa City IA 52242 and M.K. Raizada, University of Florida, Gainesville FL 32610.

We have reported that fibroblastic cultures from the skin of C57BLKs/Jm-db diabetic (D) mice have approximately 50% fewer surface insulin receptors than cultures from nondiabetic (ND) littermates. Since intracellular binding by D cells is ~1.5 times greater than by ND cells, total cellular binding in each is not significantly different. In the present study we have measured the rate of turnover of surface insulin receptors in both ND and D cells. Confluent cultures were incubated up to 36 hours in a 'heavy' medium containing ^{13}C, ^{15}N and ^{2}H labeled amino acids. ^{125}I-insulin binding was measured and bound ligand was specifically cross-linked to its receptor with the bifunctional reagent ethylene glycol bis(succinimidyl succinate). The cross-linked insulin-receptor complex was solubilized in 4% Triton X-100 and subjected to isopycnic centrifugation on a 46.7% CsCl density gradient for 24 hours. Fractions of 150 µl were collected and radioactivity counted. The half-life of surface receptors was determined by plotting the log of peak heights of 'light' receptors as a function of time of incubation in 'heavy' medium. This plot was linear, indicating that the decay of the receptor followed first order kinetics. The half-life of the receptor was estimated to be 15.04 hours for ND cells and 11.57 hours for D cells. These results indicate that the rate of turnover of surface insulin receptors is faster in D cells than in ND cells. In view of the decreased ratio of surface to intracellular receptors, this suggests that D cells have a defect in the control of internalization. Supported by AM25295 to Iowa DERC.

0671 Down-Regulation of the β-Adrenergic Receptor of the S49 Lymphoma Cell. K.A. Rich and R. Iyengar, Dept. of Cell Biol., Baylor College of Medicine, Houston, TX 77030. Prolonged exposure to hormones causes desensitization of target cells, resulting in a loss of response to further stimulation. In the S49 murine lymphoma cell line, catecholamine-induced desensitization has been shown to involve an initial phase of functional uncoupling of the receptor from the adenylyl cyclase (AC) complex, followed by down-regulation of the β-adrenergic receptors. We have investigated the conditions for down-regulation and the status of the down-regulated β-adrenergic receptors. Treatment of WT S49 cells with ISO for 16 h resulted in an 84% decrease in cell-surface β-adrenergic receptors and a 78% decrease in ISO-stimulated AC activity. Similar treatment of cyc$^-$ cells, a mutant that lacks a functional N_s, did not result in significant receptor loss despite a 50% decrease in ISO stimulation of reconstituted AC activity. Studies on the fate of the lost β-adrenergic receptors demonstrated that there was no identifiable sequestration of receptors into an intracellular membrane fraction. Furthermore, addition of cycloheximide to the culture medium of WT cells previously treated with ISO resulted in a complete blockade of the restoration of cell surface receptors which was otherwise observed within 16 h after removal of the agonist. These results provide the first evidence that the S49 cell line differs from other β-adrenergic systems in that the down-regulated receptors appear to be degraded, since protein synthesis is required for restoration of cell surface receptor levels after removal of the agonist. Furthermore, while the presence of a functional N_s is required to obtain substantial receptor loss, the receptor is selectively removed from the cell surface without any measurable change in N_s.

0672 COATED VESICLE COMPOSITION AND CELLULAR CONCENTRATION AS DETERMINED BY MONOCLONAL ANTIBODIES, Thomas F. Roth, Antonino Passaniti, Han Do Kim, and William G. Kelly, University of Maryland Baltimore County (UMBC), Catonsville, MD 21228.

Coated vesicles are carriers in a wide spectrum of specific protein transport systems, and are ubiquitous throughout the eukakryotes. Their structure is highly ordered and consists of a number of interrelated repeating subunits. How these are joined to each other is not clear, nor is their exact molecular composition well defined. To better define the polypeptides that comprise the CVs, a large number of monoclonal antibody probes were produced. Each is being characterized to a polypeptide epitope among the 20 or so proteins that comprise the coat and vesicle proteins. Both ELISA and immunoblots are used to identify and quantitate the antigens such as clathrin, the light chains and tubulins. By combining selective solubilization with these methods the relative position of each antigen can be determined. Cellular distribution by immunofluorescence is ongoing.

0673 REVERSIBLE INTERNALIZATION OF β-ADRENERGIC RECEPTORS (BAR) AFTER AGONIST-TREATMENT IN C6-GLIOMA CELLS. Cornelia Hertel, Hubert Affolter, Madeleine Portenier and Matthys Staehelin. Friedrich Miescher-Institut, P.O.Box 2543, CH-4002 Basel, Switzerland.

Short time treatment of intact C6-glioma cells with a β-adrenergic agonist, isoproterenol, induces the appearance of a low affinity binding site for isoproterenol and for the membrane impermeable antagonist CGP 12177. While the appearance for the low affinity for isoproterenol is fast ($t_{1/2}$=1min), the one for CGP 12177 appears slower ($t_{1/2}$=5min). On a sucrose density gradient receptors which possess both, low affinity for agonists and for CGP 12177 (RL) comigrate with endocytotic vesicles, as determined by labeling with horse-radish peroxidase, whereas receptors which possess high affinity for both ligands (RH) comigrate with a plasma membrane marker (5'-nucleotidase). The use of a pore-forming agent, alamethicin, reversed the low affinity of BAR for CGP 12177, but not for isoproterenol, indicating that RL is located at the inner side of the vesicle membrane.
In intact cells only BAR located at the plasma membrane bind [^{3}H]CGP 12177. Agonist incubation induced a reduction of the number of receptors accessible for [^{3}H]CGP 12177 which parallels the appearance of RL in lysates. After removing the agonist the binding sites for [^{3}H]CGP 12177 reappear rapidly ($t_{1/2}$=3min). Monovalent cation gradients, most probably for protons, and a low intracellular Ca^{2+}-concentration are necessary for the reappearance of receptors.

The results obtained so far suggest that agonist treatment of BAR induces a fast decrease in the affinity for agonists followed by a vesiculation of the receptor, which is reversible. The vesiculation may be due to reversible endocytosis of BAR.

0674 INSULIN REGULATION OF INSULIN-LIKE GROWTH FACTOR ACTION IN RAT HEPATOMA CELLS, Nancy L. Krett, Joanne H, Heaton and Thomas D. Gelehrter, University of Michigan, Ann Arbor MI 48109-0010

We have reported previously that insulin causes a complete but reversible desensitization to insulin action in HTC rat hepatoma cells in tissue culture, and that this insulin resistance is mediated by post-binding mechanisms rather than receptor down-regulation (Heaton and Gelehrter, JBC 256:12257-12262, 1981). We report here that insulin causes a similar desensitization to the induction of tyrosine aminotransferase (TAT) by the insulin-like growth factors IGF-I and IGF-II isolated from human plasma, and by multiplication stimulating activity (MSA), the rat homologue of IGF-II. The results of both competition binding studies and affinity cross-linking experiments indicate that IGFs bind primarily to IGF receptors rather than to insulin receptors. The low concentrations at which these factors induce TAT is consistent with their acting primarily via IGF receptors. This is confirmed by experiments utilizing anti-insulin receptor antibody (anti-R) which both inhibits ^{125}I-insulin binding and shifts the concentration-dependence of insulin induction of TAT to the right. This same immunoglobulin does not inhibit ^{125}I-MSA binding and only minimally inhibits ^{125}I-IGF-I binding. Anti-R also does not significantly shift the concentration-dependence for the IGFs, suggesting that IGFs induce transaminase by acting via IGF receptors. Although insulin down-regulates insulin receptors, it does not decrease IGF-I or IGF-II binding. We conclude that insulin causes desensitization of HTC cells to IGFs by affecting a post-binding step in IGF action, which may be common to the actions of both insulin and insulin-like growth factors.

0675 BIOCHEMICAL AND FUNCTIONAL CHARACTERIZATION OF THE MAMMALIAN REOVIRUS RECEPTOR ON MURINE T CELLS USING ANTI-IDIOTYPIC ANTIBODY, Glen N. Gaulton, Man Sung Co, John H. Noseworthy and Mark I. Greene, Harvard Medical School, Boston, MA 02115

Studies have been conducted to elucidate the biochemical structure, cellular distribution, and modulation of the mammalian reovirus receptor. A syngeneic monoclonal anti-idiotypic antibody (87.92.6) was prepared by injection of purified monoclonal id$^+$ antibody (G5),with specificity to the reovirus type 3 hemagglutinin (HA3). Anti-id has been shown to bind to reovirus receptors on a variety of cell types and can block viral binding. Previous work has demonstrated that this anti-id binds to 45% of Ly2$^+$ T cells and when added to T cells stimulated in vitro with Con A suppresses the proliferative response by 80%. Similar suppression observed upon reovirus exposure was mediated by the activation of Ts cells. It has also been shown that anti-id binds reovirus specific Tc and blocks the lytic activity of a reovirus specific Tc line to both reovirus infected P815 cells and to anti-id hybridoma (87.92.6) cells. We report here on the capping of reovirus receptors following anti-id binding and its linkage to cytoskeletal elements, on the kinetics of binding of ^{125}I-labeled anti-id to immune and somatic cells, and on the biochemical characterization of the reovirus receptor. Anti-id binding was abrogated by prior treatment of cells with either protease or tunicamycin suggesting that the receptor is a glycoprotein. Anti-id bound to a single band on western blots of purified cell membranes run on SDS-PAGE. Similar analysis of membrane proteins on IEF gels identified the charge characteristics. The relationship of the immune receptor on T cells to that on somatic cells will be discussed.

0676 CELL AFFINITY PURIFICATION OF A THROMBIN RECEPTOR-BINDING GROWTH FACTOR, J. S. Bergmann and Darrell H. Carney, The University of Texas Medical Branch, Galveston, TX 77550

We recently reported the partial purification of a serum factor(s) from bovine and equine sera which initiates proliferation of mouse embryo (ME) cells and inhibits binding of ^{125}I-thrombin to its high affinity receptors (J. Cell Biol. 97: 394a, 1983). This fraction eluted from a Sephacryl S-200 column in a region corresponding to an approximate molecular weight of 90 to 110 thousand daltons. From this purification alone, however, it was unclear whether the same molecule was responsible for inhibition of binding and initiation of cell proliferation. To further purify this serum factor, we have utilized cell affinity chromatography techniques to examine only molecules which bind tightly to ME cell surfaces. ME cells were cultured on Biosilon beads (18g) and cultured for 3 days in medium containing 10% calf serum. The cells (approximately 2 x 10^8 cells) were then cultured in serum-free medium for one additional day and then fixed in 3% formaldehyde. This procedure does not alter ^{125}I-thrombin binding to its high affinity receptors. These cells were then incubated with the Sephacryl fraction, rinsed exhaustively and treated with citrate saline pH 2.0 to release any bound material. The eluting fraction was composed of one major band Mr=~57,000 detected by silver stained SDS-PAGE. This fraction still inhibited ^{125}I-thrombin binding to ME cells and initiated a 50% increase in cell number at a concentration of 330 ng/ml. It appears that this growth factor initiates cell proliferation by binding to the thrombin receptor. (Supported by NIH Grants AM-25807 and CA-00805).

0677 AUTOANTIBODIES TO THE GASTRIN RECEPTOR IN PATIENTS WITH PERNICIOUS ANAEMIA, Graham S. Baldwin[1], Henry J. De Aizpurua[2], Chris Dow[2], John Pederson[2], Ban Hock Toh[2] and Berta Ungar[3], [1]Ludwig Institute for Cancer Research, [2]Monash University Medical School and [3]Royal Melbourne Hospital, Melbourne, Australia.

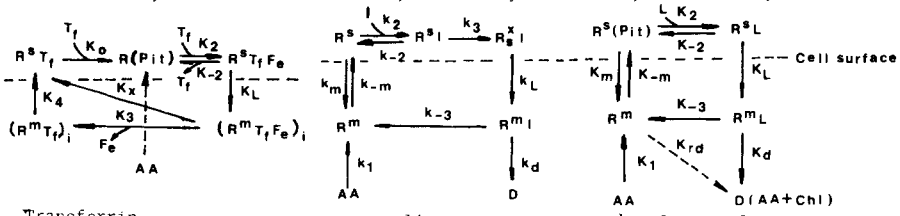
Gastrin receptors have been identified in Triton-solublised membrane preparations from dog, pig and rat stomach mucosa by covalent cross-linking of iodinated gastrin 2-17. The apparent molecular weight of the cross-linked receptor, determined by polyacrylamide gel electrophoresis in the presence of SDS and reducing agents, is 85,000.

Four lines of evidence suggest that the sera of patients with pernicious anaemia often contain antibodies directed against the gastrin receptor. The sera contain antibodies which:

i. react with the parietal cell surface (De Aizpurua et al., 1983);
ii. inhibit the binding of iodinated gastrin 17 to isolated parietal cells;
iii. inhibit the gastrin-stimulated uptake of [^{14}C]-aminopyrine by isolated parietal cells;
iv. recognise a protein of apparent molecular weight 85,000 in Western blots prepared from Triton-solublised preparations from stomach mucosa.

De Aizpurua, H.J., Toh, B.H. and Ungar, B. (1983) Clin. Exp. Immunol., 52:341-349.

0678 KINETIC MODELS FOR RECEPTOR RECYCLING, Paul W. Chun, Chan Won Lee, William F. Cantarini, and Rachel B. Shireman, University of Florida, Gainesville, FL 32610.



Transferrin (Ciechanover et al., 1983) Insulin (Based on Knutson et al., 1983) Low Density Lipoprotein (Based on Brown et al., 1983)

The macromolecular species distribution on receptor-mediated endocytotic pathways was computer simulated based on kinetic data reported in the literature. This simulation suggests that the rate limiting step of the endocytotic pathway occurs at the cell surface, controlled by the reversibility of ligand binding, and that this reversibility facilitates the translocation of surface receptors and recycling receptor, which in turn modulates the synthesis and degradation of receptor. The free receptor generated from recycling the receptor-ligand complex may differ from receptor newly synthesized from amino acid (supported by NSF PCM 81-03263).

0679 PROTEIN KINASE C MODULATES PHOSPHORYLATION AND BINDING OF THE EGF RECEPTOR, Patricia G. McCaffrey and Marsha Rich Rosner, Massachusetts Institute of Technology, Cambridge, MA 02139

The relationship between Ca^{2+}-phospholipid activated protein kinase C and epidermal growth factor (EGF) receptors was investigated. We compared the effects of an exogenous activator of C kinase, the tumor promoter 12-O-tetradecanoylphorbol 13-acetate (TPA), with those of an endogenous-type activator, the synthetic analogue 1-oleyl 2-acetyl diglycerol (OADG) in A431 cells. Previous studies in this cell type showed that TPA causes loss of EGF-induced tyrosine phosphorylation of the EGF receptor, enhancement of serine and threonine phosphorylation of the EGF receptor, and loss of EGF binding to the apparent high affinity EGF receptor. In other cell types, tumor promoters have been shown to cause association of cytosolic protein kinase C with the membrane, presumably activating the enzyme. Treatment of A431 cells with TPA results in a similar increase in membrane-associated protein kinase C activity and concurrent loss of the cytosolic enzyme activity. OADG also causes loss of cytosolic C kinase activity when added to intact A431 cells. Further, OADG, like TPA, blocks EGF-induced tyrosine phosphorylation of the EGF receptor, stimulates serine and threonine phosphorylation of these receptors, and blocks EGF binding to the apparent high affinity EGF receptor. These results indicate that protein kinase C can modulate phosphorylation and binding of the EGF receptor in A431 cells and provide strong evidence that tumor promoters act via a pathway involving this enzyme.

0680 TRANSFERRIN DEPENDENT CELL SURFACE AGGREGATION OF ITS RECEPTOR, Caroline A. Enns and James V. Staros, Vanderbilt University, Nashville, TN 37232

A membrane-impermeant cleavable crosslinking reagent, 3,3' dithio bis(sulfosuccinimidyl proprionate)(DTSSP) has been used to detect transferrin induced aggregation of its receptor on the surface of A-431 cells. Whole cells either labeled with $Na^{125}I$ or metabolically labeled with ^{35}S-methionine were incubated with ^{125}I-transferrin for a fixed period of time, washed and incubated with 1 mM DTSSP at 4°C. Following cross-linking, any residual reactive groups were quenched. The cells were solubilized, immunoprecipitated with a monoclonal antibody to the transferrin receptor and subjected to SDS polyacrylamide gel electrophoresis. Under non reducing conditions the aggregates failed to enter a 6% polyacrylamide gel. Gel electrophoresis under reducing conditions demonstrated that the aggregates were largely composed of transferrin and its receptor. The aggregation was observed to be time and temperature dependent. At room temperature it occurred within 5-10 minutes, while at 4°C aggregation was optimal at 60-90 minutes. (Supported by CA33096, AM25489, AM31880).

0681 BIFUNCTIONAL ROLE OF CYCLIC AMP IN THE EXPRESSION OF GONADOTROPIN RECEPTORS IN OVARIAN GRANULOSA CELLS, Michael Knecht, Tapio Ranta, Osamu Shinohara, and Kevin J. Catt, ERRB, NIH, Bethesda, MD 20205

Follicle-stimulating hormone (FSH) or ligands stimulating endogenous cAMP production, such as choleragen, forskolin, and prostaglandins, induce plasma membrane receptors for FSH, luteinizing hormone (LH), and prolactin during 48-72 h culture of ovarian granulosa cells. Similarly, elevation of intracellular cAMP levels with cAMP analogs, such as 8-bromo-cAMP, also increases the expression of polypeptide hormone receptors. However, a biphasic role of cAMP in receptor induction is suggested by a 2-fold inhibition of FSH-induced LH receptor formation by simultaneous incubation with large concentrations of 8-bromo-cAMP ($\geq$ 5 mM). Further, forskolin (50 µM) causes a 5-10 fold increase in FSH- or choleragen-stimulated cAMP production in granulosa cells, but inhibits LH receptor expression induced by these ligands by 5-fold. In contrast, choleragen, which enhances FSH action on cAMP levels by only 2-fold, also amplifies LH receptors by 2-fold. The inhibitory actions of forskolin on the induction of LH receptors by FSH occur whether it is added at the beginning, middle (24 hours), or near the end (42 hours) of culture. An associated large rise in cAMP levels at 48 h is detected with each addition of forskolin. These results indicate that an optimal level of cAMP production is necessary for maximal expression of gonadotropin receptors in granulosa cells. The association of excessive quantities of exogenous or endogenous quantities of cAMP with reduced receptor content is possibly due to desensitization of the cAMP response system, or to divergence of biosynthetic pathways into non-receptor related processes.

0682 PHORBOL ESTERS INHIBIT LUTEINIZING HORMONE (LH) RECEPTOR EXPRESSION IN CULTURED GRANULOSA CELLS, Osamu Shinohara, Michael Knecht, and Kevin J. Catt, ERRB, NICHD, NIH, Bethesda, MD 20205

The induction of LH receptors by follicle-stimulating hormone (FSH) in cultured granulosa cells is inhibited by gonadotropin-releasing hormone (GnRH) and its agonist analogs in a calcium-dependent manner. To evaluate if GnRH action involves stimulation of protein kinase C, the effect of 12-O-tetradecanoylphorbol-13-acetate (TPA), a potent tumor promotor and a possible substituent for diacylglycerol as an activator of protein kinase C, was studied in granulosa cells from immature, estrogen-primed rats. FSH-induced cell differentiation was assessed by the induction of LH receptors and by the production of cAMP and progesterone during culture. TPA alone (0.001-1 ng/ml) did not affect cell function during the first 48 h of culture. However, TPA suppressed FSH-induced differentiation in a dose-dependent manner when the cells were cultured with the phorbol ester for the entire 48 h period (ED_{50} = 0.02-0.05 ng/ml). A marked reduction in cAMP formation and in LH receptors was observed primarily from 24 to 48 h of culture. LH receptor expression and cAMP formation were maximally inhibited by the addition of TPA up to 30 h after initiation of culture, but were only partially suppressed when TPA was added at later time points. This inhibitory process was similar to that of a GnRH agonist. Morphologically, TPA inhibited characteristic clustering of the granulosa cells induced by FSH, and instead, flattening of the cells was evident. These results indicate the potential role of protein kinase C in granulosa cell differentiation and its inhibitory control by GnRH-related peptides.

0683 PULMONARY ALPHA-1 AND BETA ADRENERGIC RECEPTORS IN GUINEA PIGS. H.F. Takeyama, T.Sato, S.Jedruska -Witt, A. Bewtra, R. Townley, Creighton Univ., Omaha, NE 68178.

We evaluated the effect of repeated antigen or histamine (H) challenges on alpha-1 and beta areceptors in guinea pig lung. All guinea pigs were initially sensitized with 2 mg/kg i.p. ovalbumine. Two weeks later they were challenged by aerosol, five days a week for 4 weeks with isotonic saline (CONTR.): ovalbumin (OA) 10 mg/ml, 1-3 min. or (H) 0.3 mg/ml, 5 min. After two weeks of challenges with H, two distinct groups could be differentiated: H sensitive (HS) and H non-sensitive (HNS). Alpha-1 (3H-prazosin) and beta (3H_DHA) receptors were evaluated on the lung parenchyma. Lung strips and tracheal smooth muscle were studied isotonically for alpha and beta adrenergic responses. Binding study results were as follows: (Mean+/-s.e.m.).

	Alpha-1 Receptors			Beta Receptors		
Group	n	Bmax	Kd	n	B max	Kd
Contr	6	16±2	0.50±0.07	7	432±34	1.74±0.22
OA	7	23±1*	0.65±0.10	7	305±48*	1.09±0.08*
HS	4	25±3*	0.66±0.08	4	487±22	1.32±0.20
HNS	7	21±2	0.48±0.05	7	444±34	2.11±0.32

*p 0.05 vs. CONTR.: B max (fmol/mg prot.); Kd: (nM).

The ED_{50} for epinephrine relaxation in CONTR., OA, HS and HNS lung strips were $3.9x10^{-6}M$; $5.2x10^{-6}M$; $1.5x10^{-8}M$, $4.8x10^{-8}$ respectively. These findings suggest that chronic antigen or histamine induced bronchospasm and the type and duration of stress increases the number of alpha-1 receptors in the lung, while beta receptor number and affinity was reduced by the antigen challenges.

0684 INSULIN ACTIVATES THE APPEARANCE OF IGF-II RECEPTORS ON THE ADIPOCYTE CELL SURFACE, Yoshitomo Oka and Michael P. Czech, University of Massachusetts Medical Center, Worcester, MA 01605

In order to evaluate the mechanism of insulin action to increase ^{125}I-insulin-like growth factor II (IGF-II) binding to rat adipocytes, a potent rabbit antiserum was raised using purified IGF-II receptors from rat placental membranes. The antiserum elicited a positive ELISA reaction against purified IGF-II receptor at 5,000 fold dilution, and markedly inhibited ^{125}I-IGF-II binding to adipocyte plasma membrane when added prior to the growth factor. Similar immunoprecipitation lines formed between agar plate wells incubated with antiserum versus IGF-II receptor in the presence or absence of 1μM IGF-II, indicating that binding of anti-receptor immunoglobulin to the IGF-II receptor is not affected by occupancy of the IGF-II binding site. Intact adipocytes treated with or without insulin were incubated with anti-IGF-II receptor immunoglobulin, washed, and further incubated with ^{125}I-goat anti-rabbit IgG to monitor the amount of anti-receptor immunoglobulin bound. Insulin induced parallel increases in anti-IGF-II receptor immunoglobulin binding (2.4 fold) and ^{125}I-IGF-II binding (3 fold) to the isolated cells. The dose-response relationship of insulin action on ^{125}I-IGF-II binding and anti-receptor immunoglobulin binding was essentially identical with a half-maximal effect at approximately 0.07nM of insulin. That insulin does not act to expose new types of antigenic sites on IGF-II receptors was indicated by the demonstration that control adipocytes could readily adsorb the anti-receptor immunoglobulin. These data demonstrate that increased numbers of IGF-II receptors are displayed in an exposed position on the adipocyte cell surface in response to insulin.

0685 TUMOR PROMOTING PHORBOL DIESTERS MEDIATE SERINE PHOSPHORYLATION OF THE EGF RECEPTOR, Roger J. Davis and Michael P. Czech, University of Massachusetts Medical Center, Worcester, MA 01605

Phorbol diesters are tumor promoters which markedly potentiate the mitogenic effect of epidermal growth factor (EGF) on cultured cells. Recent evidence has linked the cellular receptor for phorbol diesters with a protein kinase which is sensitive to the Ca^{++}, phospholipids and diglycerides. We report that the addition of phorbol diesters to human A431 epidermal carcinoma cells causes a coordinate inhibition of the high affinity binding of EGF and an increase in the phosphorylation state of the EGF receptor. The ^{32}P content of the EGF receptor purified from phorbol diester treated cells labeled with [^{32}P] phosphate was increased by 38% compared with receptor isolated from control cells. The increase in phosphorylation was dose dependent with the half maximal effect observed between 1 and 10nM TPA. The increased phosphorylation was also observed with the less potent tumor promoter 4β phorbol 12β, 13α-dibutyrate, but not with 4β-phorbol which is inactive as a tumor promoter. Phosphoamino acid analysis indicated that the increase was mainly due to phosphoserine. HPLC analysis of tryptic phosphopeptides of the EGF receptor demonstrated that most of the increased phosphorylation could be accounted for by a single fraction eluted from a C-18 reverse phase column. These results suggest that the mechanism of phorbol diester action on the response of cells to EGF may be mediated in part by a specific phosphorylation of the EGF receptor.

0686 IDENTIFICATION OF THE GROWTH HORMONE (GH) RECEPTOR IN RAT ADIPOCYTES, Christin Carter-Su and Jessica Schwartz, Univ. Mich. Med. Sch., Ann Arbor, MI 48109

Crosslinking of ^{125}I-hGH to rat adipocytes with disuccinimidylsuberate (DSS) resulted in a prominent labeled band of Mr=134,000 and multiple less intense bands of lower Mr. To further investigate GH receptor structure, other crosslinkers were used. EGS (ethylene glycol bis(succinimidyl succinate) is longer than DSS but has the same amino group specificity and resulted in the same labeling profile as DSS. N-hydroxysuccinimidyl-p-azido-benzoate and diazopyruvoyl-p-nitrophenylester are smaller than DSS with one amino-specific group and a photoactive group with broad specificity. These two probes labeled the same proteins as DSS and EGS but overall crosslinking efficiency was decreased and the intensities of the lower Mr species were increased relative to the 134K band. Isolated plasma membranes were also incubated with ^{125}I-hGH and treated with DSS. The same proteins were labeled as when hGH was crosslinked to intact cells except the 134K species was much reduced in intensity or absent altogether. In contrast to intact cell experiments, in most membrane experiments, many lower Mr species were present even when the membranes were incubated in the presence of excess unlabeled hormone. The 134K species was also diminished compared to the other species when hGH was bound to cells prior to membrane isolation and residual bound hGH (25% of original)was crosslinked to the isolated membranes. These data support our hypothesis that all the Mr species are not disulfide linked subunits of a larger Mr receptor but may be 1. proteolyzed forms of the 134K species formed artefactually during membrane isolation or physiologically upon prolonged GH binding to its receptor, or 2.)nonreceptor proteins which are in a more favorable position for crosslinking in isolated membranes or by the photoaffinity probes.

Transforming Growth Factors and Oncogenes

0687 CLONING OF THE cDNA AND GENE FOR HUMAN TRANSFORMING GROWTH FACTOR-α, Rik Derynck and David V. Goeddel, Genentech, Inc., South San Francisco, CA 94080

Transforming growth factors (TGFs) are polypeptides able to induce anchorage-independent growth of non-neoplastic cells. Two types of TGFs have been detected and characterized: TGF-α, which binds to the receptor for epidermal growth factor (EGF), and TGF-β, which does not. The combination of both TGFs elicits an apparent phenotypic transformation of normal cells. TGFs-α have been isolated from transformed cells from humans and rodents. These growth factors are assumed to play an important role during tumorigenesis and possibly also during embryonic development. TGFs-α show a heterogeneity in size. The smallest species has been purified to homogeneity and shows a structural homology with EGF. Using RNA derived from a tumor cell line, we have isolated a cDNA clone coding for human TGF-α. We have also isolated genomic DNA sequences for TGF-α containing multiple introns from a bacteriophage λ library. Analysis of these sequences establishes the sequence of a human TGF-α of 50 amino acids long and demonstrates the existence of a larger precursor molecule, from which TGF-α is derived after proteolytic cleavage. Northern hybridization analysis shows a TGF-α mRNA of about 5.0 kbp in many transformed cell lines. The availability of these cloned sequences makes a study of the regulation and biological role of TGF-α possible.

0688 TUMOR-ASSOCIATED URINARY HIGH MOLECULAR WEIGHT (HMW) TRANSFORMING GROWTH FACTOR MAY BE HMW EPIDERMAL GROWTH FACTOR, Kurt Stromberg and David N. Orth*, National Cancer Institute, Frederick, MD 21701 and *Vanderbilt University, Nashville, TN 37232

The bulk urine (48 hr collection) of a patient bearing a highly malignant brain tumor (astrocytoma, grade IV) was absorbed on trimethylsilyl-controlled pore glass (TMS-CpG) beads to yield a high molecular weight (HMW) transforming growth factor (hTGF). This hHMW TGF promoted clonogenic cell growth in soft agar and competed for membrane receptors with epidermal growth factor (EGF). The growth factor activity eluted from TMS-CpG beads at 25-27% acetonitrile and on subsequent BioGel P-100 chromatography had a molecular weight of approximately 28,000, similar to that of a high molecular weight form of human EGF (HMW hEGF) from normal urine. Following surgical resection of tumor, no appreciable HMW hTGF activity was detectable in a comparable 48 hour urine collection. HMW hTGF generated a competitive binding curve similar to that of standard small molecular weight hEGF (SMW hEGF), and parallel to highly purified HMW hEGF. In addition, both HMW hTGF and HMW hEGF had 20-25% radioreceptor activity as compared with immunologic activity. Thus, in its apparent molecular size, receptor binding, immunologic behavior, and clonogenic activity, the brain tumor-associated HMW hTGF was indistinguishable from HMW hEGF. HMW hTGF may be of tumor cell origin or may represent a response of normal host tissue(s) to the tumor or tumor products.

0689 EFFECT OF PROTAMINE ON THE BINDING TO RECEPTORS AND MITOGENIC ACTIVITY OF HUMAN PLATELET-DERIVED GROWTH FACTOR, Paul E. DiCorleto and Earl J. Poptic, Division of Research, Cleveland Clinic Foundation, Cleveland, OH 44106

Protamine has been previously reported to be a specific competitive inhibitor of radioiodinated platelet-derived growth factor (^{125}I-PDGF) binding to cells (Huang et al, J Biol Chem 257: 8130, 1982). We have found that < 1 μg/ml of protamine is sufficient to half maximally inhibit specific binding of ^{125}I-PDGF (2 ng/ml) to confluent Swiss 3T3 cells at 4°, 22°, or 37°C. Surprisingly, however, at a concentration of protamine (25 μg/ml) that inhibits ^{125}I-PDGF binding by over 70%, the stimulation of DNA synthesis by the growth factor is greater than in the absence of the binding inhibitor. The stimulation of cell growth by PDGF as measured by cell number is also slightly enhanced by protamine. While the rate of cell-dependent degradation of ^{125}I-PDGF is reduced by protamine, the extent of degradation is comparable after 22 hr. The ability of protamine to inhibit ^{125}I-PDGF binding is stable to incubation with cells for 4 to 6 hrs at 37°C but decreases with time thereafter. When PDGF and protamine are incubated with cells at 22°C followed by rinsing and further incubation at 37°C, the stimulation in DNA synthesis by PDGF is inhibited to a similar extent to the binding of ^{125}I-PDGF to cells during the initial incubation. We conclude that protamine at nontoxic concentrations partially blocks ^{125}I-PDGF binding to its receptor, but this inhibition reduces PDGF degradation thus maintaining a higher free concentration of PDGF to interact with available receptors. The net result of the binding inhibitor is an enhanced response of the cells to the growth factor as though the integrated product over time of mitogen and free receptor concentration is the important determinant for a mitogenic response. (Supported by AHA-NE Ohio and HL-29582)

0690 STIMULATION OF Na^+/H^+ EXCHANGE BY MITOGENS. Dan Cassel, Brian Whiteley, Ying-Xin Zhuang and Paul Rothenberg, Washington University School of Medicine, Division of Biology and Biomedical Sciences, St. Louis, MO 63110, and Edward Cragoe, Merck Sharp & Dohme Res. Laboratories, West Point, PA 19486.

Addition of pure mitogens to cultured mammalian cells rapidly stimulates an amiloride-sensitive Na^+ influx and causes an elevation of intracellular pH as determined by a new fluorometric technique (Rothenberg et al., J. Biol. Chem. 258, 4883, 1983). Examples include EGF in A431 cells (Rothenberg et al., J. Biol. Chem., in press), PDGF in NR6 cells (Cassel et al., PNAS 80, 6224, 1983), and insulin in Balb 3T3 cells. Vanadate which elicits a mitogenic response in a number of cells is also a potent activator of Na^+/H^+ exchange. Studies with amiloride analogs bearing an alkyl substitution at the 5 amino group resulted in the identification of analogs that are up to 80 times more potent than amiloride as inhibitors of Na^+/H^+ exchange. These analogs will be useful for evaluating the role of Na^+/H^+ exchange in the mitogenic response to growth factors and for the assessment of Na^+/H^+ exchangers in the cell membrane. Supported by GM18405 and a grant from the Monsanto Company.

0691 Characterization of a New Growth Factor for Human Endothelial Cells from Platelets. George L. King and Sherry Buchwald. Joslin Diabetes Center, Boston, MA 02215.

The factors that are responsible for the regeneration of vascular endothelial cells are largely unknown. Although platelets appear to be involved in the process of endothelial repair, previously described platelet-derived growth factor (PDGF) does not stimulate endothelial cell growth. Recently, we have characterized a protein from human platelets which stimulates DNA synthesis and growth of human endothelial cells in culture. Supernatant derived from ultracentrifugation of platelet lysate stimulates DNA synthesis dramatically. Endothelial cell growth factor (ENDO-GF) from the supernatant is retained on a DEAE exchange column but not on a CM cellulose-Sepharose column. After elution with a continuous gradient of NaCl, a 3-fold purification of ENDO-GF was achieved. Further purification on gel chromatography of Sephacryl S-200 resulted in an additional 50-fold purification and suggests that ENDO-GFs have m.w. of 65,000 and 135,000. When the 65,000 m.w. peak is further purified by reverse phase high pressure-liquid chormatography, a single peak of biological activity is detected in the eluted fractions with an estimated 100-fold purification. Biologically, ENDO-GF appears to be a heat labile protein, since its growth effect is diminished greatly by exposure to trypsin or 56°C for 10 minutes. In the more purified preparations, ENDO-GF stimulates DNA synthesis in both human and bovine macrovascular and bovine retinal capillary endothelial cells. In the presence of low concentration of plasma or serum (5%), ENDO-GF increases the rate of human endothelial cell proliferation by 3-fold. In direct contrast to PDGF, ENDO-GF is more potent on human endothelial cells than fibroblasts. Thus, we have characterized and purified a new anionic growth factor from human platelets which appears to be specific for endothelial cells and thus may play an important role in the maintenance of endothelial cell growth and intergrity in vivo.

0692 MODULATION OF PHOSPHORYLATION OF PLASMA MEMBRANE PROTEINS IN POLYOMA VIRUS INFECTED CELLS BY THE HR-T GENE OF POLYOMA VIRUS, Kurt Ballmer-Hofer, Tom Benjamin, Dept. of Pathology, Harvard Medical School, Boston 02115 Ma.

Cell transformation by Polyoma virus is tightly linked to the ability of the virus to code for an intact middle T antigen(mt). Immunoprecipitated mt is associated with a phosphokinase activity specific for tyrosine residues, In vivo, mt is phosphorylated primarily on serine and/or threonine residues. mt is probably not a kinase itself but interacts with multiple cellular enzymes. mt is predominantly associated with the plasma membrane of infected cells. We therefore investigated its role in altering the phosphorylation of membrane proteins in isolated plasma membrane vesicles. Using SDS Acrylamide and O'Farrell 2D electrophoresis gels we found several proteins to be under or overphosphorylated in membranes from wt virus compared to hr-t mutant virus (transformation-defective) infected or uninfected cells. The changes were more prominent if the gels were treated with alkali before drying to reduce the amount of P-serine and P-threonine. mt was one of a few bands which showed alkali stable phosphate linkages presumably representing modified tyrosine residues in these membrane preparations. Interestingly some of the changes in cellular bands were only observed in intact membranes; detergent treated membranes gave identical patterns for wt as well as mutant virus infected cells. These changes in cellular protein phosphorylation therefore depend on the integrity of the membrane, and appear to depend in some manner on functions of mt being present in the plasma membrane.

0693 MONOCLONAL ANTIBODY TO THE EPIDERMAL GROWTH FACTOR RECEPTOR ENHANCES THE BIOLOGICAL ACTIVITIES OF EPIDERMAL GROWTH FACTOR, J.A. Fernandez-Pol, VA Medical Center and St. Louis University, St. Louis, MO 63125

An IgM monoclonal antibody to the epidermal growth factor (EGF) receptor has been isolated using membranes of the epidermoid carcinoma cell line A431 as immunogen. The antibody (designated 2D1) specifically immunoprecipitated the EGF receptor from biosynthetically labeled A431 cells. The specificity of the interaction between antibody 2D1 and the EGF receptor was confirmed by immunoblot analysis after transfer of A431 proteins to immobilizing matrices. Indirect immunofluorescence and electron microscopy studies showed that 2D1 recognizes a receptor antigenic determinant that is expressed on the surface of intact cells. The binding of 2D1 to the EGF receptor of A431 cells enhanced the affinity of EGF receptors towards EGF. Like EGF, 2D1 induced receptor redistribution, morphological changes, and phosphorylation of EGF receptors in A431 cells. In the absence of EGF, 2D1 also stimulated DNA synthesis and mitogenesis in A431 cells. The effects of low concentrations of EGF on morphology, phosphorylation of EGF receptors, and DNA synthesis were potentiated by the presence of 2D1. The results presented in this report support the notion that: (i) the binding of 2D1 to the EGF receptor at a different site from that to which EGF binds can initiate an effective biological response; and (ii) the determinant on the EGF-receptor recognized by 2D1 acts as an allosteric regulatory site of the effects of EGF.

0694 PRODUCTION OF A B CELL GROWTH FACTOR BY CLONED, NEOPLASTIC B CELLS: AN EXAMPLE OF ONCOGENE ACTIVATION RESULTING IN AUTOSTIMULATION? K. Brooks, J.W. Uhr and E.S. Vitetta. Department of Microbiology, University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

Clones of a B cell leukemia (BCL_1) were selected on the basis of their capacity to differentiate to antibody-forming cells (AFC) in response to T cell-derived B cell differentiation factors. These BCL_1 cells secrete a lymphokine which enhances anti-Ig-initiated proliferation of normal B cells and is thus functionally similar to T cell-derived B cell growth factor (BCGF). This BCL_1-derived BCGF is not interleukin-1 (IL-1) or interleukin-2 (IL-2) and has an apparent m.w. of 4500-7000 daltons which is different from T cell-derived BCGF. BCL_1-BCGF is not readily detectable in the culture supernatant of unsynchronized cells, but reaches maximal levels in the medium 24 hr after release of synchronized cells from a triple thymidine block. The rapid drop in BCGF levels as the cells transit through G_1 suggests that they express BCGF receptors which may absorb the factor from the medium. This autostimulatory lymphokine may be a transforming growth factor or a normal hormone.

0695 THE EFFECT OF EPIDERMAL GROWTH FACTOR ON THE CELL CYCLE Carol L. MacLeod, Andrew Luk, Janice Castagnola and John Mendelsohn, University of California, San Diego Cancer Center, La Jolla, CA, USA.

The human carcinoma cell line A431 is unusual in that epidermal growth factor (EGF) inhibits cell proliferation. This study was initiated to examine whether EGF affected the progression of A431 cells through the cell cycle. In the presence of 5 - 10 nM EGF the growth rate of logrythmically growing A431 is abruptly and markedly decreased compared to untreated control cultures with little loss of cell viability over a 4 day period. During this period of EGF treatment the incorporation of ^3H thymidine is also reduced compared to untreated control. Flow cytometric analysis of EGF-treated cells stained with mithramycin reveals a marked change in the cell cycle distribution. Both G1 and G2/M cells are increased relative to S phase cells. Since addition of the mitotic inhibitor vinblastine prevents re-entry of cells into G1 and causes accumulation of cells in mitosis, it is possible to distinguish between slow progression in G1 and G2 and blocks in those phases. EGF and control cultures were treated with vinblastine and the number of mitotic figures counted. Control cells accumulate mitotic figures as expected and show progression into S, while no mitotic figures are found among the EGF-treated cells in the presence or absence of vinblastine, nor was progression into S observed. Thus these preliminary results suggest that there are two EGF-induced blocks in cell cycle traversal; one is in late S or G2, blocking entry into mitosis and the other is in G1, blocking entry into S phase. Current experiments are underway to test the rate and EGF dose dependency of the apparent cell cycle blocks.

0696 STRUCTURAL AND FUNCTIONAL STUDIES ON THE GENES ENCODING PLATELET-DERIVED GROWTH FACTOR AND THE PLATELET-DERIVED GROWTH FACTOR RECEPTOR, Lance Fors, Richard K. Barth, Astar Winoto, Paul Tempst and Lee Hood, California Institute of Technology, Pasadena, CA 91125

We are interested in studying the PDGF-PDGF receptor mitogenic system. The recent report of the striking homology between the amino-terminal region of the PDGF protein and the predicted amino acid sequence of the v-sis oncogene product of Simian sarcoma virus has allowed us to use v-sis as a probe for the sis/PDGF gene. Initially, we examined the expression of sis/PDGF in a number of different tissues and a human osteosarcoma cell line (U-2 OS) which secretes a PDGF-like mitogen. sis/PDGF transcripts were detected in bovine pituitary (3.7 kb), mouse submaxillary gland (3.5 kb), and the osteosarcoma cells (3.9 and 3.4 kb). In addition, we have isolated sis/PDGF clones from an osteosarcoma cDNA library. We also have used v-sis to screen two genomic cosmid libraries we constructed from human sperm and from the human osteosarcoma cell line. A comparison of the sis/PDGF gene from these two sources suggests that no massive DNA rearrangements are responsible for sis/PDGF activation in the osteosarcoma cells.

In collaboration with Dr. Harry N. Antoniades we are sequencing the PDGF receptor protein. From this sequence information, we will synthesize an oligonucleotide probe which will be used to screen a human fibroblast cDNA library for a clone encoding the PDGF receptor. Once we have the clones for both PDGF and the PDGF receptor we will be able to dissect in detail the structure and regulation of this mitogenic system.

0697 COORDINATE ACTION OF TWO TRANSFORMING GROWTH FACTORS FROM FELINE SARCOMA VIRUS-TRANSFORMED RAT CELLS. Joan Massague, Elizabeth Like and Roger Davis. University of Massachusetts Medical Center, Worcester, MA 01605.
Snyder-Theilen feline sarcoma virus-transformed rat embryo (FeSV-Fre) cells release two transforming growth factors (TGFs) that when acting together induce strong anchorage-independent growth of normal NRK cells. One of these TGFs is a 24 kDa polypeptide consisting of two disulfide-linked chains of about 11 kDa as determined on SDS-polyacrylamide gels. This TGF species is a mitogen that does not induce proliferation of NRK cells in semisolid medium when acting alone. The second TGF species from FeSV-Fre cells consists of a single 7.4 kDa polypeptide chain that exhibits 20-28% amino acid sequence homology to the 30 N-terminal residues of epidermal growth factor (EGF). ^{125}I-labeled preparations of this EGF-like TGF (eTGF) interact with, and can be crosslinked to 140-160 kDa membrane receptors for EGF. Rat eTGF and mouse EGF exhibit the same affinity for eTGF/EGF receptors, and are equally potent in inducing tyrosine residue phosphorylation and down regulation of this receptor type. The interaction of eTGF and EGF with eTGF/EGF receptors is equally affected by lectins and phorbol myristate acetate. eTGF and EGF are equally mitogenic for NRK cell monolayers, and both factors exhibit a similar ability to induce limited proliferation of NRK cells in semisolid medium. However, full anchorage-independent proliferation is obtained when NRK cells are exposed to the 24 kD TGF from FeSV-Fre cells in the presence of either eTGF or EGF. The data suggest that the induction of the transformed phenotype in normal cells by polypeptide factors results from coordinated cellular stimulation by two types of TGFs, and is in part mediated through eTGF/EGF receptors.

0698 ISOLATION OF A MEMBRANE-ASSOCIATED INHIBITOR OF CELL GROWTH, Kathryn C. Stallcup and Matthew F. Mescher, Harvard Medical School, Boston, MA 02115

Lymphocytes respond to a variety of signals delivered by cell to cell contact, and in many cases isolated membranes or liposomes can replace the "stimulator" cells. For instance, cytotoxic T lymphocytes receive a proliferative signal from contact with allogeneic cells. The same signal can be delivered by membranes from allogeneic cells or by liposomes containing the appropriate antigen. We have recently found that "negative" signals can also be delivered by lymphocyte membranes, in that membranes from lymphocytes or lymphoid tumor cells can slow or stop the growth of tumor cells and inhibit the generation of immune responses. These observations suggest that cell to cell interactions regulate the growth of lymphoid cells, and that a plasma membrane component is the mediator of this regulation.

The membrane component that inhibits cell growth was found to co-purify with H-2 antigens during monoclonal antibody affinity chromatography and to retain activity when incorporated into liposomes. The inhibitor acts by slowing or stoping growth without killing cells, since inhibition was reversed when liposomes were removed. Inhibitory activity and H-2 antigens could be separated by gel filtration in deoxycholate. The inhibitor was found to be insensitive to heat or protease treatment and soluble in organic solvents. Inhibitory activity could be recovered following thin layer chromatography and thus appears to have the properties of a lipid. The lipid probably accounts for less than 0.2% of the total membrane lipid. These results suggest that recognition of a minor lipid component in cell membranes is responsible for the observed inhibition of proliferation.

0699 GROWTH CONTROL OF CEREBRAL MICROVESSEL DERIVED SMOOTH MUSCLE CELLS, Robert A. Robinson Steven A. Moore, Michael N. Hart, University of Iowa, Iowa City, IA 52242

Relatively little information is available concerning growth control mechanisms of cerebral microvessel derived smooth muscle (Sm) cells. Recently mouse cerebral microvessel derived Sm cells have been isolated in this laboratory which show smooth muscle characteristics and a nontransformed morphology in culture at low passage. Switching the Sm cells to serum free (SF) media induces growth arrest; viability remains high under these conditions for up to 7 days. At high passage the Sm cells spontaneously transform (TxSm cells). The TxSm cells do not growth arrest in SF media but their rate of proliferation is markedly reduced. If grown in the presence of serum, both cell lines are mitogenically stimulated by epidermal growth factor (EGF). ^{125}I-EGF binding assays reveal binding to be saturable and time and temperature dependent. The Sm cells show EGF binding levels 6-10 fold greater than in the TxSm cells. When growth arrested due to SF media change, the Sm cells do not respond to EGF even though preliminary data suggest there is no change in EGF binding. The TxSm cells on the other hand do respond to EGF, even if they are kept in SF media. However, if either non-mitogenic concentrations of serum or platelet derived growth factor or conditioned medium from transformed cerebral microvessel endothelial cells is added to the SF growth arrested Sm cells, EGF induced mitogenesis is restored. These studies will give insight into growth factor interactions as well as growth control mechanisms of cerebral microvasculature cells.

0700 CELL SURFACE EGF RECEPTORS ARE REGULATED BY TRANSFORMING GROWTH FACTOR-β
Richard K. Assoian and Michael B. Sporn, NIH, Bethesda, MD 20205

The binding of ^{125}I-labeled EGF to intact NRK-fibroblasts is doubled by treatment of the cells with platelet-derived transforming growth factor-β (TGF-β). The cell number is unaffected. Similar concentrations of TGF-β (5-50 pM) induce both increased EGF binding and anchorage-independent growth, but the effect on binding is an early event requiring only 6 hr of treatment with the growth factor. TGF-β does not inhibit EGF degradation nor does it increase the affinity of EGF receptors, but Scatchard plots show that cells treated with TGF-β have an increased number of cell surface EGF receptors. Similarly, both cycloheximide and tunicamycin completely inhibit the increase in EGF binding induced by TGF-β. Overall protein synthesis is not affected by TGF-β, suggesting that a selective stimulation of protein synthesis underlies its molecular action. Cells exposed to TGF-β also show a 100-fold reduction in the ability to down regulate their cell surface EGF receptors in response to ligand binding; concentrations of EGF (1-10 ng/ml) which normally result in near maximal down regulation no longer decrease the number of plasma membrane EGF receptors below the basal state. Since TGF-β does not affect the receptor binding of growth factors in general, its effect on EGF receptors agrees well with both the synergism and specificity of the EGF / TGF-β interaction.

0701 CHARACTERIZATION OF A RECEPTOR FOR TRANSFORMING GROWTH FACTOR-β IN RAT KIDNEY FIBROBLASTS, Charles A. Frolik, Lalage M. Wakefield, Diane M. Smith and Michael B. Sporn, National Cancer Institute, Bethesda, Maryland 20205

Transforming growth factors (TGFs) are a family of polypeptides that are able to reversibly confer the transformed phenotype on non-neoplastic cells. There are at least two members in this family - TGF-α, which competes with epidermal growth factor (EGF) for binding to the EGF receptor, and TGF-β which does not compete with EGF for binding to this receptor. Specific, saturable receptors for TGF-β have now been demonstrated in normal rat kidney fibroblasts. Binding is reversible, of high affinity (K_D 50-100 pM) and temperature dependent. Insulin, insulin like growth factors I and II, EGF, platelet derived growth factor and TGF-α do not compete with TGF-β for binding to the TGF-β receptor. At 37°, cell associated radioactivity is maximal at 30-45 min followed by a rapid disappearance of counts and a coincident appearance of trichloroacetic acid soluble ^{125}I-material in the medium. This decrease in binding is due to degradation of the ligand in the medium and not to disappearance of receptor sites on the plasma membrane. The decline is blocked by incubation of the cells at 4° or by treatment of the cells with 20 mM ammonium acetate. Initial evidence indicates that the TGF-β receptor may not be down regulated to the same extent as are other polypeptide growth factor receptors.

0702 PARTIAL REVERTANTS OF Ha-MuSV-TRANSFORMED MDCK CELLS EXPRESS DECREASED LEVELS OF p21 AND POSSESS A MORE NORMAL PHENOTYPE, Frederick J. Darfler*, Thomas Y. Shih# and Michael C. Lin*, LCDB, NIADDK* and LMO, NCI-FCRF#, NIH, Bethesda, MD 20205

Madin Darby canine kidney (MDCK) cells have an epitheliod morphology, respond to glucagon and vasopressin by an enhanced accumulation of intracellular cAMP, are growth stimulated by prostaglandin E_1 (PGE_1) and hydrocortisone, and produce high levels of prostaglandin E_2 (PGE_2) Harvey murine sarcoma virus-transformed MDCK cells have a fibroblastic morphology, do not respond to glucagon or vasopressin, are stimulated for growth by hydrocortisone but not by PGE_1, and produce reduced amounts of PGE_2. We have isolated four subclones of transformed MDCK cells. These subclones fall into two general classes. Two subclones have a fibroblastic morphology, have lost growth responses to PGE_1, and do not respond to glucagon or vasopressin. Two other subclones have an epitheliod morphology, are growth stimulated by PGE_1 but not by hydrocortisone, and respond to vasopressin but not glucagon. All four transformed subclones secrete reduced levels of PGE_2, grow in soft agar (unlike untransformed MDCK cells), and produce measurable amounts of the viral transforming gene product p21. Reduced levels of p21 (25-44%) are expressed in the subclones possessing a more epitheliod morphology; these subclones may represent partial revertants to a more normal phenotype. We propose that levels of p21 may have a causal relationship to the expression of discreet classes of phenotypic characteristics. These subclones may be useful in ascribing certain functions of the transforming gene product p21.

0703 TRANSMEMBRANE ORIENTATION OF A DISTINCT SUBCLASS OF SV40 LARGE TUMOR ANTIGEN, Wolfgang Deppert and Ulrich Klockmann, Dept. of Biochemistry, University of Ulm, F.R.G.

A small percentage of SV40 large T antigen in SV40 transformed cells is specifically associated with plasma membranes. This subclass of large T can be distinguished from the bulk of nuclear large T by a specific posttranslational modification, the covalent binding of fatty acid (acylation). Acylated SV40 large T is tightly bound to the plasma membrane lamina, a cytoskeletal structure directly underlying the plasma membrane. This structure links the plasma membrane with the cytoskeleton and is thought to be involved in transmembrane signaling. Using lactoperoxidase catalyzed cell surface iodination we could demonstrate that the membrane lamina associated large T is also exposed on the cell surface. Acylated plasma membrane associated large T thus exhibits properties of a transmembrane protein. The specificity of its interaction with the plasma membrane, both in regard to its orientation and its posttranslational modification, suggests that it may play an important role in transformation by modulating some plasma membrane function.

0704 DIFFERENTIATION-SPECIFIC PRODUCTION OF PDGF-LIKE MOLECULES BY RAT AORTIC SMOOTH MUSCLE CELLS, Ronald A. Seifert, Daniel F. Bowen-Pope and Stephen M. Schwartz, University of Washington, Seattle, WA 98195

Platelet-derived growth factor (PDGF) is present within platelet alpha-granules and, upon release, provides a potential source of mitogenic stimulus for fibroblasts and smooth muscle cells. Although thought to stimulate cellular proliferation and synthesis of extracellular matrix during wound healing and to have a role in the pathogenesis of atherosclerosis, PDGF may have additional roles as a growth factor. It has been suggested that PDGF-like molecules may play a role in transformation and PDGF has recently been identified as the c-onc gene related to the simian sarcoma virus onc-gene, v-sis. We present evidence that a mitogenic PDGF-like molecule is expressed by normal cells in a differentiation-specific manner. As measured by radioreceptor assay, cultured aortic smooth muscle cells (ASMC) isolated from 2-week-old rat pups secrete 0.5-3.5 ng PDGF/10^6 cells/2 days into culture media while ASMC from 3-month-old adult secrete at least 25 fold less. Pup ASMC-conditioned medium is mitogenic for Balb/c 3T3 cells and this mitogenic activity, as well as the competitive binding activity observed by radioreceptor assay, is removed by prior adsorption of conditioned medium with anti-PDGF antibodies. ASMC cultured from 3-month-old rats express 35-60,000 PDGF receptors per cell, while cultured pup ASMC appear to express 4-40 fold fewer PDGF receptors. It is possible that the decreased receptor number on pup ASMC is due to downregulation of PDGF receptors following binding of endogenously synthesized PDGF-like molecules in an autocrine type system. The binding phenotype of rat pup ASMC is mimicked by rat adult ASMC incubated at 37° C with PDGF or rat pup ASMC-conditioned medium before addition of ^{125}I-PDGF, and in neither case can binding be restored by brief acid dissociation of prebound PDGF.

0705 BIOSYNTHESIS OF THE HUMAN EGF-RECEPTOR, Cathleen Carlin and Barbara Knowles, The Wistar Institute, Philadelphia, PA 19104

Using antisera specific for the human EGF-receptor, we have identified several protein species that bear a precursor relationship to the 145/165Kd form of the receptor that is displayed at the cell surface. The EGF receptor is initially synthesized as a 70Kd protein that is rapidly processed to a 95Kd species by N-linked glycosylation. This 95Kd species is susceptible to digestion with Endo H. The 95Kd protein appears to be converted to a 135K protein, perhaps by association with another peptide, as has been hypothesized for the biosynthesis of the LDL receptor. Maturation of the molecule from 135K to the 145Kd/165Kd involves the addition of negative charges to the molecule which includes but is not limited to the addition of phosphate to serine and threonine residues. The complexity of the biosynthesis of the EGF receptor raises questions as to a relationship between the molecular processing of the protein and its biological function.

0706 INTRACELLULAR PROCESSING OF EPIDERMAL GROWTH FACTOR, Bruce E. Magun, L.M. Matrisian, and S.R. Planck, University of Arizona, Tucson, AZ 85724

Previously we reported that ^{125}I-epidermal growth factor (EGF) was processed intracellulary to more acidic derivatives in Rat-1 fibroblasts (J. Cell Biochem. 20:259-276, 1982). We now report that the processing occurs by proteolytic cleavages of the carboxyl terminus. The first processed derivative (pI 4.2) lacked one or two terminal amino acid residues and was formed at the cell surface and within vesicular organelles as determined by subcellular fractionation on Percoll gradients. The EGF was then processed in vesicular organelles to a pI 4.35 derivative by a trypsin-like cut between residues 48 and 49. Cleavage of at least the carboxy-terminal arginine of the pI 4.35 species generated a pI 4.0 species which was localized to the lysosome peak of Percoll gradients. All processed derivatives were immunoreactive, but the pI 4.35 and 4.0 species demonstrated reduced affinity for the EGF receptor. The lysosomotropic amines, methylamine and ammonium chloride, completely blocked the progression of EGF processing to the pI 4.0 species and caused an accumulation of the 4.2 species within vesicular organelles. These lysosomotropic compounds also markedly inhibited EGF-induced stimulation of DNA synthesis. We suggest that EGF processing may be involved in targeting of the EGF receptor to intracellular sites, and that processing of EGF to lower affinity derivatives may be related to uncoupling of the receptor-EGF complex.

0707 INTERACTION OF THE V-SIS GENE PRODUCT WITH PDGF RECEPTOR Jean Y.J. Wang*, Patrice Tremble#, Jeremy S. Garrett#, and Lewis T. Williams#. *Dept. Biology, UCSD, La Jolla CA 92093, #Howard Hughes Medical Inst. and Dept. Medicine, UCSF, San Francisco, CA 94132

The protein encoded by the v-sis oncogene of simian sarcoma virus (SSV) has been found to be homologous with a subunit of platelet-derived growth factor(PDGF). We have identified in the plasma membrane of fibroblasts a 170K MW protein which specifically binds PDGF and that binding stimulates the phosphorylation of tyrosine residues in the receptor protein. To examine whether the v-sis protein can interact with this receptor, we tested the ability of this protein to compete for PDGF-binding. The v-sis protein was obtained from E. coli by expressing the v-sis coding sequence from the inducible P_R promoter of phage lambda:the promoter is repressed at 30° but can be induced by raising the temperature to 40°. Soluble extracts from bacteria containing the v-sis expression plasmid grown at 40° were found to block the binding of ^{125}I-PDGF to membranes whereas those from bacteria grown at 30° did not contain this inhibitory activity. However, neither of these extracts could stimulate tyrosylphosphorylation of the 170K MW protein. We also found that SSV-transformed NRK cells could release into the medium an inhibitor of PDGF-binding. This inhibitor, similar to the v-sis protein produced in bacteria, did not induce tyrosine phosphorylation of the receptor protein. Nontransformed NRK cells did not produce this inhibitor for PDGF-binding. These results suggest that the v-sis gene product may bind to the PDGF-receptor but it does not stimulate receptor phosphorylation.

0708 EARLY GROWTH-FACTOR- AND DEXAMETHASONE-INDUCED PROTEINS IN SWISS 3T3 CELLS: PERTINENCE TO DNA SYNTHESIS, Richard Levenson, Ken Iwata and Donald A. Young, University of Rochester, Rochester, NY 14642

Quiescent cells incubated with growth factors (GF's) rapidly initiate the synthesis of specific proteins. Some of these have been implicated in the control of the cell cycle. However, in addition to DNA synthesis, GF's also induce other metabolic perturbations including increases in RNA and protein synthesis, ion fluxes and phospholipid turnover. Many of the induced proteins may reflect these or other, non-DNA-pertinent, processes. Dexamethasone (dex) can positively and negatively modulate GF-induced DNA synthesis. We sought to correlate these dex effects with patterns of protein inductions detected on giant 2-D gels. Assayed on Swiss 3T3 cells, dex enhanced cartilage-derived growth factor (CDGF) stimulation by 100%, had no effect on platelet-derived growth factor (PDGF) action, and inhibited serum stimulation by 40%. We examined over 3,300 (35-S)-labelled protein spots induced within 3 h of stimulation and identified 34 GF- and 12 dex-induced proteins, as well as 5 induced in common. Using 2-D gel densitometry, we were able to correlate the dex-CDGF synergism seen at the DNA level with synergistic induction of these 5 proteins. However, since dex alone can induce them, their presence appears necessary but not sufficient for initiation of DNA synthesis. All other GF-inductions were strongly inhibited by dex--even while DNA synthesis was enhanced. Thus most early GF-induced proteins are dissociable from mechanisms of growth control.

0709 EFFECTS SERUM FREE CULTURE CONDITIONS UPON HEMATOPOIETIC AND STROMAL CELLS IN HUMAN LONG TERM MARROW CULTURES, A.J. Salvado, R.M. Meagher P. Young, A.I. Meierovics, D.G. Wright, Walter Reed Army Institute of Research, Washington, D.C. 20307

Growth of hematopoietic stem cells in human long term marrow cultures (LTMC) is regulated by adherent "stromal" cells which proliferate during the first 2-3 weeks of culture. We have studied the establishment of human LTMC under serum free conditions. Fetal bovine serum (FBS) and horse serum (HoS) were fractionated by ultracentrifugation into lipoprotein (L) and non lipoprotein (NL) fractions. Protein analysis and thin layer chromatography indicated 90-95% recovery of starting protein with only .4-3.6% in the L fraction and > 90% of the lipoprotein in this fraction. Human LTMC were initiated with FBS, HoS, L&NL fractions, or L&NL fractions remixed in the original proportions. All cultures contained 25% serum or serum substitute. L fractions also contained 1% deionized, delipidated BSA, 300 µg/ml iron saturated transferrin, 10^{-7}M selenium, and 5 µg/ml insulin. Only, L fed cultures showed no evidence of attachment, or proliferation of adherent cells at 1-2 weeks. Wright's stained preparations showed that non adherent cells surviving for up to two weeks in L fed cultures are predominantly well differentiated cells of multiple lineages. Methyl cellulose cultures showed persistence of BFU-E and CFU-GM in all cultures at one week. By week 2, cell counts are too low to plate in the L fed cultures. These progenitors continue to persist in all other cultures. When cultures initiated with lipoprotein were switched to complete serum, no adherent foci were established in cultures which had been serum free for 2 or more days. We conclude that bone marrow "stromal" cells fail to adhere under the serum free conditions described although hematopoietic progenitors can survive for longer periods under these serumless conditions.

0710 PROTEOLYSIS OF EPIDERMAL GROWTH FACTOR (EGF) RECEPTORS BY PURIFIED CALCIUM ACTIVATED NEUTRAL PROTEASE (CANP), Ronald E. Gates, and Lloyd E. King, Jr., Vanderbilt Univ. and V.A. Medical Center, Nashville, TN 37232.

An intracellular, calcium-activated protease converts the M_r=180K to the 160K form of the EGF receptor-kinase (both autophosphorylate) when A-431 carcinoma cells and some normal tissues are lysed in calcium solutions at neutral pH. While this endogenous protease is probably the ubiquitous CANP, other proteases can produce a similar conversion of the EGF receptor-kinase. To further establish and characterize this interaction, CANP was purified essentially to homogeneity from beef lung using multiple chromatographic steps. CANP activity was calcium (mM) dependent, sulfhydryl sensitive, had a pH optimum of 7-8.0 and was inhibited by iodoacetate, EDTA, EGTA, leupeptin and a partially purified, heat stable, specific protein inhibitor of CANP. By SDS-PAGE only two bands at M_r=80K and 30K were identified. This purified CANP hydrolyzed the covalent ^{125}I-EGF-receptor complex from M_r=180K to labeled bands at 160K and 130K. Hydrolysis of the autophosphorylated 180K form of the EGF receptor resulted in previously unidentified phosphorylated fragments of M_r less than 25K. Since these phosphorylated fragments were produced during conversion of the 180K to 160K form of the EGF receptor, the autophosphorylated sites are not identical in these forms. Furthermore, trypsin digestion of the 180K and 160K phosphorylated forms of the EGF receptor followed by peptide mapping showed that their $^{32}PO_4$-labeled peptides were different. Conclusion: Beef lung CANP had the reported properties of other previously studied CANPs. While converting the M_r=180K form of the autophosphorylated EGF receptor-kinase to 160K, this CANP generated phosphorylated peptides. These peptides may be important biological process mediators.

0711 Mutagenesis of the Abelson Murine Leukemia Virus Genome, Ron Prywes, J. Gordon Foulkes, Naomi Rosenberg* and David Baltimore, Whitehead Institute, Massachusetts Institute of Technology, Cambridge, MA 02139 and *Tufts University Medical School, Boston, MA 02111

We have investigated the sequences of Abelson MuLV required for transformation of fibroblast and lymphoid cells by in vitro mutagenesis of the viral genome. Our previous work (Cell, 34:569-79) has identified the 5' 1.2 kb of the 4.3 kb v-abl gene as being sufficient for fibroblast transformation. We have made further deletions into both the 5' (in frame) and 3' ends of this region. These deletions abolish transforming activity. To determine whether the entire region is necessary we randomly inserted 12 bp linkers, causing 4 amino acid insertions. Five of six of these proved to be transformation defective mutants indicating that practically the entire region is required for transformation. We have established cell lines containing the defective genomes and have analyzed the mutant proteins for tyrosine kinase activity. Each defective mutant lacks detectable tyrosine kinase activity. This correlates with the ability of this region, when expressed in E. Coli, to code for a tyrosine providing strong evidence that the kinase domain of the gene product is essential for transforming activity. In addition it is this region of v-abl that contains strong amino acid homology to v-src.

0712 SEPARATING THE FUNCTIONS OF POLYOMA'S TRANSFORMING GENES, Robert L. Garcea*, Leda Raptis ≠, and Tom Benjamin ≠, Dana-Farber Cancer Institute* and Dept. of Pathology ≠, Harvard Medical School, Boston, MA 02115.

The host-range transforming (hr-t) mutants of polyoma virus demonstrate that the middle and small T (tumor) antigens are responsible for cell transformation and tumor induction, as well as normal lytic viral growth. The primary role of these transforming genes may be viewed teleologically as stimulating virus production, and cell transformation as a side-effect of recruiting cellular functions for this goal. Therefore, we have approached the function of the transforming genes by studying their role in virus growth. Using hr-t mutant viruses we have shown that middle and small T antigens stimulate viral DNA replication 3-fold and encapsidation of the viral minichromosome 10-fold in non-permissive 3T3 cells. The effect on virus encapsidation appears to be mediated by an increase in post-translational modifications of the major capsid protein, VP_1. We have used additional mutant viruses and cells transfected with either middle or small T genes to separate the effects of these proteins on virus growth and the transformed phenotype. The individual contributions of middle and small T antigens to these processes will be described.

0713 THE GROWTH FACTOR ACTIVATABLE Na^+/H^+ ANTIPORT CONTROLS GROWTH OF FIBROBLASTS BY REGULATING INTRACELLULAR pH. Pouysségur, J., Franchi, A., L'Allemain, G. and Paris, S., Centre de Biochimie, CNRS, Parc Valrose, 06034 Nice, France.

We have previously characterized in Chinese hamster lung fibroblast a growth factor activatable and amiloride-sensitive Na^+/H^+ antiport. Here we present the results of two approaches to analyze its physiological role. First, we found that in HCO_3^--free medium and 25 mM $[Na^+]_o$, amiloride and a variety of analogs (K_I varying from 0.04 to 20 µM) inhibited reinitiation of DNA synthesis with the same rank order as that for Na^+/H^+ antiporter inhibition. Over a range of 3 logs of concentration, a tight correlation was established between IC_{50} for the blockade of the Na^+/H^+ exchange and IC_{50} for inhibition of serum-induced DNA synthesis. In a second approach, using the Na^+/H^+ antiport as a H^+ vector killing device, we selected fibroblast mutants specifically lacking Na^+/H^+ exchange activity. The wild type cells reinitiate DNA synthesis and grow exponentially in HCO_3^--free medium over a wide range of external pHs (6.6 to 8.3). In contrast, growth and mitogen-induced DNA synthesis are precluded at neutral and acidic pHs (<7.4) in the mutant cells. Measurements of pH_{in} in wild type and mutant cells clearly demonstrated that the Na^+/H^+ antiport plays a major role in pH_{in} homeostasis (pH_{in} is 0.2 to 0.3 pH unit more acidic in mutant cells). Therefore, we conclude that the Na^+/H^+ antiport as a major pH_i-regulating system is of crucial importance in growth factor action.

0714 REGULATION OF EGF RECEPTOR, Peter J. Parker, Silvia Stabel and Michael D. Waterfield, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX.

The interactions between growth factor receptors, the phorbol ester receptor and certain hormone systems are under investigation. Monoclonal antibodies directed against the EGF receptor have provided a means of purification of this receptor for structural analysis. These monoclonal antibodies have also been used to study interactions between the EGF receptor and the phorbol ester receptor in vitro. For this purpose, protein kinase C (Ca^{++}/phospholipid dependent protein kinase) has been purified to homogeneity (82,000 daltons) and shown unequivocally to be the phorbol ester receptor; phorbol esters and factors that alter the turnover of phosphatidylinositol could regulate EGF receptor function through protein kinase C. Reconstitution experiments in vitro provide circumstantial evidence for this. Interaction of the EGF receptor with hormonal systems functioning through the cyclic-AMP pathway has indicated that in vitro the cyclic-AMP dependent protein kinase may also regulate EGF receptor function through chaning its phosphorylation state; this effect however may be indirect. The interactions between these different regulatory pathways in vitro, reflect very complex antagonisms/synergisms that may exist in vivo. An understanding of the molecular events involved will provide a valuable insight into the mechanism of action of growth factors and tumor promoters.

0715 EMBRYONAL CARCINOMA DERIVED GROWTH FACTOR(S): SPECIFIC GROWTH PROMOTING AND DIFFERENTIATION INHIBITING ACTIVITIES, Aya Jakobovits and G.R. Martin, Department of Anatomy, University of California, San Francisco, California 94143

We have previously shown that concentrates of serum-free medium in which teratocarcinoma stem cells (embryonal carcinoma cells; EC cells) have been cultured (EC conditioned medium, EC-CM) can promote the growth of pluripotent, EC-like "embryonic stem cell" lines from normal preimplantation mouse embryos (Martin, G.R. (1981) Proc. Nat. Acad. Sci. USA 78, 7634). This led us to suggest that EC-CM contains a factor(s) that stimulates the proliferation of pluripotent cells in the early embryo and/or inhibits their differentiation, and that such a factor might have an autostimulatory mitogenic function in the EC cells that produce it. Our results indicate that EC-CM does indeed contain a factor(s) with specific growth-promoting and differentiation-inhibiting activities. EC-CM not only stimulates the proliferation of the pluripotent cells that produce it (autostimulatory activity) but affects the growth and differentiation of developmentally restricted cell types: it stimulates the proliferation of fibroblasts and induces them to express a transformed phenotype; it stimulates the proliferation of Friend erythroleukemia cells and also inhibits their induced differentiation. Our data further suggest that EC-CM activities are not related to those of epidermal growth factor (EGF), platelet-derived growth factor (PDGF), or tumor growth factors (TGFs) types α or β, thus suggesting that EC-CM contains a hitherto unknown class of embryo-derived growth factors which may play a unique role in the control of proliferation and differentiation of the cells in the early embryo as well as in the adult.

0716 POST-ENDOCYTOTIC PROCESSING OF EPIDERMAL GROWTH FACTOR IN HUMAN FIBROBLASTS, *William E. Van Nostrand, H. Steven Wiley, and Dennis D. Cunningham,* Department of Microbiology, University of California, Irvine, CA 92717

Utilizing a pulse-chase procedure in combination with native gel electrophoresis we have been able to detect intact and three sequentially processed forms of ^{125}I-EGF located intracellularly after binding to cell surface receptors and subsequent internalization. It was shown kinetically that the primary processing of internalized ^{125}I-EGF precedes the intitiation of the lysosomally mediated degradation of the polypeptide. We purified the processed forms of ^{125}I-EGF in order to investigate whether they were still capable of interacting with cell surface receptors. The first processed form of ^{125}I-EGF still possessed the ability to bind to cell surface receptors and was internalized at nearly the same rate as intact ^{125}I-EGF. The next smaller form did not detectably bind to cellular EGF receptors. The last form was small and lost upon dialysis, but it is doubtful that it would possess any binding ability. Utilizing various inhibitors of polypeptide ligand degradation we can selectively modulate the sequential processing at specific sites in the pathway.

0717 INITIATION OF DNA SYNTHESIS BY THROMBIN INVOLVES TWO SEPARATE CELL SURFACE INTERACTIONS, Darrell H. Carney and Janet Stiernberg, The University of Texas Medical Branch, Galveston, TX 77550

We have recently demonstrated that early amiloride-senstive Na^+ influx is not necessary or sufficient for thrombin initiation of DNA synthesis but that a later amiloride-sensitive event is necessary (J. Cell. Physiol. 117: 272-281, 1983). We have also examined the involvement of receptor binding and proteolytic activity in stimulation of early and late ion flux into hamster NIL cells using various derivatives of human α-thrombin. High concentrations of nitro-thrombin and γ-thrombin stimulated early and late $^{86}Rb^+$ uptake, respectively, almost as well as native α-thrombin, but did not initiate DNA synthesis. Since these derivatives do not bind the thrombin receptor, this suggests that proteolytic activity alone may be sufficient to stimulate ion fluxes. In contrast, concentrations of DIP-thrombin, which bind and saturate the thrombin receptor, did not significantly stimulate $^{86}Rb^+$ influx. Thus, receptor binding by DIP-thrombin is not sufficient to stimulate these ion fluxes. This derivative also does not initiate DNA synthesis. When DIP-thrombin and nitro-thrombin were added to cells at the same time, however, DNA synthesis was stimulated to levels equivalent to maximal stimulation by native α-thrombin. These results indicate that two separate types of transmembrane signal may be required for thrombin to initiate DNA synthesis. (Supported by NIH Grants AM-25807 and CA-00805).

0718 INSULIN AND CYCLIC NUCLEOTIDES IN GROWTH REGULATION OF REUBER H35 CELLS. John Koontz, Univ. of Tennessee, Knoxville, TN 37996 and Ben H. Leichtling, Natl. Jewish Hospital, Denver, CO 80206

The interaction between insulin and agonists which elevate intracellular cAMP is frequently antagonistic in nature. The putative 2nd messengers of insulin action can be detected by virtue of inhibiting cAMP dependent protein kinase (1), adenylate cyclase (2), or stimulating low k_m cAMP phosphodiesterase (3). Furthermore, cAMP agonists have been reported to inhibit insulin binding, decreasing the number of accessible receptors (4).

Using the Reuber H35 rat hepatoma cell line, in which insulin stimulates a growth response via the insulin receptor (5), we asked whether insulin would alter cyclic nucleotide levels in quiescent cells and whether cyclic nucleotides would inhibit the cellular response to insulin. Neither cAMP nor cGMP is altered significantly during the first three hours of exposure to insulin (or to serum). Also, cholera toxin or dibutyryl cAMP do not inhibit the progress of insulin or serum stimulated cells from G1 into S phase. It is concluded that alterations in the level of cAMP or cGMP do not contribute significantly to the regulation of the transition from quiescent to growing state in this cell line.

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0719 STRUCTURAL RELATIONSHIPS BETWEEN SOMATOMEDIN CARRIER PROTEINS AND BINDING PROTEINS, E. Martin Spencer, Childrens Hospital, San Francisco, Ca 94118

Somatomedins are potent mitogens which are also called insulin-like growth factors. Their principal physiologic role is in mediating the growth-promoting effect of growth hormone. Contrary to other polypeptide hormones, they are transported in plasma bound to a large molecular weight carrier protein(s) whose function is not only transport but also inactivation of the somatomedins on complexing with them. We are probing the structure of the carrier protein(s) in order to perform structural and genetic comparisons with their plasma membrane receptors.

0720 THE PRESENCE OF NONFUNCTIONAL EPIDERMAL GROWTH FACTOR RECEPTOR IN VIRUS TRANSFORMED CELLS, Chu Chang Chua, Deborah E. Geiman, Alain B. Schreiber, Roger L. Ladda, Pennsylvania State University, College of Medicine, Hershey, PA 17033.

The cell membrane receptor for epidermal growth factor (EGF) appears to be a glycoprotein of M_r 170,000 and mediates the mitogenic and metabolic responses of cells with EGF receptors (EGF-R). Normal rat kidney cells (NRK) have about 3×10^5 EGF-R per cell. Upon transformation of NRK cells by Kirsten sarcoma virus, the transformed derivative (KNRK) loses the ability to bind ^{125}I-EGF. Membranes from NRK and KNRK cells were included in EGF-dependent phosphorylation reactions to search for evidence of the EGF-R. Phosphorylated protein of M_r 170,000 was detected in both NRK and KNRK membranes. The level of phosphorylation of the M_r 170,000 band in the NRK membrane preparations responded to increasing doses of EGF. In contrast, the intensity of the M_r 170,000 band in KNRK cells remained unchanged with increasing amount of EGF. The M_r 170,000 protein was identified to be EGF-R by immunoprecipitation with monoclonal antibody to the receptor. Furthermore, two-dimensional peptide mapping using trypsin and chymotrypsin digestions of the iodinated receptors from both NRK and KNRK cells showed essentially identical patterns. These data indicate that the EGF-R is present in KNRK cells with apparently the same protein structure as the NRK counterpart.

0721 IGF-II AND INSULIN STIMULATE PROLIFERATION OF H-35 CELLS THROUGH THE INSULIN RECEPTOR, Cristina Mottola and Michael P. Czech, University of Massachusetts Medical Center, Worcester, MA 01605

It is possible to induce proliferation in rat H-35 cells by preincubation with insulin (optimal concentration 10^{-9}M) or IGF-II (optimal concentration 10^{-7}M). Affinity crosslinking with labeled hormones indicates that the plasma membrane of these cells is very rich in IGF-II receptor, contains insulin receptor, and appears to be devoided of IGF-I receptor. Binding studies suggest that the receptor involved in the cell growth promotion is that one for insulin (Massague, J., Blinderman, L.A., and Czech, M.P., J. Biol. Chem., 257, 13958-13963, 1982). Further insight into this question has been now provided employing a polyclonal antibody specifically directed against IGF-II receptor. This antibody, elicited in rabbit with purified rat placenta IGF-II receptor, inhibits the binding of IGF-II to its receptor in rat (5 cell types were screened) and with lower affinity in mouse, but not in human. The antibody does not inhibit the binding of any hormone either to the insulin or the IGF-I receptor in all the species tested. The inhibitory effect on IGF-II binding to its receptor in H-35 cells is unaffected by incubation for 17 hrs at 37°C. In conditions in which the binding of IGF-II to its receptor is completely blocked, the stimulation of [^{3}H] thymidine uptake induced by insulin (10^{-9}M) or IGF-II (10^{-7}M) is unchanged. The antibody itself does not mimic the hormone action, while a specific human anti-insulin receptor antibody (B-9) increases [^{3}H] thymidine uptake to the same extent as insulin. All these data agree with a model implicating that insulin and not IGF-II receptor is responsible for triggering cell growth in H-35 cells.

Cell-Cell Interactions in Regulatory Responses

0722 CELL IMMUNE RECOGNITION AND GROWTH REGULATION OF HUMAN MAMMARY CARCINOMA : ISOLATION AND CHARACTERIZATION OF THE CELL MEMBRANE GLYCOPROTEIN. Anwar A. Hakim and Charles E. Joseph. Loyola Univ. of Chicago Med. Cntr. Maywood, Ill. and Unive. Southern Calif. Los Angeles, Calif.

Earlier studies showed that treatment with vibrio cholera neuraminidase (VCN) reduced the oncogenicity and increased the immunogenicity of human mammary carcinoma cells (Hakim, A.A. Immunol. Commun. 7: 25-39, 1978). A non- oncogenic, immunogenic and protective mammary carcinoma cells were prepared by VCN-treatment (Hakim, A.A. Experientia 33: 518-520, 1977). The present studies were aimed at the chemical nature of the receptor(s) of cytotoxic afferent cells. VCN-pretreated cells were extracted with 2% perchloric acid. The extracts were neutralized to pH 7.6, then applied onto Concanavalin A-Sepharose - 6B columns. A glycoprotein that was retained on the column was eluted with -methyl-D-mannoside showed the following characteristics: (a) Disc electrophoresis revealed one major band stained by Amido black 10B. Detailed structural studies of the oligosaccharides liberated from the glycoprotein by hydrozinolysis contained one biantennary complex-type sugar chain. (b) Subcutaneous implantation of the tumor cells HMCC-GM cells into New Zealand rabbits produced both blood lymphocytes and immunoglobulins cytotoxic to HMCC-GM cells. When preincubated with the membrane glycoprotein, the rabbit cytotoxic lymphocytes lost their killing activity against the HMCC-GM target cells. (c) When incubated with sheep red blood cells (SRBC), the cytotoxic lymphocytes formed rosettes. Preincubated with the membrane glycoprotein, neither SRBC nor the lymphocytes formed rosettes. Threfore, the membrane glycoprotein acted as receptor in rosette formation.

0723 CELL-CELL INTERACTION BETWEEN FIBROBLASTS AND A MUSCLE CELL LINE, Jeffrey Boone Miller, University of California, San Francisco, CA 94143

Fibroblasts and myoblasts normally coexist in developing muscle. I studied the effects of fibroblast co-culture on muscle cell lines. The most spectacular interaction occurred between rat or mouse muscle fibroblasts and myoblasts of the L6 cell line. L6 myoblasts normally fused to form wide, flat, sheet-like myotubes with a two-dimensional array of nuclei. When primary lower leg muscle fibroblasts were present during fusion, however, the resulting L6 myotubes were long and thin and the nuclei were arranged in single file - an arrangement similar to myotubes *in vivo*. In addition, some of the normally unclustered acetylcholine receptors on L6 cells were found in clusters after fibroblast co-culture. Fibroblasts affected myotube development only when added before myoblast fusion had begun. Neither medium conditioned by fibroblasts nor fixed fibroblasts affected L6 development.
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0724 CELL-CELL INTERACTIONS REGULATING GENE EXPRESSION IN DICTYOSTELIUM. Edward A. Berger, Judy M. Clark, Marcia B. Berman, and Jennifer A. Morgenthaler, Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

We have been studying the relationship between specific cell-cell contact and the expression of the genes of discoidin I, an endogenous lectin that has been implicated in the cell cohesion process. By performing RNA gel blot and dot hybridization analyses using a cloned discoidin I cDNA probe, we have found: 1) During normal development on filters, discoidin I mRNA levels rise during the early aggregation stage but then decline precipitously as specific cell-cell contacts occur and tight aggregates form, 2) When tight aggregates are disaggregated, discoidin I mRNA levels rise dramatically, 3) When cells are developed in suspension (conditions that interfere with the formation of tight cell aggregates), discoidin I mRNA accumulates to abnormally high levels, and these persist well after the levels in filter-developed cells have declined. These results strongly suggest that cell-cell contact is a developmental signal to deactivate discoidin I gene expression, making this the first contact-deactivated gene for which a cloned DNA probe is available. Furthermore, we have found that exogenous cAMP nearly completely blocks the disaggregation-induced reactivation of discoidin I gene expression. This, coupled with preliminary analyses indicating a drop in intracellular cAMP levels upon cell disaggregation, support the emerging notion that cAMP might serve as an intracellular "second messenger" mediating the expression of contact-regulated genes. We are now seeking to identify the initial molecular events at the cell surface that trigger the contact-regulated switches in developmental gene expression.

0725 NEOPLASTIC B CELLS EXPRESS RECEPTORS FOR B CELL DIFFERENTIATION FACTORS, Gyan C. Mishra, Peter Krammer, Jonathan W. Uhr and Ellen S. Vitetta, University of Texas Health Science Center, Dallas, Texas 75235 and the Institute for Immunology and Genetics, German Research Cancer Center, Heidelberg, Germany ...

B cell growth and differentiation into immunoglobulin-secreting cells are regulated in part, by cytokines derived from macrophages and T cells. T cell-derived B cell growth factor (BCGF) sustains the proliferation of anti-Ig stimulated B cells. T cell-derived differentiation factors (BCDF) induce activated B cells to secrete IgM (BCDFμ) and IgG (BCDFγ). These lymphokines probably act by binding to specific receptors on their target cells. In the present study, cells from the in vitro adapted murine B cell tumor, BCL_1, were subcloned by limiting dilution and subclones expressing receptors for BCDFμ and BCDFγ were selected based on their ability to absorb the respective lymphokines from supernatants of the Con A-induced alloreactive PK 7.1 T cell line. Subclones which absorbed either BCDFμ, BCDFγ, or both were obtained. In preliminary experiments, it appears that these subclones did not absorb BCGF. These results suggest the existence of specific receptors for each of the BCDFs on activated B cells.

0726 NEURAL CELL SURFACE POLYPEPTIDES BIND SPECIFICALLY TO THE HEPARIN/HEPARAN CLASS OF GLYCOSAMINOGLYCANS. Richard Akeson, Children's Hospital, Cincinnati, Ohio 45229

Cell responses to many stimuli including growth factors are modulated by cell-extracellular matrix interactions. Although cellular interactions with polypeptide matrix components have been extensively studied, cellular interactions with the glycosaminoglycan (GAG) components are less well understood. We have tested the interaction of neural cell surface components with GAG's by lactocperoxidase iodinating cell surface polypeptides, solubilizing the cells in NP40, and examining the "binding" of the labelled polypeptides to solid phase GAG agarose beads. At physiological conditions 15-25% of the added radioactivity from iodinated rat embryonic neurons can be bound to heparin or heparan sulfate beads. Lesser levels are bound to dermitan sulfate and polygalacturonic acid beads and little specific binding to chondroitin sulfate or hyaluronic acid beads is seen. Binding to heparin beads is competed by soluble heparin/heparan but not chondroitin or keritan sulfate or hyaluronic acid. When the heparin bound polypeptides are electrophoresed on SDS gels, the majority of major iodinated bands in the initial NP40 soluble preparation were also observed in the heparin bound fraction. In a mixed cell type primary culture each cell type may contribute only one or two of the observed heparin binding species. Therefore the clonal rat neuronal line PC12 was surface iodinated. Again heparin beads had the greatest binding and the electrophoretic profile of peptides bound to heparin beads was qualitatively similar to that of the initial NP40 soluble preparation. These results indicate that a number of neuronal cell surface components have affinity for heparin/heparan like carbohydrate sequences.

0727 THE SUCCESS AND FAILURE OF AXONS TO REGENERATE AROUND LESIONS IN ADULT RAT BRAIN. Anne P. Foerster. Dept. of Neurosciences, McMaster Univ., Hamilton, Ontario L8N 3Z5
A new lesioning technique, involving a fine cutting wire (suspended from 2 vertical support wires) that is lowered vertically through the brain, reveals an unexpected capacity for rapid and substantial regeneration of cut axons in the adult rat brain. The device remains implanted during healing; the original cut is thus known to have occurred between the vertical supports. Surprisingly, the regenerating fibers do not cross the lesion, but grow alongside it, then forming a detour around the support wires to reconnect their own severed pathways, eventually reaching their former target region, where new evidence indicates function can be restored. When cut axons would have to bend aside from more than about 1 mm to grow to reach the end of the cut, they show no signs of attempted growth but generally remain (for months, and probably longer) in an arrested state, facing the cut, with their ends somewhat withdrawn from it. They are still connected to their cell bodies, and axoplasmic flow continues; this may represent the chronic condition of non-regeneration so often reported in the injured mammalian central nervous system. The unknowns include: a) the signal that evokes growth; b) why it is ineffective or absent when the lesion is extended; c) why the majority of axons grow around, rather than across, the lesion.

0728 A CLONAL MODEL FOR T LYMPHOCYTES BEARING FC RECEPTORS FOR IGA, Katie R. Williams, and Richard G. Lynch, University of Iowa, Iowa City, IA 52241

T lymphocytes bearing receptors for the Fc portion of IgA, (IgA FcR T cells), may play an important role in regulation of immunoglobulin expression. We have previously shown that large numbers of IgA FcR T cells (20-30% of mouse splenic T lymphocytes) can be induced both in vivo and in vitro by high levels of polymeric IgA. In order to identify a monoclonal source of IgA FcR T cells for biochemical and functional studies, we screened a series of BALB/c T lymphomas. One lymphoma, BALENTL8 (BAL8) (JEM 147:1267, 1978) was shown to constitutively express Fc receptors for IgA and therefore is a potential model for IgA FcR T cells.

Fc receptors on the BAL8 cells are assayed by decorating the cell with polymeric IgA, washing away unbound IgA, and then visualizing IgA FcR T cells by their ability to form rosettes with the appropriate hapten conjugated erythrocytes. IgA-decorated BAL8 cells rosette at a frequency of 50-80%. Rosette formation can be competitively inhibited with other IgA proteins. Only weak, variable inhibition is observed with either IgG or IgM. Additionally, the Fc receptor of BAL8 cells is able to bind to membrane IgA present on IgA producing myeloma cells. This is visualized by a myeloma-lymphoma rosette formed directly between cells without addition of IgA protein. NP40 lysates of BAL8 cells have been prepared after biosynthetic labelling with ^{3}H-leucine. Several proteins can be isolated from these lysates by affinity chromatography on polymeric IgA-Sepharose columns. (Supported by PHS #CA09119 and CA32277)

0729 THREE CLASSES OF SIGNALLING MOLECULES ON B CELL MEMBRANES, R.B. Corley, N.J. LoCascio, T. Kuhara, L.W. Arnold, P.S. Pillai, D.W. Scott and G. Haughton. Duke Medical Center, Durham, NC 27710 and University of North Carolina, Chapel Hill, NC 27514

The question of whether surface immunoglobulin (sIg) and Ia molecules have a signalling function in helper T (Th) cell-dependent activation of resting B cells is being evaluated. Two sources of B cells have been used, one a purified population of hapten-binding B cells, the other a B cell lymphoma, CH12, with known antigen specificity. CH12 has the activation properties of a resting B cell. The requirement for antigen in B cell activation was studied using Th cells selected such that they interacted with B cells in the absence of the sIg-antigen binding. B cells were not activated when normal ratios of Th cells and B cells were used unless the B cell antigen was also present in culture, demonstrating that sIg-ligand interactions contribute to B cell activation. The requirement for sIg-ligand binding could be overcome using higher multiplicities of T cell help. However, Th cell recognition of B cell Ia was still essential. Preliminary evidence that Th cell-Ia interactions result in the transmission of an activation signal is provided from the study of CH12. Only Th cells that interact with I-E molecules on CH12 activate the lymphoma, even though CH12 express both I-A and I-E molecules. Whether only one Ia molecule is functional on normal resting B cells is unknown and currently under investigation. Taken together, these data indicate that both sIg and Ia molecules function as signalling receptors in B cell activation, possibly by inducing the expression of a third class of membrane receptors, in particular those that bind the factors that promote growth and differentiation of responding B cells.

0730 TRANSFERRIN RECEPTOR INDUCTION IS REQUIRED FOR B CELL ACTIVATION, L.M. Neckers, G.Y. Yenokida, and S. James, NCI, NHLBI and NIAID, NIH, Bethesda, MD 20205.

Transferrin receptors are expressed on proliferating cells and are required for their growth. Transferrin receptors can be detected after, but not before, mitogenic stimulation of peripheral blood T and B cells. T cells demonstrate a functional requirement for transferrin receptors in the activation process. These receptors, in turn, are induced to appear by TCGF. In the experiments reported here we have examined the regulation of transferrin receptor expression on activated human B cells and whether these receptors are necessary for activation to occur. Activation was assessed by studying both proliferation and immunoglobulin secretion. We have determined that transferrin receptor expression on B cells is regulated by a factor contained in supernatants of mitogen-stimulated T cells. This expression is required for proliferation to occur, since antibody to the transferrin receptor blocks B cell proliferation. Induction of immunoglobulin secretion, however, although dependent on PHA-treated T cell supernatant, is not dependent on transferrin receptor expression and can occur in mitogen-stimulated cells whose proliferation has been blocked by anti-transferrin receptor antibody. These findings support a model for B cell activation in which mitogen (or antigen) delivers two concurrent but distinct signals to B cells: one, dependent on BCGF and transferrin receptor expression for proliferation; and a second, dependent on T cell derived factors and not requiring transferrin receptors, which leads to immunoglobulin secretion. B cell activation is apparently regulated by the interaction of several different groups of cell surface receptors which can function independently of each other.

0731 PHYSICO-CHEMICAL PROPERTIES OF THE N-FORMYL PEPTIDE RECEPTOR OF HUMAN NEUTROPHILS. Rodger A. Allen, Algirdas J. Jesaitis, Larry A. Sklar, Charles G. Cochrane, and Richard G. Painter. Dept. of Immun., Res. Inst. of Scripps Clinic, La Jolla, CA 92037

To investigate the physico-chemical properties of the N-formyl receptor of human neutrophils, the receptor was covalently labelled with an iodinated photoactivatable derivative of the chemotactic hexapeptide (F-Nle-Leu-Phe-Nle-[^{125}I]Tyr-Lys). After labelling isolated neutrophil membranes, the receptor was extracted with octyl glucoside or Triton X-100 and subjected to gel filtration on a calibrated Ultrogel AcA 34 column equilibrated with 0.1% Triton. The receptor had a Stoke's radius (r_S) of 38Å. Sedimentation equilibrium analysis was carried out in H_2O and D_2O-H_2O mixtures containing 0.1% Triton, in a Beckman airfuge. An apparent M.W. of 62,000 was obtained for the receptor-ligand detergent complex, which agrees closely with the reduced SDS-PAGE value of 50-60,000. Thus the receptor is monomeric in Triton. This value together with the r_S value suggests a receptor-detergent particle that is globular in shape. A second peak containing specifically labelled material also eluted in the void volume of the Ultrogel AcA 34 column which, when electrophoresed in the absence of β-mercaptoethanol on SDS-PAGE, retained its high molecular weight and after reduction migrated with a MW of 50-60,000. When subjected to isoelectric focusing either under reduced or non-reduced conditions, the high molecular weight material focused with a pI of 6-6.5, which is the same as the monomeric form of the receptor. Thus, the receptor extracted from membranes of unstimulated cells appears to be monomeric with a tendency to form disulfide linked homo-oligomers in Triton. Similar analysis is underway on membranes isolated from cells stimulated at 37°C.

0732 EVALUATION OF HUMAN T CELL GROWTH FACTOR (TCGF) RECEPTOR EXPRESSION USING RADIOLABELED MONOCLONAL ANTI-TAC ANTIBODY. J.M. Depper, W.J. Leonard, T.A. Waldmann, and W.C. Greene. NCI, NIH, Bethesda, Md. 20205

We have shown that monoclonal anti-Tac recognizes the human receptor for TCGF. We have tritiated this antibody to high specific activity by reductive methylation with $NaBH_4$, and have used it to develop a sensitive binding assay to evaluate cell surface TCGF receptor expression. TCGF receptors appear within 4-8 hours after PHA stimulation of normal peripheral blood T cells. This expression requires new RNA and protein, but not DNA synthesis. After 48-72 hours of lectin stimulation, T cells maximally bind 30,000-60,000 molecules of antibody per cell with a K_D of 1-3 x 10^{-10} mol/L. Thereafter, despite optimal culture conditions, a progressive decline in receptor number is observed, to levels of 5-10,000 molecules bound/cell after 10 days. Receptor expression is increased by reexposure to PHA, phorbol diesters, or the initial stimulating antigen (in the case of antigen stimulated cells). Thus, TCGF-mediated immune responses may be regulated not only by the amount of TCGF secreted, but also by the number of TCGF receptors expressed. Moreover, our studies indicate 10-200 fold more receptors on various cell populations than evaluated by previously reported studies employing ^{3}H-TCGF. Preliminary data suggest that there may be two classes of TCGF receptors which differ in their binding affinity for TCGF but not in their binding affinity for anti-Tac.

0733 INTERFERON RECEPTORS AND REGULATION OF HUMAN MONOCYTE FUNCTION, Susanne Becker, University of North Carolina, Chapel Hill, NC 27514

The interaction of interferons with human monocytes leads to changes in the expression of receptors for C3b, C3bi and Fc as well as in the expression of HLA-D determined antigens (Ia). Interferons α and β have similar effects in that they downregulate the complement receptors but have little or no effect on the Fc receptor and Ia. At the same doses of antiviral units (300 u/ml) and 10 fold lower interferon γ upregulates the Fc receptors and Ia and strongly inhibits complement receptor expression. Monocytes treated with interferon γ are better accessory cells in immune responses, while α and β treated monocytes tend to be suppressive.

These observations have led to the instigation of studies comparing the necessity of binding, internalization and degradation of α and γ interferon for the expression of their effects on monocytes. Early effects are monitored by assays for CAMP and 2'-5' oligo(A)polymerase. Later effects are expressed as the changes in receptors and antigens mentioned above.

0734 SEPARATION OF SIGNALS INVOLVED IN LYTIC FUNCTION AND CELLULAR PROLIFERATION IN CLONED CYTOTOXIC LYMPHOCYTES, John H. Russell, Dept. of Pharmacology, Washington University School of Medicine, St. Louis, Mo. 63110

The interaction between the cytotoxic T lymphocyte (CTL) antigen receptor and its antigen bearing target cell initiates two functionally distinct events. These are: 1) target cell lysis; and 2) CTL proliferation. We have isolated cloned CTL that will allow us to study the physiologic events within the CTL that are associated with these individual functional pathways. This clone is apparently cross-reactive between two different class I major histocompatibility antigens. It is unique in that the "strength" of their antigenic stimulus depends upon the functional assay used. That is it lyses cells bearing H-2D^k better than cells bearing H-2D^d, while H-2D^d cells provide a stronger proliferative stimulus than H-2D^k cells. We have used this clone to demonstrate that accelerated $^{86}Rb^+$ release from CTL as a result of CTL-target interaction appears to be associated with the lytic function rather than the proliferative response. Replacement of extracellular Na^+ with K^+ or Rb^+ selectively blocks CTL-mediated lysis at a site in the lytic process that is beyond the initial binding event. The physiological requirements for antigen specific $^{86}Rb^+$ release from CTL are consistent with the interpretation that it measures a late event in the lytic process. Supported by grant numbers CA 28533 and CA 34817 awarded by the National Cancer Institute, Dept. of Health and Human Services.

0735 A NOVEL METHOD FOR RADIOLABELING ANTIGEN-BINDING CELLS AND RECEPTORS. Y. S. Choi, G. C. Higgins and A. J. Rosenspire, Sloan-Kettering Institute, Rye, NY 10580

Specific radiolabeling of antigen-binding cells and receptors is accomplished by utilizing a novel technique employing lactoperoxidase (LPO) covalently linked to antigen (Ag). The surfaces of antigen-binding cells were first bound to the Ag-LPO conjugates through specific receptors and the bound LPO was then allowed to catalyze radioiodination of the receptors. Using this technique, we are now able to radiolabel antigen-binding cells with such high selectivity that we can easily discern them via autoradiography. Furthermore, when whole cell lysate was analyzed in one- and two-dimensional SDS-PAGE, the most intensely labeled protein was the immunoglobulin receptors. These results suggest that the receptors are selectively radiolabeled. An additional polypeptide of mol. wt. 55K was also selectively labeled and isolated with immunoglobulin. Since this molecule was also isolated by myosin-affinity chromatography, this may be a molecule that links the immunoglobulin receptors with actin.

0736 SIALIC ACID RESIDUES ON NERVE GROWTH FACTOR RECEPTORS, Ronald D. Vale, Markus Hosang and Eric M. Shooter, Stanford University School of Medicine, Stanford, CA 94305

We have previously shown that the lectin wheat germ agglutinin (WGA) alters the binding properties of NGF receptors on PC12 cells. Normally, two populations of NGF receptors with rapid and slow dissociation kinetics are present; however, WGA acts to convert rapidly dissociating NGF receptors into a slowly dissociating form. In order to identify the sugar moeity on the receptor which interacts with WGA, sugar specific glycosidases were tested for their abilities to inhibit the effects of WGA on NGF receptors. Neuraminidase pretreatment of PC12 cells inhibited the WGA-induced receptor conversion by 50%, indicating that a sialic acid residue is involved. N-Acetyl-D-glucosaminidase as well as four other glycosidases had no effect. Combinations of glycosidases did not produce a greater inhibition than neuraminidase alone. In addition, a succinylated derivative of WGA which binds N-acetyl-D-glucosamine but not sialic acid residues does not affect NGF binding even at high concentrations. Crosslinking of ^{125}I-NGF to PC12 cells using a heterobifunctional crosslinking reagent also reveals the presence of two receptor species with molecular weights of 160 K and 100 K which correspond to the slowly and rapidly dissociating forms of the receptor respectively. Neuraminidase treatment alters the migration of both NGF receptor species in a SDS polyacrylamide gel. The apparent molecular weights of both were lowered slightly as a result of neuraminidase treatment due to the removal of highly charged sialic acid residues. Other glycosidases did not alter the molecular weights of the receptor. Removal of sialic acid by neuraminidase does not, however, affect the ability of the receptor to bind NGF.

0737 FLOW CYTOMETRIC ANALYSIS OF HUMAN MONOCYTE-MACROPHAGE SURFACE RECEPTOR AND ANTIGEN DISTRIBUTION, Stephen Haskill, University of North Carolina, Chapel Hill, NC 27514

Fluorescence resonance energy transfer has been successfully used by the Jovins (PNAS, 80:5985-5989, 1983) as a tool to determine the proximity of both ConA receptors and H-2k antigens on the surface of leukemia cells. Distances less than 100A can be assessed by this technique when fluorescein-rhodamine pairs of probes are employed. In addition to information about proximity and quantitation of receptor number, information about surface distribution can be obtained from analysis of the pulse shapes produced when the cell passes through the 2μ beam. We have recently modified an ORTHO SYSTEM 50 CYTOFLUOROGRAF to investigate receptor and an antigen distribution and interaction during monocyte differentiation and activation. Preliminary data on ConA receptors and HLA.DR antigen sites on human monocytes will be presented.

0738 KAINIC ACID, A HETROCYCLIC ANALOG OF THE NEUROTRANSMITTER L-GLUTAMIC ACID, EVOKES A CHEMOTACTIC RESPONSE IN HUMAN PERIPHERAL MONOCYTES, J. David Malone, Michael Richards, and Arnold Kahn, V.A. Med. Center, St. Louis Univ., and Washington Univ., St. Louis, MO. 63106.

We have previously reported that L-γ-carboxyglutamic acid (L-gla) evokes a dose dependent chemotactic response in human peripheral mononuclear cells (HPM's), (Malone, et al ASBMR 1982 abst.). The response is optimum at 10^{-10} M and is not evoked in the absence of a concentration gradient. No chemotactic response is observed when polymorphonuclear leucocytes are used as the test cell. Neither the D isomer of gla or L-glutamic acid evoke directed cell movement when tested against HPM's. To our knowledge there have been no previous reports of a single amino acid exciting a chemotactic response in mammalian cells. However L-glutamic acid (L-glu) and its hetrocyclic analogues have been extensively studied as excitatory neurotransmitters in mammalian CNS systems. Despite the absence of a response to L-glu in our chemotaxis assay we were curious to know if any of the hetrocyclic analogues could evoke a chemotactic response. One such analogue, kainic acid, when tested in our chemotaxis assay evoked a dose dependent response over the range 10^{-11} M to 10^{-6} M, with maximum cell movement observed at 10^{-10} M. No response was observed in the absence of a concentration gradient. Competition experiments were performed with L-gla and demonstrated that L-gla blocked the chemotactic response to kainic acid. Reciprocally, kainic acid blocked the chemotactic response to L-gla. These findings suggest that L-gla and kainic acid share a common receptor on HPM's. If these findings are confirmed the system offers a rapid and readily available method for screening and analyzing potentially active neurotransmitter substances.

0739 BRIDGING OF RECEPTORS FOR IMMUNOGLOBULIN E ON THE CELL SURFACE TRIGGERS THEIR INTERACTION WITH THE CYTOSKELETAL ARCHITECTURE, Barbara Baird, Anant Menon, Deborah Robertson, and David Holowka, Cornell University, Ithaca, NY 14853.

Two separate lines of evidence indicate that external crosslinking of immunoglobulin E (IgE) bound to receptors on the surface of rat tumor basophils induces subsequent interactions of receptors with the cellular cytoskeleton. In one study, the distribution of small oligomers of IgE bound to RBL cells was examined with fluorescence microscopy. The oligomers were seen to become clustered into large visible patches on the cell surface at 4°C, and at higher temperatures internalization was also observed. In contrast, cells labeled with monomeric IgE remained uniformly labeled under identical conditions, and no clustering was observed when oligomers were bound to protrusions of membranes (blebs) which lack normal cytoskeletal associations.
In a second study, visible clusters of fluorescent IgE-receptor complexes on the cell surface were induced by crosslinking with anti-IgE, and these clusters were found to remain associated with the cellular cytoskeleton after the cell membranes had been solubilized by Triton X-100. Unclustered IgE-receptor complexes were completely solubilized under these conditions. The receptor-cytoskeleton interactions revealed in these studies may be important in generating the transmembrane triggering signal of IgE-receptor mediated degranulation.

0740 ISOTYPE SPECIFICITY OF Fc RECEPTOR-MEDIATED REGULATION OF B CELL ACTIVATION. Nancy E. Phillips and David C. Parker, Molecular Genetics and Microbiology, University of Massachusetts Medical Center, Worcester, MA.
We are using a polyclonal in vitro model of B cell activation to study the mechanism by which immune complexes and anti-idiotypic antibodies can regulate B cell activation. This system utilizes rabbit IgG anti-mouse μ-chain specific antibodies and antigen nonspecific helper factors. In the presence of antigen nonspecific helper factors, these antibodies do not induce DNA synthesis or Ig secretion unless the Fc region has been removed by pepsin digestion, and the whole anti-μ inhibits $F(ab')_2$anti-μ-induced proliferation and Ig secretion. The inhibition is reversible, occurs during the first 48 hrs of culture independently of adherent cells or suppressive factors, and can be blocked by a monoclonal anti-Fc_γ receptor antibody (2.4G2). Recently we have examined the Fc receptor isotype specificity of this inhibition. This has been accomplished by coupling arsanilic acid (ars) to the $F(ab')_2$anti-μ and investigating the effect of mouse monoclonal anti-ars antibodies of different isotypes. Our results indicate that IgG1, IgG2b, or IgG2a anti-ars will mediate this inhibition, but not IgG3 or IgM anti-ars. Furthermore, inhibition by IgG2a as well as by IgG1 and IgG2b, can be blocked by Fab fragments of 2.4G2, suggesting that binding of IgG2a to B cell Fc receptors, unlike binding to macrophage Fc receptors, is blocked by 2.4G2 Fab.

0741 PHOSPHORYLATION OF ACTIN-ASSOCIATED PROTEINS IN B LYMPHOCYTES CAN BE MODULATED BY THE CROSS LINKING OF MEMBRANE IMMUNOGLOBULIN. A. J. ROSENSPIRE AND Y. S. CHOI, Sloan-Kettering Institute for Cancer Research, Rye, New York 10580
The cross linking of membrane immunoglobulin (mIg) on B-lymphocyte surfaces by anti-Ig antibodies has been shown to be a mitogenic signal to B cells. Because in many instances growth control appears to be mediated by phosphokinases, we asked the question of whether cross linking mIg will also lead to the specific phosphorylation of lymphocyte proteins.

Utilizing a myosin affinity technique, we directly examined proteins that are associated with actin in the chicken B cell. We found that some of these proteins are phosphorylated, and that in some instances, the level of their phosphorylation can be modulated by cross linking mIg.

When B cells were biosynthetically labeled with ^{32}P orthophosphate and the mIg subsequently cross linked, the ^{32}P radioactivity associated with actin-binding proteins increased 2-4 fold that of the radioactivity associated with protein derived from non-crosslinked cells. In SDS gels, much of this radioactivity could be accounted for by a very heavy band barely entering the (7.5%) gels, whose phosphorylation was clearly dependent upon the cross linking of mIg.

In most cases, protein radioactivity was sensitive to alkali, probably indicating serine-linked phosphorylation. However, we detected alkali-resistant radioactivity associated with two proteins of 130K and 38K mol. wt., possibly indicating tyrosine-associated phosphorylation. For both proteins, it appears that the level of their phosphorylation may also be dependent upon the cross linking of mIg.

0742 REGULATION OF ERYTHROID PROGENITOR PROLIFERATION BY INSULIN-LIKE GROWTH FACTOR-II (IGF-II) AND PLATELET DERIVED GROWTH FACTOR (PDGF), Nicholas Dainiak and Sandra Kreczko, St. Elizabeth's Hospital and Tufts University, Boston, MA 02135

Human erythroid progenitor cells can be induced to proliferate in vitro by erythropoietin and other apparently erythroid specific growth factors termed burst-promoting activities. PDGF (Dainiak et al, JCI 71:1206, 1983) and a variety of classic hormones, including insulin may also influence erythroid colony formation (ECF). Since mitogenic activity of insulin is attributable to crossreactivity with receptors for structurally related peptides (IGFs), we tested purified rat IGF-II for erythropoietic activity in human marrow cultures that contained erythropoietin plus charcoal-adsorbed platelet poor plasma-derived serum (PDS) or adsorbed whole blood serum (WBS). IGF-II stimulated ECF at nanogram concentrations in cultures prepared with either PDS or WBS. While ECF was reduced by nearly one half in PDS containing cultures, dose response curves for IGF-II were similar to those obtained in cultures prepared with WBS. The addition of purified PDGF at concentrations capable of stimulating 50% and 100% maximal growth to PDS-containing cultures did not alter responses to IGF-II. Colony stimulation was similar at all serum concentrations (9, 18 and 27%), and it was unaffected by the addition of an optimal concentration of burst-promoting activity. Stimulatory activity was not abrogated by increasing the erythropoietin concentration in culture and IGF-II did not stimulate ^{59}Fe incorporation in the hypertransfused, polycythemic mouse bioassay for erythropoietin. We conclude that IGF-II stimulates ECF in vitro, an effect that is independent of PDGF concentration and that is distinct from that of erythroid-specific growth factors.

0743 Dihydrocytochalasin B inhibits N-formyl chemotactic peptide receptor modulation, endocytosis and cytoskeletal association in human granulocytes. Algirdas J. Jesaitis, James O. Tolle, Larry Sklar, Charles G. Cochrane, and Richard G. Painter. Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

When N-formyl chemotactic peptides bind to granulocyte receptors at 37°C they rapidly form a high affinity complex whose coisolation with cytoskeletal residues of Triton X-100 extracted cells is under cellular control. (Jesaitis et al, J. Cell. Biol., in press). When granulocytes were preincubated with dihydrocytochalasin B (dhCB) for 10 min at 37°C and then stimulated with 50 mN N-formyl-Met-Leu-[^{3}H] Phe, receptor conversion to a high affinity form, cytoskeletal colocalization, and uptake of the radioligand were all inhibited. The inhibition was 50% effective at 0.25 μg dhCB/ml. Maximal inhibition (80-90%) occurred at doses of dhCB>1 μg/ml. These results support the hypothesis that coisolation of the high affinity receptor-peptide complexes with granulocyte cytoskeletons occurs because of specific association of the complexes with the cytoskeleton at the cell surface. In addition, the results suggest the necessary participation of cytoskeletal-mediated receptor modulation in the process of receptor-ligand endocytosis. Preliminary experiments were also performed to evalute other functional consequences of cytoskeletal disruption on chemotactic peptide-stimulated functions. Chemotaxis as measured by under agarose migration of granulocytes toward F-Met-Leu-Phe demonstrated a 84% inhibition by cytochalasin B, whereas, F-Met-Leu-Phe stimulation of O_2^- production was potentiated due to a prolongation of the response with the same dose dependency as the inhibition of receptor modulation.

0744 RECOGNITION OF AN LDL-LIKE PARTICLE ISOLATED FROM HUMAN ATHEROSCLEROTIC PLAQUES BY BINDING SITES ON MOUSE PERITONEAL MACROPHAGES, Henry F. Hoff, Beverly A. Clevidence, and Richard E. Morton, Cleveland Clinic, Cleveland, Ohio 44106.

A lipoprotein demonstrating many of the characteristics of plasma low density lipoproteins (P-LDL) was isolated from homogenates of human atherosclerotic lesions by affinity chromatography followed by gel filtration. This particle termed A-LDL, differed from P-LDL in that the surface charge of A-LP was more electronegative, and the apoprotein of A-LDL (apoB) showed numerous bands on SDS-PAGE with molecular weights less than the B-100 band in P-LDL. In addition most of these bands showed apoB immunoreactivity by immunoblotting. We wished to determine whether A-LDL, in contrast to P-LDL, would be recognized by a high affinity binding site on mouse peritoneal macrophages (MPM). Incubation of MPM with A-LDL resulted in stimulation of cholesterol esterification, showing dose-dependent saturation kinetics (half-maxima around 50 μg/ml LDL cholesterol). This stimulation resulted in a 50 fold increase in intracellular cholesteryl ester mass when MPM was incubated with A-LDL at 100 μg/ml cholesterol for 48 hrs. Since acetylation of P-LDL was shown to render the P-LDL more electronegative and to be recognized by high affinity binding sites on MPM, we tested whether excess non-labeled A-LDL could compete with ^{125}I-acetyl LDL for binding sites on MPM. However, A-LDL at 20 fold excess concentrations did not compete with binding of ^{125}I-acetyl LDL. Cumulatively, these results show that an in vivo-modified LDL is present in the human aorta which is recognized by a binding site separate from one recognizing acetyl LDL. This modification, possibly the result of apoB degradation, could explain why some macrophages in the arterial intima become foam cells, resulting in the formation of the fatty streak lesion.

0745 A MONOCYTE CHEMOTACTIC FACTOR FROM ATHEROSCLEROTIC LESION-PRONE AREAS OF SWINE AORTA, Ross G. Gerrity, Jennifer A. Goss, and Lynn Soby, Research Division, Cleveland Clinic Foundation, Cleveland, OH 44106 USA.

Aortic areas predisposed to early atherogenesis in swine, demarcated by Evans blue uptake (blue areas) show preferential intimal penetration by blood monocytes prior to, and during lesion formation, compared to adjacent non-blue (white) areas, which are not lesion-prone. To examine mechanisms controlling monocyte migration into these areas, extracts of aortic tissue from blue and white areas of normal (N) and hypercholesterolemic (H) swine (prior to lesions) were tested for chemotactic activity against monocytes from N- and H-swine. Extracts of H-swine blue areas elicited a chemotactic response. Those from H-swine white areas, or N-swine blue and white areas did not. Moreover, only monocytes from H-swine responded chemotactically, whereas N-swine monocytes responded chemokinetically to H-swine blue area extracts as determined by chequerboard assay. H-swine blue extracts were fractionated by gel filtration, and two peaks of activity eluted with molecular weights of ≈68,000 and ≈5,000. The higher molecular weight fraction was further purified using Blue Sepharose chromatography. Activity was associated with the bound fraction, and shown to be heat and trypsin sensitive. It is feasible that the large fraction may be albumin to which the smaller active molecule is bound. The presence of a monocyte chemotactic factor(s) in the aortic wall at sites predisposed to atherogenesis may provide a mechanism controlling preferential monocyte migration into these areas prior to lesion formation. The results also suggest a specific alteration of monocyte function in the hyperlipemic state to enable response to the chemotactic factor.

0746 B CELL ACTIVATION: PROVISION OF SECOND SIGNALS FOR G_0 TO G_1 TRANSITION BY ANTI-Ia ANTIBODIES. John C. Cambier, Kenneth M. Coggeshall, Lesley K. Harris and Olivera J. Finn, National Jewish Hospital, Denver, CO 80206 and Duke University Durham, NC 27710.
It is well established that generation of humoral immune responses to most protein antigens requires interaction of B cells with antigen, antigen specific Ia restricted T cell help, and nonspecific macrophage and T cell derived factors. Interaction of quiescent B cells with antigen and restricted T cell help promotes entry of these cells into cell cycle, i.e. G_0 to G_1 transition. Upon subsequent interaction of G_1 cells with nonspecific T cell derived factors, cells proliferate and differentiate into antibody forming cells. Palacios et al. (PNAS 80:3456) have recently reported that certain anti-Ia antibodies promote normally thymus dependent anti-SRBC antibody responses in the absence of T cells. These findings suggest that anti-Ia antibody mimic the T cell signal in its action on B cells. Here we report further studies of the ability of anti-Ia antibodies to "replace" T cells in in vitro B cell immune responses. Results demonstrate that certain monoclonal anti-DR/DC antibodies which crossreact with mouse I-A/E determinants promote anti-SRBC responses in vitro. Optimal promotion of B cell responses by anti-Ia requires presence of antigen, as well as supernatants containing IL1, IL2, BCGF and BCDF. The anti-Ia antibodies partially inhibit anti-SRBC responses generated in the presence of SRBC primed and irradiated helper T cell populations. Finally, these antibodies synergize with receptor immunoglobulin crosslinking ligands in stimulating quiescent (G_0) B cells G_0 enter G_1. Results indicate that membrane associated Ia molecules may play an important signal transducing role in the antigen and T cell dependent promotion of G0 to G1 by B lymphocytes transition during humoral immune responses.

0747 EMBRYONIC GLYCANS INVOLVED IN CELLULAR ADHESIONS, Raymond J. Ivatt, Department Tumor Biology, M.D. Anderson Hospital, Texas Medical Center, Houston, TX 77030.

Embryonal carcinomas and early embryonic cells express an unusual class of carbohydrates on their cell surfaces. These carbohydrates are lost in a programmed way during early embryogenesis and have a very restricted distribution in the adult. Their disappearance during development coincides with the major period of histogenesis. In the adult, they are associated with cells of the reticuloendothelial system. Therefore in both the embryo and the adult, this unusual class of carbohydrate is associated with cellular interactions which are transient in nature. There are several lines of evidence which implicate these carbohydrates in cellular recognition in the early embryo. We have explored this role using the embryonal carcinoma system as a model. Embryonal carcinoma cells in culture demonstrate the ability to adsorb and recognize exogenous carbohydrates. We have investigated this property by characterizing the radiolabeled carbohydrates, prepared from embryonal carcinoma cells, which were adsorbed by other cells. On the basis of these studies, we have selected embryonal carcinoma variants with altered levels of expression of these carbohydrates. The variant embryonal carcinoma cells which were selected because of a decreased level of expression reaggregate more slowly than the parental population from single cell dispersion and the aggregates which are formed are easily disrupted mechanically. Other variants which were isolated on the basis of their very high level of expression of these carbohydrates reaggregate rapidly and have unusually tight cellular interactions. These variants show varying degrees of stability. The low expressors are less stable than the high expressors. We are currently exploring the cellular interactions which regulate the expression of these carbohydrates, and, in complementary studies, we are selecting embryonal carcinoma variants which are unable to bind these carbohydrates.

0748 INTERFERON RECEPTOR ON HUMAN CELLS, Tom Evans and D.S. Secher, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England

^{125}I-labelled pure human IFN-A binds to high affinity specific sites on human lymphoblastoid cells. This binding progressively diminishes in the presence of cycloheximide, with a half-life of ~4 hours. In the continuous presence of saturating amounts of labelled interferon the total cell-associated radioactivity peaks after ½ hour and then declines to approximately 20% of this value after 4 hours; this decay is inhibited by Monensin.

A fraction of the cell-associated radioactivity is trypsin-resistant. This internalised pool reaches a maximum after 1 hour and then declines with similar kinetics to the total cell-associated counts. These results show the interferon receptor is turning over rapidly on the cell and mediates endocytosis of surface bound interferon. However, as is the case with EGF, there appears to be no recycling of the receptor.

0749 PROTEIN PHOSPHORYLATION IN THE ACTIVATION OF RESTING B CELLS THROUGH SURFACE Ig. Ian M. Zitron, Department of Biology, University of Pennsylvania, Philadelphia,PA19104

Surface immunoglobulin (sIg) molecules are the antigen-specific receptors on B lymphocytes. Two isotypes, sIgM and sIgD, are present on resting, primary B cells. In a number of systems these molecules have been shown to deliver intracellular signals to the cell. Amongst the effects observed are activation from G_0 to G_1, which initiates events culminating in DNA synthesis and cell division. The biochemical mechanism(s) by which sIg-mediated signals are delivered are unknown.

Experiments have been undertaken to ask whether sIgD-mediated signalling occurs via protein phosphorylation, particularly on tyrosine residues, in a similar way reported for growth factor and hormone receptors on other cell types. Resting B cells have been loaded with ^{32}P and stimulated via sIgD using a monoclonal anti-δ antibody, which has been previously shown to induce DNA synthesis in these cells. After brief periods of exposure to antibody, cells have been lysed, nuclei pelleted and the extract analyzed by both SDS-PAGE and 2D gel electrophoresis. Under the conditions used, there is clear enhancement of phosphorylation of species in the range 35-38kd. The enhanced labelling is alkali-resistant, suggesting that it may represent phosphotyrosine. 2D gel analysis shows that extracts from sIgD-stimulated cells contain molecules of the same molecular weight range, showing a heterogeneous charge distribution. Most, but not all, of these are absent from extracts of cells which had been exposed to a control monoclonal antibody which does not bind to sIg.

0750 EVIDENCE FOR NEGATIVE COOPERATIVE INTERACTIONS OF FORMYL PEPTIDE CHEMOTAXIS RECEPTORS ON THE RAT NEUTROPHIL. Wayne A. Marasco, Douglas E. Feltner and Peter A. Ward, Dept. of Pathology, University of Michigan Medical School, Ann Arbor, 48109.

Steady-state binding of f Met-Leu-(^{3}H)Phe to intact rat neutrophils and purified plasma membranes was measured at 4°C using a silicone oil centrifugation assay. The equilibrium binding data were subjected to statistical analysis and to computer curve fitting using the NONLIN computer program. Curvilinear, concave upward Scatchard plots were obtained. NONLIN and statistical analysis of the binding data indicated that both the 2 saturable site model and the negative cooperative model were preferable to a 1 saturable site model and statistically valid by the F-test ($p < .01$). The characteristics of the 2 site model for intact cells were: a high affinity site with a K_D value of 0.687 ± .208 x 10^{-9}M and a low affinity site with a K_D value of 54.78 ± 9.81 x 10^{-9}M. 8% of the two sites detected were of the higher affinity (599 ± 98 sites/cell) and 92% were of the lower affinity (7,030 ± 836 sites/cell). The negative cooperative model was characterized by a K_D (at (1/2 saturation) raised to the Hill coefficient) of 10.74 ± 0.33 nM, a Hill coefficient of 0.77 ± 0.05 and 5,350 ± 372 binding sites per cell. Site-site interactions of the destabilizing type (negative cooperativity) were demonstrated by investigating the kinetics of dissociation. The rate of dissociation of f Met-Leu-(^{3}H)Phe from intact cells was dependent on the fold excess of unlabelled f Met-Leu-Phe used in the dilution medium and at the highest concentration tested (10,000 fold excess), the dissociation rate was more than doubled the dissociation rate seen with dilution alone. Ligand induced-negative cooperative interactions of formyl peptide receptors may serve as a regulatory control in modulating several receptor-mediated biological responses.

0751 EARLY EVENTS IN SURFACE IMMUNOGLOBULIN MEDIATED STIMULATION OF B CELLS, Tore Godal and Reino Heikkilä, The Norwegian Radium Hospital, Montebello, Oslo 3, Norway

Previous studies showed early increased influx of the K^+ analogue $^{86}Rb^+$ to be associated with subsequent proliferation in $F(ab)_2$ anti-Ig stimulated monoclonal B cells (human lymphoma cells) in vitro (Heikkilä et al. Expti Cell Res 136:447-54, 1981). Further experiments have been performed to determine the nature and requirements of this anti-Ig (including monoclonal antibodies) induced $^{86}Rb^+$ response. The response was susceptible to the calmodulin antagonist trifluoperazine, but independent of extracellular Ca^{++}. Studies with the fluorescent Ca^{++}-chelator quin 2 confirmed that anti-Ig increased the intracellular free (cytosol) Ca^{++} concentration $(Ca^{++})_i$, even in the absence of extracellular free Ca^{++}, although the change was then weaker and of shorter duration. Increases in $^{86}Rb^+$ flux was always associated with an increase in cytosol Ca^{++} concentration, but many lymphomas and leukemias responded to anti-Ig by increases in cytosol Ca^{++}, without an increase in $^{86}Rb^+$ flux. It is concluded that increases in cytosol Ca^{++} is a required, but not sufficient, condition for the $^{86}Rb^+$ response.

0752 THROMBIN RECEPTOR CHARACTERIZATION BY MONOCLONAL ANTIBODIES, Gloria J. Herbosa, William C. Thompson and Darrell H. Carney, The University of Texas Medical Branch, Galveston, TX 77550

Thrombin interaction with its receptors appears to be necessary for thrombin to initiate cell proliferation. Early photoaffinity labeling studies demonstrated ^{125}I-thrombin binding to a Mr=50,000 molecule on the surface of mouse embryo cells (J. Biol. Chem. 254: 6244, 1979). More recent analysis with a proteolytically inhibited ^{125}I-thrombin photoaffinity label indicated a receptor with Mr=150,000 on hamster lung cells (J. Biol. Chem. 258: 3996, 1983) suggesting that the 50,000 Mr component could be a proteolytic fragment of the receptor or an adjacent molecule. To characterize and identify the thrombin receptor, we immunized Balb C mice with whole human fibroblasts, fused the immunized spleen cells with P_3 myeloma cells and selected 9 hybridoma clones for inhibition of ^{125}I-thrombin binding and immunofluorescent staining of human and mouse embryo cell surfaces. One clone in particular (TR9) appeared to have a staining pattern which closely resembled that of thrombin on the surface of mouse embryo cells. This antibody selectively binds octylglucoside solubilized ^{125}I-thrombin receptor complexes, but does not bind soluble ^{125}I-thrombin or ^{125}I-thrombin complexed to protease-nexin. TR9 affinity chromatography of solubilized ^{125}I-labeled cell surface proteins revealed 3 peaks with approximate Mr=150,000, 94,000 and 40,000. Immunoblotting analysis of total ME cell proteins electrophoresed on SDS gels, however, revealed that TR9 binds to a single peak of Mr=~150,000. Additional studies are underway to determine whether this component represents the mitogenic thrombin receptor and whether antibody binding alone can initiate cell proliferation. (Supported by NIH Grants AM-25807 and CA-00807).

0753 MUSCARINIC CHOLINERGIC RECEPTORS ON HUMAN NEUTROPHILS (PMNs) DURING THEIR DEVELOPMENT AND FUNCTION Daniel G. Wright, Anda I. Meierovics and Diane L. Lucas, Dept. of Hematology, Walter Reed Army Institute of Research, Washington, D.C. 20307

Cholinergic agents have been shown to enhance functional responses of PMNs (chemotaxis and secretion) and to interact with PMNs via specific receptors analogous to those on neuromuscular and secretory cells. To evaluate cholinergic receptor availability on human PMNs during their maturation and function, PMNs and PMN precursors were isolated from aspirated marrow and venous blood of volunteers and from exudates produced in skin chambers. Cells were incubated with the muscarinic antagonist, quinuclidinyl-benzilate (^3H-QNB), at 22°C for 20 min with and without atropine. QNB bound to cells collected on glass fiber filters was then measured. Specific, atropine-displaceable ^3H-QNB binding (SQB) by blood PMNs was saturable with $0.8\text{-}1.5 \times 10^5$ binding sites per cell and a Kd for binding of 8-14 nM. Neutrophilic cells isolated from marrow by density gradient/sedimentation (enriched with bands and metamyelocytes) or by counter-flow elutriation (enriched in blasts, promyelocytes and myelocytes) had 2 to 4 times more SQB sites/cell than did blood PMNs. The human promyelocytic cell line, HL-60, also had greater receptor numbers ($>10^6$/cell). In contrast, SQB by exudate PMNs and by blood PMNs exposed to the chemotactic peptide, f-met-leu-phe (10^{-7} M), was 30 - 50% less than that of control PMNs. These studies indicate that muscarinic cholinergic receptors are acquired early in the development of these cells, are most numberous on PMN precursors in the marrow, and are lost or become less available for binding once PMNs are engaged in an inflammatory response. These findings suggest that a cholinergic response apparatus may be particularly important to PMNs during their development and storage in the marrow, a richly innervated organ.

0754 Transferrin Receptor Expression by Murine Peritoneal Macrophages Is Modulated by Gamma Interferon. Thomas A. Hamilton, James E. Weiel and Dolph O. Adams, Dept. of Pathology, Duke University Medical Center, Durham, N.C. 27710

The expression of the transferrin receptor on murine peritoneal macrophages varies depending on their stage of functional activation. Both monocytes and macrophages from sites of sterile inflammation possess 15-20 x 10^3 binding sites/cell whereas macrophages from sites of immunologically mediated inflammation have 10-20 fold fewer sites/cell. The down regulation of receptor expression which accompanies activation can be reproduced in vitro following a minimum 2 hour exposure of inflammatory macrophages to gamma-interferon (gamma-IFN) produced by a T-cell hybridoma or by monkey COS-7 cells transfected with a plasmid containing the murine gamma-IFN gene under control of the SV40 promoter. We have used this change as an experimental model to study the molecular and cellular features of gamma-IFN action in macrophage activation. The downshift of transferrin binding activity can be mimicked by exposure to the calcium ionophore A23187 but the gamma-IFN induced downshift is not inhibited by Verapamil, a blocker of membrane Ca^{++} channels. This indicates that gamma-IFN may induce intracellular Ca^{++} mobilization. Tumor promoting phorbol-diesters, which are known to elevate cellular Ca^{++} from intracellular stores and which bind to and activate protein kinase C could also down regulate transferrin receptor expression. These results, taken together, suggest that gamma-IFN may function at least in part by stimulating the phosphatidyl inositol cycle culminating in the activation of protein kinase C.

0755 EXPOSURE OF FIBRINOGEN RECEPTORS ON PLATELET SURFACE BY ADP AND PROTEOLYTIC ENZYMES, Stefan Niewiarowski, Elizabeth Kornecki, George P. Tuszynski and Diane Hershock, Temple University Health Sciences Center, Philadelphia, PA 19140

Fibrinogen (fg) plays a major role in platelet aggregation. Resting platelets do not interact with fg. Platelets stimulated by ADP or proteolytic enzymes (chymotrypsin or pronase) expose similar number of low (N ~ 80,000; Kd ~ 10^{-6} M) and high (N ~ 2,000; Kd ~ 10^{-8} M) affinity fg receptors. The velocity of fg induced platelet aggregation depends on the occupancy of these receptors. The fg receptors are associated with surface glycoproteins IIb/IIIa complex (GPIIb/GPIIIa) which is deficient in Glanzmann's thrombasthenia. Exposure of fg receptors on enzyme-treated platelets correlated with the appearance on platelet surface of a 66 K Mr component derived from GPIIIa. A number of polyclonal and monoclonal antibodies interacting with GPIIb/GPIIIa complex blocked ^{125}I-fg binding to ADP-stimulated and to enzyme-treated platelets and inhibited fg induced aggregation of these platelets. However, proteolytic enzymes, but not ADP, exposed high affinity fg receptors on thrombasthenic platelets with a partial deficiency of GPIIb/GPIIIa. Fg induced aggregation of thrombasthenic platelets, mediated by these receptors, was blocked by anti-GPIIb/GPIIIa antibodies. It can be suggested that ADP and proteolytic enzymes, acting by an entirely different mechanism, expose fibrinogen receptors associated with the same domaine of GPIIIa molecule.

0756 COMPARISON OF T CELL STIMULATION BY CELL- OR LIPOSOME-ASSOCIATED CLASS I ALLO-ANTIGENS, Anne-Marie Schmitt-Verhulst, Francoise Albert, Claude Boyer, Christine Hua, and Claire Langlet, Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille cedex 09, FRANCE.

Differences were observed between T cells stimulated with the cell-associated or with the liposome-associated H-2K^b molecule. H-2K^b-specific alloreactive T cell lines and clones maintained in long term cultures with cell-associated H-2K^b all expressed Lyt-2 and were L3T4 negative. They were dependent on the expression of H-2K^b, but not of Ia on the stimulating cells, in addition to exogenous interleukins (Il-1 or Il-2 dependent on a given T cell line) for their stimulation to growth. Their H-2K^b induced proliferation was inhibited by anti-H-2K^b or by anti-Lyt-2 monoclonal antibodies (mAb), but not by anti-Ia or anti-L3T4 mAb. For most T cell clones tested the integrity of both the first and the second N-terminal domains of the H-2K^b seemed required for "recognition" by T cells. T-ese T cell clones could not be stimulated with immunopurified H-2K^b inserted in liposomes, even in the presence of exogenous interleukins. T cell lines initiated in vitro with H-2K^b-liposomescould proliferate in response to H-2K^b-liposomes and feeder cells. Such T cell lines contained T cells bearing the Lyt-2 marker as well as cells bearing the L3T4 marker and their H-2K^b-induced proliferation was inhibited by anti-H-2K^b or by anti-Ia, as well as by anti-Lyt-2 or anti-L3T4 mAb. Characterization of these cells at the clonal level and analyses of the H-2K^b epitopes involved in recognition by these T cells are under investigation.

0757 HUMAN T CELL HYBRIDS EXPRESSING NATURALLY OCCURRING HLA-DR RESTRICTED RECEPTORS THAT RECOGNIZE EBV-INFECTED B CELLS. Mary A. Valentine, Constantine D. Tsoukas, John H. Vaughan, and Dennis A. Carson. Scripps Clinic and Research Foundation, La Jolla, California 92037.

Receptor interactions of T cells that react with Epstein-Barr Virus (EBV) infected B cells are not well defined. Several types of recognition have been described in cells during the course of EBV infection, but clonal examination of naturally occurring receptors showing the actual requirements for HLA restriction has not been reported. Therefore, we utilized somatic cell hybridization to create cells whose surface molecules would pertain directly to those expressed *in vivo* during various phases of EBV infection. The parent lymphoma was a double mutant of the human cell line, JM, a cell which can release IL-2 on mitogenic stimulation. Patient cells used for direct fusion with JM were acquired from Viral Capsin Antigen (VCA) negative donors, or from a patient during the acute and convalescent phases of mononucleosis. Fusion products were then characterized as hybrids by phenotypic and karyotypic analysis, as well as by growth in selective medium and the acquisition of functional receptors. Receptor activity was monitored by production of IL-2 following co-culture with irradiated autologous or allogeneic B cells which were Epstein-Barr Nuclear Antigen (EBNA) positive or negative. The derived hybrid clones recognized specifically autologous EBV-infected B lymphoblasts, or allogeneic EBNA positive cells sharing HLA-DR antigens. Stimulator cells with no shared HLA-DR or evidence of EBV infection could never induce IL-2 release. These recognition requirements clearly define a population of T cells that arise *in vivo* during acute phase EBV infection that interact with EBV transformed cells in a HLA-DR restricted manner.

0758 SUGGESTIVE EVIDENCES FOR THE PRESENCE OF FIBRONECTIN RECEPTORS ON THE SURFACE OF MACROPHAGES. Janos Molnar, Department of Biological Chemistry, College of Medicine, University of Illinois, Chicago, IL. 60612

Plasma fibronectin (pFN) promotes phagocytosis of gelatin coated particles by macrophages (MΦ). In the present studies ^{125}I-gelatin-coated latex beads (gLtx*) were used. Incubation of MΦ with high doses of pFN prior to exposure to gLtx* did not cause decreased phagocytosis unless pFN was added simultaneously with the particles. When gLtx*, preopsonized with a saturating amount of pFn was incubated with MΦ, addition of free pFN did not cause inhibition of phagocytosis suggesting that particulate-bound pFN was preferentially recognized by the MΦ receptors. Free gelatin interacted with pFN and caused strong inhibition of pFN mediated uptake of gLtx* by MΦ. Free gelatin, however, did not inhibit phagocytosis of gLtx* when the particles were previously opsonized with pFN. Soluble complexes of pFN-gelatin did not cause any inhibition of phagocytosis of preopsonized particles (pFN - gLtx*). These observations suggest that the MΦ receptors react preferentially with particulate bound pFN-gelatin and have weaker interactions, if any, with soluble pFN-gelatin complexes. The pFN-mediated phagocytosis of MΦ can be diminished by brief exposure to pronase. Trypsin, chymotrypsin, plasmin and thermolysin treatment of MΦ did not have any effect on pFN-gLtx* uptake. MΦ - treated with pronase recovered their ability to phagocytize pFN·gLtx* after 2-3 hr of incubation in fresh culture media. Cycloheximide prevented this recovery. Chloroquine protected MΦ against the pronase effect. The data are consistent with the theory that MΦ contain receptors which recongnize pFN only when it coats partculate-bound gelatin. The receptor is a protein and it is a recycling type and protected against proteolysis by pronase only when it is present internally.

Tyrosine Kinases and Phosphatases

0759 CELL MEMBRANE-ASSOCIATED p36 TYROSINE PROTEIN KINASE SUBSTRATE IS REORGANIZED AFTER DRUG-INDUCED REDISTRIBUTION OF CYTOKERATIN. Jorma Keski-Oja, Department of Virology, University of Helsinki, SF-00290 Helsinki, Finland.

Epithelial PtK_2 cells were used as a model to study the organization of the major tyrosine protein kinase substrate, p36 as a consequence of the redistribution of keratin filaments. The distribution of cytokeratin type intermediate filaments of these cells was altered by treatment of the cells with colchicine and cytochalasin D (Krapp et al., Science 219, 501, 1983). PtK_2 cells grown on glass cover slips were treated with colchicine, cytochalasin D or a combination of these two. After the incubation the cells were fixed with methanol and double-stained with rabbit antibodies against p36 (Courtneidge et al. Molec. Cell Biol. 3, 340, 1983) and mouse monoclonal antibodies against keratin.
In control PtK_2-cells p36 was distributed characteristically at the cell membrane and in the cytoplasm. Neither colchicine nor cytochalasin D alone were able to cause major reorganization of cytokeratin or p36. Their combined effect resulted in vigorous blebbing of the cell surface. The membrane-associated p36 antigen, together with keratin, was reorganized and heavily concentrated at several star-like formations at the dorsal cell surface and at the cell-cell junctions (cytokeratin organization centers). These observations, for the first time, show a drug-induced redistribution of p36. These results suggest a relationship between membrane-associated p36 and the cytokeratin intermediate filaments.

0760 MULTIPLE FORMS OF THE RSV src PROTEIN FOUND IN TRANSFORMED CELL LYSATES, Marc S. Collett and Susan K. Wells, Department of Microbiology, University of Minnesota Medical School, Minneapolis, MN 55455

The protein $pp60^{v\text{-}src}$ is responsible for the oncogenic capabilities of Rous sarcoma virus (RSV). $pp60^{v\text{-}src}$ possesses at least one enzymatic activity, that of a tyrosine-specific protein kinase. $pp60^{v\text{-}src}$ itself contains two major phosphorylated residues: a phosphoserine located near the NH_2-terminus of the protein (ser-17) and a phosphotyrosine residue located in the COOH region of the protein (tyr-416). The potential regulation of $pp60^{v\text{-}src}$ function by phosphorylation-dephosphorylation modifications of the $pp60^{v\text{-}src}$ polypeptide has been our concern. We recently reported that extensive phosphorylation in vitro (autophosphorylation) of purified $pp60^{v\text{-}src}$ resulted in the generation of an electrophoretic variant of $pp60^{v\text{-}src}$ possessing previously unrecognized amino-terminal phosphotyrosine (Virology 128, 285[1983]), and that this could be correlated with a significant increase in the specific activity of the src protein kinase (Mol Cell Biol 3, 1589[1983]). We report here the identification of a structurally analogous form of $pp60^{v\text{-}src}$ in RSV-transformed cell lysates. Our ability to observe this transient form of $pp60^{v\text{-}src}$ required the appropriate manipulation of the cell cultures and cell lysis conditions prior to immunologic detection. The use of phosphotyrosyl-protein phosphatase inhibitors (vanadium ions) and/or protein kinase activators (Mg^{+2}, ATP) was required. After purification of the modified $pp60^{v\text{-}src}$ protein from these transformed cell lysates, evaluation of its enzymatic activity confirmed the correlation between extensive amino-terminal tyrosine phosphorylation and increased $pp60^{v\text{-}src}$ protein kinase activity.

0761 SITE-DIRECTED MUTAGENESIS OF THE SRC GENE OF ROUS SARCOMA VIRUS. Victoria W. Wilkerson, Debra L. Bryant, and J.T. Parsons, Department of Microbiology, University of Virginia, Charlottesville, VA 22908

The transformation of cells by Rous sarcoma virus (RSV) is mediated by the product of the RSV src gene, $pp60^{src}$. To define structural and functional domains of $pp60^{src}$, we have used site-directed mutagenesis techniques to introduce defined deletions and point mutations into the src gene of Prague A RSV DNA. The effects of these mutations on the functional expression of $pp60^{src}$ have been investigated using DNA transfection of chicken cells. Deletion of sequences encoding amino acid residues 82-169 (tdCH121) yielded transformation-defective virus whose src gene product retained kinase activity. Deletion of sequences encoding amino acids 169-225 yielded virus temperature-sensitive for morphological transformation. These data indicate that $pp60^{src}$ contains an amino-terminal functional domain which is essential for src-induced alteration of cellular morphology. A second domain has been demonstrated by the use of bisulfite mutagenesis to introduce single base mutations within a sequence encoding the highly conserved amino acid sequence ala_{439}-ala_{443}. Mutations resulting in either an ala_{439}-val, pro_{441}-ser, lys_{442}-glu, or ala_{443}-thr yield transformation-defective virus that encodes a functional inactive src protein. We suggest that this conserved sequence is part of a functional domain essential for tyrosine kinase activity. Additional mutations which alter structural and functional properties of $pp60^{src}$ will be discussed.

0762 PHOSPHORYLATION ON TYROSINE OF A 42,000 M_r PROTEIN IN RESPONSE TO MITOGENS, Ricardo Martinez, Richard Bishop, Kenji D. Nakamura, and Michael J. Weber, Department of Microbiology, University of Illinois, Urbana, IL 61801.

We have identified proteins which become phosphorylated on tyrosine following treatment of cells with mitogens, by electrophoresing total extracts of ^{32}P-labeled cell cultures on SDS-polyacrylamide gels and analyzing the phosphoamino acid content of the separated proteins. Exposure of chicken embryo fibroblasts or 3T3 cells to a variety of mitogens, including serum, EGF, PDGF, MSA, or TPA, resulted in the phosphorylation on tyrosine of a protein peak of 42,000 M_r. This phosphorylation was the only tyrosine-specific phosphorylation detected. The phosphorylation of the 42,000 M_r protein peak was quite rapid, reaching a maximum within 5 minutes, and then decaying with a half-time of 60 - 180 minutes. NR6/3T3 cells, which lack detectable EGF receptors, did not display phosphorylation of the 42,000 M_r peak when treated with EGF, indicating the necessity of specific receptors in order for this phosphorylation to occur. TNR9/3T3 cells, which do not respond mitogenically to TPA but which have TPA receptors, also did not respond to TPA with increased phosphorylation of the 42,000 M_r peak. These data suggest that phosphorylation on tyrosine of the 42,000 M_r protein peak may be an essential, early step in the integration of a variety of mitogenic signals.

0763 CHARACTERIZATION OF A SERUM/EGF-STIMULATED RIBOSOMAL PROTEIN S6-KINASE ACTIVITY IN SWISS 3T3 CELLS, Ilse Novak-Hofer and George Thomas, Friedrich Miescher-Institut, P.O. Box 2543, CH-4002 Basel, Switzerland

Stimulation of quiescent Mouse Swiss 3T3 cells with serum or growth factors leads to rapid phosphorylation of ribosomal protein S6. Soluble extracts from stimulated cells show up to 30-fold higher incorporation of ^{32}P into S6 than extracts from quiescent cells. It is shown that the increased S6 phosphorylation observed in extracts from stimulated cells is not due to the inactivation of a S6-phosphatase but to the activation of S6-kinase(s). Activation can be observed 2 min after serum-stimulation, reaches 50% by 10-15 min and is maximal by 60 min serum stimulation. Extracts prepared from cells stimulated with Epidermal Growth Factor (EGF) show partial activation of S6-kinase(s) (56% of serum). The dose-response curve for EGF-stimulation roughly parallels the one observed in intact cells. In intact cells the fully phosphorylated form of S6 contains multiple phosphorylation sites. The phosphorylation in vitro with crude S6-kinase(s) activity from extracts of serum-stimulated cells leads to the phosphorylation of most of the phosphopeptides labeled in vivo. Further development of this system is directed towards elucidating the sequence of events leading from receptor-binding to the activation of S6-kinase(s).

0764 Regulatory Features Affecting the Phosphorylation of Protein Tyrosine Residues. T.M. Martensen, NIH, NHLBI, Bethesda, MD 20205

Phosphotyrosine (Tyr-P) content of cellular phosphoprotein is governed by a dynamic equilibrium determined by activities of specific protein tyrosine kinases (PTK) and specific protein phosphotyrosine phosphatases (PTPase). Both of these activities were assayed in normal cells platelets, human skin) and cancerous cells (Ehrlich ascites [EAT]). PTPase activity was assayed and semipurified using an alternative substrate [^{32}P]phosphotyrosyl-glutamine synthetase (PTGS). PTK activity was assayed by measurement of [^{32}P]Tyr-P after base hydrolysis and isolation of Tyr-P from reaction mixtures. The predominant amount of PTPase activity in platelets, skin, and EAT cells is cytosolic and specific for phosphotyrosine residues residues of proteins. The primary sequence about the Tyr-P residue does not appear to be critical since the enzyme semi-purified using PTGS as the substrate readily dephosphorylates vinculin phosphorylated by pp60^{src}. The specific activity of the EAT cell PTPase in extracts increases with dilution in a manner suggesting the presence of endogenous inhibitors. Boiled cells contain heat stable nondialyzable material which inhibits the PTPase, and is unlike skeletal muscle phosphatase inhibitors. The heat stable material may modulate PTPase activity. The PTK in cells appears to be predominantly membranous and detergent soluble. Maximal expression of activity in extracts is pH and divalent cation dependent. These conditions can be correlated with the inhibition of PTPase activity. Casein and tubulin can be be used as exogenous substrates.

0765 **EGF-DEPENDENT PROTEIN PHOSPHORYLATION IN DIGITONIN-TREATED CELLS,** Terrence D. Giugni, Leslie C. James, and Harry T. Haigler, University of California, Irvine, CA 92717

Epidermal growth factor (EGF) stimulates a c-AMP-independent, tyrosine-specific protein kinase activity that has been extensively studied in isolated membranes. Although it is speculated that this tyrosine kinase activity is involved in the regulation of cell growth, its role in intact cells is not known. We have investigated this activity in cultured diploid human fibroblasts that have a well characterized mitogenic response to EGF. Small effects of EGF on the incorporation of phosphate into certain proteins could be detected by two-dimensional gel electrophoresis of proteins extracted from cultures labeled with ^{32}P-orthophosphate. These effects were difficult to demonstrate over the high background of radioactivity due to extended prelabeling in large amounts of ^{32}P-orthophosphate. To overcome this problem a method to permeabilize cells was developed that permits the rapid labeling of cellular proteins with exogenously added [gamma^{32}P]ATP. Cells were made permeable by incubation with digitonin (25 ug/ml) for 5 min at room temperature. This treatment caused approximately a 25% loss of cellular protein, marker enzymes from the cytosol, and EGF binding capacity. In digitonin-treated cells there was a dramatic effect of EGF on the the incorporation of radioactivity from [gamma^{32}P]ATP into certain cellular proteins.

0766 ENHANCEMENT OF THE CELLULAR SRC GENE PRODUCT TYROSYL KINASE ACTIVITY FOLLOWING POLYOMA VIRUS INFECTION AND TRANSFORMATION, Joseph B. Bolen,[1] Wes Yonemoto,[2] Joan S. Brugge,[2] and Mark A. Israel[1], [1]Pediatric Branch, NCI, NIH, Bethesda, MD 20205, [2]Department of of Microbiology, State University of New York at Stony Brook, Stony Brook, New York 11794
The polyoma virus (Py) middle tumor antigen (MT Ag) is thought to play a central role in Py-mediated oncogenesis and has an associated protein kinase activity which phosphorylates tyrosine on MT Ag in in vitro kinase assays. Current evidence suggests that MT Ag may not possess intrinsic protein kinase activity, and it has been reported that MT Ag is associated with the cellular src gene product, pp60$^{c\text{-}src}$ (c-src), a known tyrosyl kinase. Recently, we reported that monoclonal antibodies which recognize c-src immunoprecipitate both c-src and MT Ag from extracts of Py-infected or Py-transformed cells and that both of these proteins are phosphorylated on tyrosine residues in the presence of ATP and Mg^{2+}. We have now analyzed the effect of Py-infection and Py-transformation on c-src kinase activity. Our results demonstrate that infection of mouse cells with wild-type Py or Py mutants which are capable of transforming rodent cells stimulates c-src kinase activity 5-10 fold over that observed in uninfected cells or cells infected with transformation-defective Py mutants when evaluated by in vitro kinase assays measuring casein or [val^5] angiotensin II phosphorylation. Similar results were obtained with Py-transformed hamster, rat, and mouse cells. Stimulation of c-src kinase activity was not observed in SV40 transformed cells. While the mechanism by which Py infection or transformation stimulates c-src kinase activity is unknown, our current data suggests that the specific activity the kinase may be increased, since no detectable difference in the amount of pp60$^{c\text{-}src}$ synthesis or in vivo phosphorylation was observed.

0767 THE PHOSPHORYLATION OF RIBOSOMAL PROTEIN S6 IS INCREASED FOLLOWING MICROINJECTION OF pp60$^{v\text{-}src}$ INTO XENOPUS LAEVIS OOCYTES, Jordan G. Spivack, Raymond L. Erikson* and James L. Maller, University of Colorado School of Medicine, Denver, CO 80262 and *Harvard University, Cambridge, MA 02138.

Rous sarcoma virus (RSV) is able to transform cells through the expression of a single gene product, termed pp60$^{v\text{-}src}$, which is a protein kinase that phosphorylates tyrosine residues. One clue to the interaction between pp60$^{v\text{-}src}$ and the host cell is that ribosomal protein S6 is phosphorylated in RSV-transformed cells even in the absence of serum (Decker, S., PNAS 78: 4112, 1981). Normally, S6 is not phosphorylated in serum-starved cells in culture, but is rapidly phosphorylated in response to many stimuli that promote protein synthesis and cell division. We have microinjected purified pp60$^{v\text{-}src}$ into Xenopus oocytes and observed an increase in S6 phosphorylation on serine residues. Two-dimensional gel electrophoresis of ribosomal proteins confirmed an increase in the absolute phosphate content of S6. Since the pp60$^{v\text{-}src}$ preparation lacked S6 kinase activity in vitro, we propose that pp60$^{v\text{-}src}$ directly or indirectly activates a kinase and/or inactivates a phosphatase for S6. Microinjection of pp60$^{v\text{-}src}$ into oocytes also led to a 2-3 fold increase in the level of phosphotyrosine and a slight elevation in total protein phosphorylation. Additionally, pp60$^{v\text{-}src}$ dramatically shortened the time course of progesterone-induced maturation of oocytes, but appeared unable to cause maturation by itself. These experiments suggest that microinjection of oocytes may prove useful in identifying the pathway(s) by which growth is regulated by pp60$^{v\text{-}src}$ and the role of tyrosine phosphorylation in early development.

0768 ISOPROTERENOL-INDUCED UNCOUPLING OF THE INSULIN RECEPTOR TYROSINE KINASE ACTIVITY Jeffrey E. Pessin, Wendy Miller, Yoshitomo Oka, and Michael P. Czech, University of Massachusetts Medical Center, Worcester, MA 01605

We have previously demonstrated that β-adrenergic receptor agonists, acting via elevated levels of cyclic AMP, inhibit insulin and EGF receptor binding activities in isolated rat adipocytes. The number of insulin receptors was found to be unchanged by several criteria including, (i) competition of binding analysis, (ii) removal of cell surface ligand by acid-extraction, (iii) specific binding of anti-insulin receptor antibodies, (iv) insulin receptor distribution after subcellular membrane fractionation. In vivo $H_3{}^{32}PO_4$ labeling indicated that isoproterenol has little or no effect on the amount of total phosphate incorporated into the β-subunit (M_r=90,000) of the insulin receptor. However, isoproterenol appears to increase the relative proportion of phosphoserine with a concomitment decrease in phosphotyrosine. Further, when adipocytes were incubated with isoproterenol subsequent to insulin addition no increase in the phosphorylation of the β-subunit was found. Under identical conditions insulin alone was able to enhance the phosphorylation of the insulin receptor approximately 2 fold. This uncoupling of the insulin-stimulated tyrosine kinase activity of the insulin receptor was also observed in vitro. The initial rate of γ-^{32}P ATP autophosphorylation of the β-subunit in the absence of insulin (basal conditions) was similar for the insulin receptors isolated from both control and isoproterenol-treated cells. However, the insulin-stimulated tyrosine kinase activity (at saturating insulin concentrations) was markedly reduced in the insulin receptors isolated from isoproterenol-treated cells compared to control cells.

0769 PHOSPHORYLATION OF THE INSULIN RECEPTOR BY CASEIN KINASE II, Gary M. Hathaway*, Jolinda A. Traugh,* Dennis T. Pang+, and Jules A. Shafer+, University of California*, Riverside, CA 92521 and The University of Michigan+, Ann Arbor, MI 48109.

Human, placental insulin receptor, purified by wheat germ agglutinin-agarose chromatography, was phosphorylated by the multipotential protein kinase, casein kinase II. Phosphate was incorporated into the 95,000 dalton β subunit which was identified by comigration with the insulin-stimulated autophosphorylated band. Unlike autophosphorylation, which was insulin and Mn^{2+}-dependent and resulted in modification of tyrosine, casein kinase II catalyzed phosphorylation of serine and threonine residues, required Mg^{2+}, and was insulin independent. This phosphorylation was inhibited by 5 μg/ml heparin, a diagnostic test for casein kinase II. The time course of the reaction revealed biphasic kinetics with a rapid, initial reaction followed by a second, slower phase. The biphasic kinetics taken together with the serine/threonine determinations suggest that a minimum of two types of sites were phosphorylated by casein kinase II. At least two times as much phosphate was incorporated into the insulin receptor by casein kinase II relative to that observed with the insulin-stimulated autophosphorylation.

0770 PHOSPHORYLATION OF THE SOLUBILIZED INSULIN RECEPTOR BY THE GENE PRODUCT OF THE ROUS SARCOMA VIRUS, $pp60^{src}$, Morris F. White, Diane K. Werth, Ira Pastan and C. Ronald Kahn. Joslin Diabetes Center and Harvard Medical School, Boston, MA 02215 and NCI, Bethesda, Maryland 20205

We have shown that the solubilized insulin receptor in a glycoprotein-enriched fraction obtained from a hepatoma cell line (Fao) by wheat germ agglutinin-agarose affinity chromatography is a protein kinase. Insulin stimulates an intramolecular autophosphorylation reaction of this insulin receptor and stimulates the phosphorylation of certain other proteins reconstituted with this system. Trypsin digestion of the phosphorylated β-subunit yielded at least 5 phosphopeptides when this mixture was separated by HPLC on a reverse phase column. Insulin stimulated the phosphorylation of each peptide, but they varied in their rate and degree of phosphorylation. The gene product of the Rous sarcoma virus (RSV), $pp60^{src}$, is a tyrosine kinase which is responsible presumably for the transformation of cells infected with RSV through abnormal phosphorylation of cellular components. Physiologically relevant substrates for the insulin receptor kinase and $pp60^{src}$ have not been identified; however, both of these kinases catalyze phosphorylation of similar proteins such as IgG directed against $pp60^{src}$, histones and casein. Reconstitution of the insulin receptor with purified $pp60^{src}$ stimulated the phosphorylation of the β-subunit of the insulin receptor in the absence and presence of insulin. This phosphorylation does not alter the insulin dose response for autophosphorylation of the receptor. Increasing concentrations of $pp60^{src}$ increased the phosphorylation of the receptor. Whether phosphorylation of the insulin receptor by $pp60^{src}$ occurs at a unique site or alters the specificity and activity of the receptor kinase are current subjects of investigation.

0771 A COMPARISON OF THE INSULIN- AND EGF-STIMULATED PROTEIN KINASES IN HUMAN PLACENTA Linda J. Pike, Elizabeth A. Kuenzel, John Casnellie and Edwin G. Krebs. Univ. of Washington, Howard Hughes Medical Institute, Seattle, WA 98195

Wheat germ lectin Sepharose-purified preparations of solubilized human placenta membranes contain both insulin- and EGF-stimulated tyrosine protein kinase activity. The properties of these two growth factor-stimulated kinases were compared. Using a synthetic tyrosine-containing peptide as the phosphorylatable substrate, the insulin-stimulated kinase exhibited a K_m for the peptide of 2.0 mM with a specific activity of 72 nmol/min/mg. Similarly, the EGF-stimulated kinase demonstrated a K_m of 1.4 mM for the peptide with a specific activity of 312 nmol/min/mg. The higher specific activity of the EGF-stimulated kinase is most likely due to the fact that there are 3 to 5 times more EGF than insulin receptors in this preparation. Both the insulin- and EGF-stimulated kinases were active in the presence of either Mg^{2+} or Mn^{2+} but showed the greatest activity when a combination of the two was used to fulfill the divalent cation requirement. The two kinases showed similar nucleotide specificities and were both inhibited by treatment with N-ethyl maleimide. When a number of different proteins were tested for their ability to serve as substrates for these two kinases, it was found that the insulin- and EGF-stimulated protein kinases exhibited similar but not identical substrate specificities. Such similarity at the molecular level may be the basis of the similarity between the actions of insulin and EGF at the cellular level.

0772 SOLUBILIZATION AND PARTIAL PURIFICATION OF RECEPTORS FOR PLATELET-DERIVED GROWTH FACTOR, Lewis T. Williams, A.Raymond Frackelton, and Patrice Tremble, Howard Hughes Medical Institute and Dept.of Medicine, UCSF, San Francisco, CA. 94132

Platelet derived growth factor (PDGF) initiates cell division by binding to a 170 kd receptor which has been identified in intact cells and in membrane preparations. We have developed methods for solubilizing and partially purifying this receptor. The binding of ^{125}I-PDGF to the soluble sites was measured by precipitating the soluble receptors onto liposomes in the presence of lectin. The solubilized 170kd receptor sites had the specificity and kinetic, characteristics of PDGF receptors found in membrane preparations and intact cells. Native PDGF at a concentration of .03 nM half-maximally occupied the soluble sites, whereas reduced PDGF, which is biologically inactive, did not bind to the sites. We have shown that PDGF stimulates the tyrosine phosphorylation of a 170 kd protein in intact 3T3 cells. This phosphoprotein co-purifies with the soluble PDGF binding sites on wheat germ agglutinin-sepharose columns and on anion exchange resins. In cells stimulated by PDGF, the 170 kd protein labeled with ^{35}S-methionine could be immunopurified by affinity chromatography using a monoclonal antibody to phosphotyrosine. The 170 kd protein from unstimulated cells did not bind to the anti-phosphotyrosine antibody. These data show that the PDGF receptor can be solubilized in an active form and that it co-purifies with the 170 kd protein which is phosphorylated in response to PDGF.

0773 Phorbol Ester Tumor Promotors Stimulate Tyrosine-Specific Phosphorylation of EGF Receptors, A. Christie King and Soon Hahn, University of Illinois, Chicago, Illinois 60607

New results from my laboratory indicate that the potent tumor promotor phorbol myristate acetate (PMA) stimulates phosphorylation of the EGF receptor in A$_{431}$ membranes. The phosphorylation occurs at 4°C and is dependent on the presence of manganese, a known activator of the tyrosine protein kinase activity associated with the EGF receptor. Phosphorylation measured under these conditions is apparently calcium-independent, since the presence of the calcium-specific chelator EGTA has no effect of the extent of receptor phosphorylation as measured by autoradiography. These very exciting results suggest that protein kinase C (PK-C), a calcium-dependent enzyme known to be activated by phorbol esters, is not indirectly modulating the activity of the tyrosine-specific protein kinase associated with the EGF receptor. Instead, it appears that PMA can directly stimulate phosphorylation of the EGF receptor, probably by directly activating the tyrosine-specific kinase activity associated with the EGF receptor. Interestingly, in the presence of calcium, a new and unique substrate is phosphorylated in A$_{431}$ membranes when either EGF or PMA are presnt, and two other substrates are phosphorylated to a lesser extent. These data suggest that phorbol ester tumor promotors like mitogens may activate multiple types of kinases. Each of these may be involved in generation of a biochemical signal required to induce quiescent cells to divide.

0774 INSULIN RECEPTOR PROTEIN KINASE: THE SEARCH FOR PHSYIOLOGICAL SUBSTRATES, Perry J. Blackshear, Raphael A. Nemenoff, Yan C. Kwok,A. Raymond Frackelton Jr., Joseph Avruch, Massachusetts General Hospital, Diabetes Unit, Fruit St, Boston, MA 02114

The fact that the insulin receptor is an insulin-activated, tyrosine specific protein kinase immediately suggests that the activation of this kinase might be the first step in insulin action. However, in common with the polypeptide growth factors and certain retrovirus transforming proteins, physiologically relevant substrates for this kinase have not been identified. We addressed this problem in a number of ways including: 1-Phosphorylation of membrane proteins from rat liver microsomes by endogenous and exogenous insulin receptor kinase. Insulin promotes the phosphorylation of at least 6 proteins in these extracts of M_r 60,55,52,28,23 and 20,000, some of which have been partially purified and characterized. 2-Evaluation of autoradiograms of alkali-treated two-dimensional gels of ^{32}P-labelled phosphoproteins from cells exposed to insulin. Of the approximately six proteins whose phosphorylation is promoted by insulin on alkali-stable residues, all investigated to date have been phosphorylated on serine or threonine residues. 3-Attempted purification of phosphotyrosine-containing proteins from cell homogenates using a monoclonal antibody which recognizes phosphotyrosine. These studies have resolved primarily the insulin receptor itself as the major phosphotyrosine containing protein to undergo affinity purification. 4-Phosphorylation of proteins of potential interest using purified insulin receptor kinase. Results from these studies, and their implications for the physiological relevance of the insulin receptor kinase, will be discussed.

0775 STIMULATION OF TYROSINE-SPECIFIC PHOSPHORYLATION IN INTACT FIBROBLASTS BY PDGF.
B. Ek and C-H. Heldin. Dept of Med Chem, Box 575, University of Uppsala, Sweden.
Platelet-derived growth factor is the major growth promoting activity in serum for connective tissue-derived cells. The factor has a M_r of about 30 000 and is composed of two disulphide linked polypeptide chains. Amino acid analysis of the protein has revealed a strong homology with the transforming gene product of simian sarcoma virus, $p28^{sis}$, suggesting a role for a PDGF-like factor in SSV-mediated transformation. PDGF exerts its effect on cells via binding to a specific cell surface receptor. The receptor is associated with tyrosine kinase activity which probably is an intrinsic part of the receptor molecule itself. Experiments with membranes prepared from human foreskin fibroblasts indicates that the kinase becomes activated upon binding PDGF. This in turn results in autophosphorylation of the receptor molecule. In order to identify substrates for the receptor associated kinase in intact cells we have used an antiserum against phosphotyrosine. Immunoprecipitation of lysates from PDGF-stimulated cells labeled either with ^{32}P-orthophosphoric acid or ^{35}S-methionine showed that a 185 kDa component, identified as the PDGF-receptor, was the major phosphorylated component. The phosphorylation was rapid with a maximum 5 minutes after addition of PDGF. Phosphoamino acid analysis of the immunoprecipitated 185 kDa component showed that it contained, in addition of phosphotyrosine, also phosphoserine. This suggests that some characteristics of the receptor might be regulated by phosphorylation on serine residues. In addition to the PDGF-receptor other, less pronounced, components with M_r's of 115 000, 88 000 and 35 000 were also specifically precipitated by the antiphosphotyrosine immune serum.

0776 SUBUNIT STRUCTURE OF THE ADIPOCYTE INSULIN RECEPTOR PROBED USING AUTOPHOSPHORYLATION,
Gonul Velicelebi and R. A. Aiyer, Harvard University, Cambridge, MA. 02138

It has been well-established by covalent labeling with ^{125}I-insulin and its analogs that the insulin receptor has insulin binding subunit of 135,000 daltons, the α-subunit. More recently, it has been documented that the receptor undergoes insulin-stimulated autophosphorylation that predominantly labels a 95,000-dalton subunit, the β-subunit. We have solubilized rat adipocyte insulin receptors in Triton X-100 and partiallly purified the protein on wheat germ-Sepharose affinity column. Subsequently, we labeled the two subunits of the receptor independently using ^{125}I-insulin for the 135,000 and ^{32}P for the 95,000-dalton polypeptide. Sucrose density gradient sedimentation and SDS-PAGE were used to characterize the native, oligomeric structure of the receptor.

In 0.1 % Triton X-100, the receptor sedimented as a single species of $s_{20,w}$ = 10.2 S as detected by ^{125}I counts and the SDS-PAGE profile of the 95,000 phosphorylated band under reducing conditions. SDS-PAGE under nonreducing conditions revealed a large species that appeared to be $\alpha_2\beta_2$ and to a lesser extent $\alpha\beta$. Treatment of the solubilized, partially purified receptor with 10 mM DTT led to the conversion of the 10.2 S species to a samller one sedimenting at 6.6 S. The composition of this species was determined to be $\alpha\beta$ by SDS-PAGE under non-reducing conditions.

These results indicate that the ability to label the two subunits of the receptor separately should facilitate detailed study of its oligomeric structure both in solution as well as in the membrane.

0777 TYROSYL PROTEIN KINASES IN HUMAN SERUM, Ming-Fong Lin, Tina Q. Tan, Pat Lobelle-Rich, and Gail M. Clinton, Louisiana State Medical Center, New Orleans, LA 70112.

Human serum was tested for the presence of tyrosyl-protein kinases in order to evaluate their expression during normal human development and to evaluate possible changes in their expression in malignant diseases. Tyrosyl kinase activity was detected in human serum using the peptide angiotensin II, pp60src specific IgG, or endogeneous proteins as substrates. Analyses of tyrosyl kinase activities in over 150 serum samples indicate that levels of the enzyme relative to serum volume or relative to overall protein kinase activity vary in different age groups. High activity was observed in serum obtained from the cord blood of newborns. The levels dropped during early development and increased again in the third age decade. These results point to a role for these enzymes in human development. Fractionation of the serum enzymes by column chromatography revealed the presence of at least three tyrosyl kinases. We are currently determining which of these enzymes are differentially expressed in the serum from individuals of different ages. These studies were supported by Grant CA-34517 (GC).

0778 REGULATION OF INSULIN RECEPTOR KINASE ACTIVITY BY COVALENTLY-LINKED INSULIN DIMERS. Richard A. Roth, David Morgan, Delanie J. Cassell, Michele A. Tatnell[+], Richard H. Jones[+], Achim Schuttler[*] and Dietrich Brandenburg[*], Mt. Zion Hospital and University of California, San Francisco, CA 94143, [+]St. Thomas' Hospital Medical School, London, U.K., and [*]Deutsches Wollforschungsinstitut, Aachen, F.R.G.

Three covalently-linked insulin dimers were prepared by crosslinking insulin monomers with a suberoyl chain at either the amino group of the B1 Phe or B29 Lys. The resulting 3 dimers (B1-B'1, B1-B'29 and B29-B'29) exhibit a discrepancy between their ability to inhibit the binding of ^{125}I-insulin to adipocytes and their ability to stimulate lipogenesis (Hoppe-Seyler's Z. Physiol. Chem. (1982) 363, 317-330). The B29-B'29 dimer had the greatest discrepancy; this dimer had a 10-fold greater ability to bind to the receptor than to stimulate lipogenesis. These three dimers were tested for their ability to stimulate the kinase activity of purified insulin receptors and to inhibit the binding of insulin to its purified receptor. Again, B29-B'29 exhibited the greatest discrepancy in binding potency and potency in stimulating the receptor kinase. Although this dimer inhibited the binding of ^{125}I-insulin with one-third the potency of native insulin, it had only 1% the ability of native insulin to stimulate the receptor kinase. These results indicate that occupancy of the receptor binding site is not sufficient to activate the receptor kinase and that the insulin dimers may be useful tools for studying the signals required for activation of the receptor kinase. In addition, these results are consistent with the hypothesis that the stimulation of the receptor kinase mediates the metabolic effects of insulin.

0779 IDENTIFICATION OF pp60src TYROSINE KINASE SUBSTRATA BY PHOSPHOTYROSINE ANTIBODIES. M.F. Di Renzo, L.Naldini, F.Giancotti, P.C.Marchisio and P.M.Comoglio . University of Torino, 10126 Italy.

The v-src oncogene product pp60src is known to trigger the acquisition of the transformed phenotype by phosphorylating target molecule(s) at tyrosine residues. In order to identify phosphotyrosine containing proteins, rabbit antibodies were raised against the synthetic hapten p-azobenzene-phosphonate (ABP) that specifically cross-reacts with phosphorylated tyrosine. By immunodecoration of proteins extracted from RSV transformed mouse fibroblasts and transferred to nitrocellulose sheets, phosphoproteins of 130, 70 and 60 Kd were identified. These molecules were found to be associated with the cellular fraction insoluble in non-ionic detergent. Moreover, ABP antibodies precipitated detergent-insoluble proteins of 130, 70, and 60 Kd -plus two additional components of 85 and 65 Kd- that had been phosphorylated in vitro by 32P-v-ATP under conditions allowing the kinase reaction catalyzed by pp60src. The radioactivity comigrated with authentic phosphotyrosine in two-dimensional chromatography. In RSV transformed fibroblasts -fixed and detergent permeabilized- ABP antibodies stained diffusely the cytoplasm and specifically decorated restricted areas of the ventral plasma membrane corresponding to adhesion plaques at the level of cell-cell contacts.

0780 PHOSPHORYLATION OF INSULIN RECEPTOR IN CONTROL AND DESENSITIZED HEPATOMA CELLS. M. Crettaz and C.R. Kahn, Joslin Diabetes Center, Boston, MA 02215

In intact cells insulin stimulates the phosphorylation of its own receptor. This is due in part to the stimulation of protein kinase activity associated with the receptor. Prolonged incubations of hepatoma cells with 10^{-6}M insulin produced a 70-80% decrease in receptor number (receptor down-regulation). In down-regulated cells, insulin failed to stimulate the activity of both glycogen synthase and tyrosine aminotransferase indicating desensitization of cells to hormonal action. In order to know whether insulin's inability to produce its biological effects in desensitized cells could be related to an abnormal receptor phosphorylation, insulin effect was studied in intact cells. Short incubations (30 min) with insulin produced a 6-fold increase in receptor phosphorylation. Prolonged exposure to the hormone was associated with a progressive decreased effect such as after 24h exposure to insulin--time at which total desensitization occurred - no insulin effect could be detected. To know whether this decrease was due to a defect of the receptor kinase per se, insulin receptors were partially purified from control and desensitized cells and receptor phosphorylation was measured in a cell-free system. When expressed per mg of protein, basal and insulin-stimulated phosphorylation was lower in receptors from desensitized cells when compared to control cells. However, when expressed per insulin-binding sites, receptors of desensitized cells were phosphorylated to the same extent than control receptors. These results indicate that in desensitized cells 1: the kinase activity of insulin receptors is normal; 2: the decrease in receptor phosphorylation observed in intact cells is mainly accounted for by the decrease in receptor numbers.

0781 RESOLUTION OF MULTIPLE SPECIES OF PHOSPHOTYROSYL-PROTEIN PHOSPHATASE, T. S. Ingebritsen, J. W. Johansen and L. C. Dally, Univ. of Colorado Health Sci. Ctr., Denver, Colorado 80262.

Previous studies (Ingebritsen, T.S. and Cohen, P., Science 221, 331-338 (1983)) have shown that the protein phosphatase activity towards a large number of phosphoseryl- and phosphothreonyl-proteins is due to four enzymes, termed protein phosphatase 1 (Type 1 protein phosphatase) and protein phosphatases 2A, 2B and 2C (Type 2 protein phosphatases). In contrast, little is known concerning the nature of the protein phosphatases acting on a novel class of phosphoproteins in which phosphorylation occurs on tyrosine residues. In this report the nature of phosphotyrosyl-protein phosphatases in extracts of bovine brain has been investigated using as a substrate, ^{32}P-labeled casein phosphorylated exclusively on tyrosine residues by pp60^{src}. Approximately 70% of the phosphotyrosyl-casein phosphatase activity in the extract was present in the cytosolic fraction. Following successive chromatographies of this fraction on DEAE-cellulose and phosphocellulose, and HPLC gel filtration on a TSK-G3000SW column, 8 species of phosphotyrosyl-casein phosphatase were resolved. Six of the species, accounting for 80-85% of the total activity, were separated from the major alkaline phosphatase activities and the major phosphorylase phosphatase activities (i.e., protein phosphatases 1 and 2A). The remaining phosphotyrosyl-casein phosphatase activity was accounted for by protein phosphatase 2A and by a further species which also contained a significant amount of alkaline phosphatase activity (5-10% of the total activity in the cytosolic fraction). These results indicate that the majority of the phosphotyrosyl-casein phosphatase activity present in bovine brain cytosol is due to a new class of protein phosphatases which is distinct from alkaline phosphatases and from type 1 and type 2 protein phosphatases. (Supported by grants from the NIH, BRSG-05357 and P50-NS09199, and the American Cancer Society, IN-5W).

0782 TYROSINE PHOSPHORYLATION OF THE INSULIN RECEPTOR β SUBUNIT ACTIVATES THE RECEPTOR-ASSOCIATED TYROSINE KINASE ACTIVITY, Kin-Tak Yu and Michael P. Czech, University of Massachusetts Medical School, Worcester, MA 01605

The regulation of insulin receptor tyrosine associated kinase activity by phosphorylation and dephosphorylation has been examined using partially purified receptor immobilized on insulin-agarose. Prior phosphorylation of the insulin receptor preparation with increasing concentrations of ATP, followed by washing to remove the unreacted ATP, resulted in a progressive activation (4 fold) of the receptor kinase activity when assayed in the presence of histone and [γ-^{32}P] ATP. High pressure liquid chromatographic analysis of tryptic hydrolysates of the ^{32}P-labeled insulin receptor β subunit reveals three domains of phosphorylation (designated peaks 1, 2 and 3). Phosphotyrosine and phosphoserine residues are present in these three domains while peak 2 contains phosphothreonine as well. Thus, at least seven sites are available for phosphorylation on the β subunit of the insulin receptor. Incubation of the phosphorylated insulin receptor with alkaline phosphatase at 15° results in the selective dephosphorylation of the phosphotyrosine residues on the β subunit of the receptor. The dephosphorylation of the receptor is accompanied by a marked 65% inhibition of the receptor kinase activity. Almost 90% of the decrease in ^{32}P-phosphate content of the receptor after alkaline phosphatase treatment is accounted for by the decrease in phosphotyrosine content in peak 2. These results demonstrate that the extent of phosphorylation of tyrosine residues in receptor domain 2 closely parallels the receptor kinase activity state, suggesting phosphorylation of this domain may play a key role in regulating the insulin receptor tyrosine kinase.

Regulation of Intracellular Enzymes and Phospholipids

0783 MONOCYTE ACTIVATION IS PRECEDED BY AN INCREASE IN CYTOPLASMIC IONIZED CALCIUM. Sean P. Scully, George B. Segel and Marshall A. Lichtman, University of Rochester, Rochester, NY 14642

We have examined the putative role of calcium (Ca) as a second messenger in the regulation of monocyte activation. The production of superoxide after exposure to Con A was used to assess activation of the monocytes. The analysis of the uptake and exodus of 45Ca was indicative of a three compartment model. Non-linear regression analysis used to determine pool sizes and exchange rates was compatible with ectocellular plasma membrane, cytoplasmic and organelle-related pools. Treatment with Con A (100 μg/ml) caused a 75% increase in the exchange rate of the cytoplasmic pool (from .040 to .070 min^{-1}, $p<.05$) and an increase of 65% in the size of this pool (from .080 to .13 fmoles/cell, $p<.05$). No changes occurred in the ectomembrane or organelle pools. Con A increased the cytoplasmic free Ca from 83 to 163 nM as measured with the fluorescent probe Quin 2. The time of increase in free Ca (31±6 sec) preceded the production of super-oxide (61±9 sec) in each cell population tested ($p<.001$). Further, the magnitude of the increment in cytoplasmic free Ca, 80 nM, doubled the activity of the Ca transport ATPase in monocyte plasma membrane vesicles. The increase in ATPase activity was similar to the increase in Ca exodus from intact monocytes treated with Con A. These data indicate that activation of human monocytes leads to a rapid increase in plasma membrane permeability to Ca, accelerating Ca influx and contributing to both internally bound and free cytoplasmic Ca. The increase in free Ca stimulates Ca extrusion and limits the increment in free Ca to nanomolar quantities, sufficient to contribute to the modulation of cellular enzymes that are closely associated with activation of the cell.

0784 ENDOCYTOSIS OF ERYTHROCYTE MEMBRANE PROTEINS DURING DIFFERENTIATION OF HUMAN ERYTHROLEUKEMIC CELLS, Richard C. Hunt and Linda M. Marshall, The University of Mississippi Medical Center, Jackson, MS 39216 and University of Texas Health Science Center, San Antonio, TX 78284

When K562 erythroleukemic cells are induced to differentiate, glycophorin is excluded from internalization via clathrin-coated pits and is distributed on the cell surface over regions that are lined by spectrin. In contrast, transferrin receptors are concentrated into coated pits and are internalized after hemoglobin synthesis has been induced. The amount of surface-associated transferrin receptor is diminished as differentiation proceeds. Two-dimensional gel electrophoresis reveals five forms of the receptor that are separable by virtue of differences in their isoelectric points and all of which are amenable to lactoperoxidase-catalyzed iodination of intact cells and, therefore, located on the cell surface. Alkaline phosphatase digestion shows that four of these forms are phosphorylation variants of a single polypeptide and it has been found that differentiation is accompanied by a change in the relative amounts of the various forms with the more phosphorylated forms becoming more predominant as differentiation proceeds. Incubation of cells with trifluoperazine, a drug that inhibits calmodulin-dependent phosphorylation of some proteins, converts the receptors to less phosphorylated forms and results in diminution of the amount of the receptor that is labeled by iodination. Since there is no reduction in the total amount of receptor, inhibitor treatment probably results in accumulation at an intracellular location. It is suggested that phosphorylation of the transferrin receptor may be involved in internalization and recycling of the receptor through the cell.

0785 F-MET-LEU-PHE INDUCES REVERSIBLE CHANGES IN F-ACTIN CONTENT OF HUMAN BLOOD NEUTROPHILS Paul J. Wallace, Robert P. Wersto, Charles H. Packman, and Marshall A. Lichtman, University of Rochester School of Medicine, Rochester, New York 14642

Motile responses of human neutrophils (PMN) to chemotactic stimuli appear to require formation of F-actin filaments from cytoplasmic stores of unpolymerized actin molecules. We have studied the effect of the chemotactic peptide f-Met-Leu-Phe (f-MLP) on actin conformation in PMN using fluorescent NBD-Phallacidin (NBD-P) which binds specifically to F-actin oligomers and filaments. After incubation with NBD-P, PMN F-actin was measured by fluorescence with a flow cytometer. Uptake of NBD-P was saturable and inhibited by fluid phase F-actin but not G-actin. Stimulation of PMN by $\geq$1nM f-MLP resulted in a dose dependent increment in F-actin content in 80-85% of PMN by 30 sec. In spite of the continued presence of f-MLP, F-actin content began to decrease by 1 min. and approached baseline levels at 5 min. The f-MLP induced increase in F-actin was blocked entirely by 30μm cytochalasin B or an excess of a t-Boc peptide that competitively inhibits f-MLP binding. Under fluorescence microscopy, NBD-P staining of unstimulated PMN gave homogeneous cytoplasmic fluorescence while staining of PMN 1 min. after addition of f-MLP revealed accentuated subcortical fluorescence. In the continued presence of an initial stimulatory dose of f-MLP, PMN could respond within 1 min. with increased f-actin content after the addition of a 3-fold or greater increased concentration of f-MLP. Thus, incubation of f-MLP with PMN results in a rapid transient conversion of unpolymerized actin to subcortical F-actin filaments that can be restimulated within 1 min. by increased f-MLP concentration. The directed movement of PMN may involve similar rapid reversible changes in actin conformation in response to chemoattractant gradients.

0786 SYSTEMS FOR BULK PHASE ENDOCYTOSIS AND THEIR REGULATION, Mojtaba Esfahani and Louis Scerbo, Hahnemann University, Philadelphia, PA 19102.

Bulk phase endocytosis (pinocytosis) by a human macrophage-like cell line, U937, which requires cholesterol for growth, is low but can be induced at least 50-fold by concanavalin A (con A) when the cells are grown in a medium containing fetal calf serum (fcs). Supplementation of this medium with 10 μg/ml of cholesterol enriches cellular cholesterol three-fold but has no effects on pinocytosis. By contrast, cells incubated in a medium containing delipidated serum (dlfcs) become depleted of cholesterol and fail to acquire an optimal pinocytotic capacity even with con A stimulation. Supplementation of dlfcs with cholesterol, while replenishing the cellular cholesterol content and supporting full growth, further inhibits the already diminished pinocytotic activity. Thus the lower pinocytotic activity is neither due to depletion of cellular cholesterol nor due to impaired growth. Furthermore, supplementation of the medium containing dlfcs with lipids extracted from serum failed to raise pinocytotic activity significantly. Based on two criteria, specific activity of pinocytosis and susceptibility to cholesterol inhibition, we propose the existence of two systems for pinocytosis, both capable of functioning in growing cells. Our results also suggest that a serum factor(s), which is not required for growth, is required for regulation of bulk phase endocytosis. This factor(s) is destroyed or depleted by delipidation of serum but is not a pure lipid.

0787 INTERACTION OF THROMBIN RECEPTORS WITH THE CYTOSKELETON AFFECTS THE RATE OF THROMBIN DISSOCIATION, William C. Thompson, Mary L. Bradley and Darrell H. Carney, University of Texas Medical Branch, Galveston, Texas 77550.
Thrombin receptors appear to cluster on the surface of mouse embryo (ME) fibroblasts prior to thrombin binding (J. Cell Biol. 95:697, 1982), but do not associate with coated pits or participate in "receptor mediated endocytosis" (J. Cell. Biochem. 20:247, 1982). To determine whether there is cytoskeletal anchorage of these receptor clusters we incubated ME and hamster NIL cells with ^{125}I-thrombin before or after extraction of the cells with 0.5% Triton X-100 in microtubule stabilizing or destabilizing buffers. From 30 to 50% of the ^{125}I-thrombin specifically bound to the cells prior to extraction remained bound to the insoluble cytoskeletons. Likewise, when extracted cytoskeletons were incubated with ^{125}I-thrombin, approximately 50% of the original receptor sites remained with the cytoskeletons. The percentage of receptors remaining with cytoskeletons was increased by preincubation of the cells with taxol prior to extraction, and was decreased by extraction in microtubule destabilizing buffer, suggesting a possible microtubule involvement in receptor anchoring. When ^{125}I-thrombin was chased from receptor sites by incubating unextracted cells with unlabeled thrombin, dissociation curves showed an initial rapid dissociation or exchange, followed by a much slower dissociation. Cells detergent-extracted subsequent to the rapid dissociation retained 70-80% of the ^{125}I-thrombin with their cytoskeletons, indicating that thrombin dissociates slowly from anchored receptors and rapidly from unanchored receptors. (Support: AM25807, CA00805).

0788 PHORBOL ESTERS INDUCE Na^+/H^+ EXCHANGE AND DIFFERENTIATION IN A PRE-B LYMPHOCYTE CELL LINE, Philip M. Rosoff and Lewis C. Cantley, Harvard University, Cambridge, MA 02138.
The chemically transformed, murine pre-B lymphocyte cell line, 70Z/3, may be induced to differentiate from a surface immunoglobulin (-) to a surface immunoglobulin (+) phenotype with both phorbol esters (PE) and the non-specific B cell mitogen, lipopolysaccharide (LPS). We have previously shown that LPS works by raising the intracellular $[Na^+]$ through an amiloride-sensitive, ouabain-resistant transport system: this is the critical, rate-limiting step for differentiation in this cell line (Rosoff and Cantley, Proc. Natl. Acad. Sci. USA: in press). The increase in $[Na^+]_i$ is accompanied by a concomitant increase in pH_i via a putative Na^+/H^+ antiporter. Tumor promoting phorbol esters also induce 70Z/3 cell differentiation by activating a similar amiloride-sensitive mechanism. A rise in cellular Na^+ content is first detectable 10 to 30 minutes after exposure to PE and is maintained for at least 2 hours. Increased pH_i is detectable via an increase in 490 nm absorbance of intracellular 6-carboxyfluorescein within 2.5 minutes at 37°C. 1 mM amiloride blocks both Na^+/H^+ exchange as well as PE-induced differentiation. Retinoic acid (as all-trans and 13-cis) also blocks PE-induced differentiation. We suggest that PE works by activating protein kinase C which either directly or indirectly activates the Na^+/H^+ antiporter to initiate the differentiation process.

0789 CALCIUM REGULATION OF IMMUNOGLOBULIN SECRETION, R.C.McGlennen, J.B.Trepel, J.Cossman, E.S.Jaffe, and L.M.Neckers, Lab. of Pathology, NCI, NIH, Bethesda, MD 20205
Many secretory events are Ca^{2+} dependent. In these studies we demonstrate the Ca^{2+} dependence of immunoglobulin (Ig) secretion in (1) spontaneously secreting nodular lymphoma cells (NL), (2) TPA induced chronic lymphocytic leukemia cells (CLL), and (3) normal peripheral blood B cells (PBL). In vitro cultures of CLL are unable to secrete Ig spontaneously. When treated with the tumor promotor TPA, however, these cells are stimulated to secrete Ig of the same heavy and light chain isotype as the isotype we detected on the cell surface by flow cytometry. Ig secretion in CLL was completely inhibited by the calmodulin antagonists trifluoperazine, chlorpromazine, and dibucaine, at IC_{50}'s ranging from 9nM to 30uM, without loss of cell viability. We compared TPA stimulated secretion in CLL to two spontaneously secreting cases of NL, in which TPA had no measureable effect on Ig secretion, and found that, similarly to TPA stimulated Ig secretion, the calmodulin inhibitors eliminated spontaneous Ig secretion, and with the same IC_{50}'s. We also found that both spontaneous and TPA stimulated Ig secretion were inhibited in a dose-dependent manner by the chelation of extracellular Ca^{2+} ions with EGTA. In these experiments, 2.5mM EGTA completely inhibited Ig secretion in both CLL and NL. At lower EGTA concentrations, the inhibition can be partially reversed by Ca^{2+} addition.To determine if Ca^{2+} regulation of Ig secretion is restricted to malignant B cells, we induced Ig secretion in normal PBL by addition of PHA-stimulated T cell supernatant and Staph protein A, and again found a dose dependent inhibition of Ig secretion by calmodulin inhibitors and EGTA. These results suggest that both extracellular Ca^{2+} and an intracellular Ca^{2+}-calmodulin complex are required for Ig secretion.

0790 MECHANISM OF PHORBOL ESTER-INDUCED IMMUNOGLOBULIN SECRETION IN LEUKEMIC B CELLS, J.B.Trepel, R.C.McGlennen, J.Cossman, E.H.Lipford, L.M.Neckers, NCI, NIH, Beth.,MD
The phorbol ester receptor has been identified in several systems as the calcium-activated phospholipid dependent protein kinase C. We have been using the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) to induce immunoglobulin secretion in chronic lymphocytic leukemia cells (CLL). We have observed a rapid elevation in the level of immunoglobulin mRNA followed by the appearance of intracytoplasmic translated protein. Secretion of immunoglobulin was not detected until several days after these events can be observed. We questioned whether all these events were mediated via the same mechanism. We examined the requirement for calmodulin and/or extracellular calcium. We found that the calmodulin inhibitor trifluoperazine (10uM), as well as EGTA (1-2mM) and the membrane stabilizer ethanol (1%) all completely inhibited TPA induced immunoglobulin secretion without affecting cell viability. None of these agents, however, affected the TPA-induced elevation of immunoglobulin mRNA level. We also examined the protein phosphorylation pattern in CLL after TPA treatment. TPA induced the phosphorylation of specific proteins as detected by SDS slab gel electrophoresis. We found that trifluoperazine and other calcium/calmodulin antagonists had multiple effects on the pattern of TPA stimulated phosphorylation. We conclude, therefore, that in a clonal B cell population we can distinguish at least two TPA-induced phenomena: 1) immunoglobulin message induction and translation, a rapid calcium/calmodulin independent event, and 2) immunoglobulin secretion, an event probably requiring the calcium/calmodulin dependent phosphorylation of certain membrane proteins by protein kinase C.

0791 A SERUM LIPASE THAT HYDROLYZES PHORBOL ESTERS, Myles C. Cabot, Medical and Health Sciences Division, Oak Ridge Associated Universities, Oak Ridge, TN 37830

Rat serum contains lipase activity that is specific for hydrolysis of the long chain tetradecanoate moiety of 12-O-tetradecanoylphorbol-13-acetate (TPA). This enzyme activity can be enhanced 8-fold by removal of serum lipids and in turn drastically inhibited by re-addition of these lipids to the reaction. Phosphatidylcholine, alone, is a potent inhibitor of TPA hydrolysis, although the delipidated enzyme preparation is devoid of phospholipase activity, and will not catalyze the hydrolysis of cholesteryl esters. The enzyme displays two pH optima (5.5 and 8.0) and is completely destroyed by heating (56°C, 30 min). NaF (5 mM), protamine (250 µg/ml), or NaCl (0.5 M) inhibited the formation of phorbol acetate by 35, 25, and 80%, respectively. Analysis of whole serum showed it to be rich in phospholipids [30% (phosphatidylcholine, lysophosphatidylcholine, sphingomyelin)] and neutral lipids [70% (cholesterol, triacylglycerols, cholesteryl esters)]. It is thus likely that TPA lipase activity is masked in native serum by either substrate competition (acylglycerols) or by binding of TPA to serum lipids. TPA is membrane-targeted and its action may in part be expressed through mimicry of a specific type of membrane-resident lipid. Two questions currently being investigated may provide clues to the mechanism of phorbol ester/membrane interaction: what is the true substrate of the serum TPA lipase, and in cells could the action of phorbol esters be influenced by membrane acylglycerol lipase? This work was supported by the Office of Energy Research, US Department of Energy (Contract No. DE-AC05-76OR00033).

0792 REGULATION OF PHORBOL ESTER RECEPTORS IN RAT PITUITARY CELLS. SUSAN JAKEN, LABORATORY OF CELLULAR CARCINOGENESIS AND TUMOR PROMOTION, NCI, BETHESDA, MD AND THE JOHNS HOPKINS ONCOLOGY CENTER, BALTIMORE, MD
Phorbol ester receptors have been characterized in GH4C1 cells using [3H]phorbol dibutyrate (PDBu). Recovery of receptors in cell lysates was 91 ± 13% of that measured in intact cells. In cell lysates, receptors were distributed between the membrane and cytosol fractions; 78% of the total recovered receptors were in the cytosol. This implies that the cytosolic receptor, which is dependent on phospholipids for binding, is a major component of the the total receptors measured in intact cells. PDBu treatment before cell lysis caused a redistribution of receptors; the cytosolic receptor concentration was decreased 58% and the membrane receptor concentration was increased 77%. The close association between PDBu binding activity and protein kinase C activity suggests that diacylglycerol may be the endogenous ligand for PDBu receptors. Phospholipase C-treatment of cultures was used to generate diacylglycerol in the membrane in order to test the effect of diacylglycerol on PDBu binding. Phospholipase C-treatment caused a decrease in affinity from K_d = 11 ± 3 nM to 31 ± 4 nM with no apparent change in the number of total binding sites. This is consistent with diacylglycerol being a competitive inhibitor of PDBu binding. Phopholipase C-treatment also caused a redistribution of receptors to the membrane fraction; cytosolic receptor concentration was decreased 45% and membrane receptor concentration was increased 200%. These results suggest that PDBu dissolved in the membrane or diacylglycerol generated in the membrane can mediate membrane stabilization of the cytosolic receptor.

0793 RECEPTORS FOR TUMOR PROMOTING PHORBOL ESTERS: REGULATION BY PHOSPHOLIPIDS AND OTHER MEMBRANE COMPONENTS, Bernhard Koenig and Peter M. Blumberg, Molecular Mechanism of Tumor Promotion Section, LCCTP, NCI, NIH, Bethesda, MD 20205

Biological effects of phorbol esters are mediated by specific receptors which are found both in membranes and in the cytosol. The quantitatively major receptor appears to be a complex between lipid and protein kinase C. Using the partially purified apo-receptor from mouse brain and [^{3}H]phorbol 12,13-dibutyrate, we studied the contribution of the lipid to the binding activity of the lipid/protein complex. Phospholipids differed in both their potency and efficacy. The negatively charged phospholipids phosphatidylserine (PS), phosphatidylinositol (PI) and phosphatidic acid (PA) were most effective. Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) by themselves were ineffective but could reconstitute binding when used as a mixture (e.g. PC/PE, 1:1). The concentrations required for half-maximal binding activity were 2 µg/ml with PA, 7 µg/ml with PS or PI and 80 µg/ml with a mixture representing the phospholipid composition of erythrocyte membranes (PL-Mix). Increasing the concentration of Ca^{++} in the assay lowered the amount of phospholipid required for reconstitution of binding (e.g. PS: 7 - 0.1 µg/ml at half-maximal activity). Phospholipids also modulated the affinity of the receptor for phorbol esters. K_D values varied from 0.7 nM with PS to 20 nM with PL-Mix. The affinity for diacylglycerol, a competitive inhibitor of phorbol ester binding, was shifted in a similar manner. Diacylglycerol stimulates protein kinase C and is believed to play an important role in signal transmission. Our results suggest that alterations in the lipid domain may represent a regulatory mechanism for modulation of the receptor.

0794 SORTING OF SURFACE SIALOGLYCOPROTEINS IN HeLa CELLS DURING INTERNALIZATION AND RECYCLING, J. S. Cook and J. B. Fishman, Biology Division, Oak Ridge National Laboratory and U.T.-Oak Ridge Graduate School of Biomedical Sciences, Oak Ridge, TN 37830

Surface sialoglycoproteins (GP's) of HeLa cells, labeled by sequential treatment with $NaIO_4$ and $NaB[^3H]_4$, exchange with an intracellular pool from which material recycles to the cell surface (Fishman and Cook, 1982, JBC 257:8122). The labeled material can be visualized fluorographically after SDS-PAGE. From homogenates of labeled cells we observe internalized GP's at 10 min in an endosomal compartment, subsequently associated with the lysosomal compartment with a peak at 30-60 min, and entering the Golgi complex at 1-3 h. SDS-PAGE analysis shows that sorting occurs at internalization and in movement from lysosomes to Golgi. Surface labeled GP's do not appear to be further sorted with respect to each other at the Golgi. They pass through an intermediate compartment (GERL?) on recycling to the surface. Chloroquine or NH_4Cl leads to overaccumulation in the lysosomes and blocks transfer to Golgi and subsequent recycling. (Research sponsored by the Office of Health and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with the Union Carbide Corporation. J.B.F. is supported by grant CA 09104 from the NIH.)

0795 ENDOCYTOSIS OF SOLUBLE IgG IMMUNE COMPLEXES BY ACTIVATED MURINE MACROPHAGES, David S. Finbloom, Walter Reed Army Institute of Research, Washington, D.C. 20307.

Complexes of IgG antibody bind to and are internalized by cells bearing receptors that recognize the Fc portion of antibody(FcR). Whereas binding and uptake of monomeric IgG is limited, endocytosis of large soluble immune complexes proceeds exceedingly well. Since macrophages exist in various stages of activation, it is important to determine whether functional differences accompany changes in receptor number at each stage. The ability of resident(RES), thioglycolate(TG), and BCG elicited peritoneal macrophagaes to bind, endocytose, and metabolize radiolabeled dimers or heavy oligomers(5-6 IgG molecules) was studied. FcR recycling was estimated by measuring the amount of radiolabeled complex bound following an endocytic load. RES cells bound 5-10 fold less oligomer than either TG or BCG cells.In spite of adequate binding, dimers were considerably less efficient than heavy oligomers at initiating endocytosis(5% vs 40% intracellular at 30min). This difference was due to rapid dissociation of bound dimer from the cell surface upon warming(90% vs 30% dissociated at 30min). Heavy oligomers, which were endocytosed well(40% at 30min), were metabolized rapidly with TCA soluble products appearing within 60min of warming. Following an endocytic load, TG and BCG cells were unable to rebind maximum amounts of labeled complexes within 4hr. This suggests that rapid recycling of FcR does not occur. Thus dimers in contrast to heavy oligomers do not appear to provide an adequate signal for internalization in any cell studied; nor do FcR appear to recycle. Whereas FcR number may vary with the state of activation, the function of the receptor is similar in all cell types studied.

0796 GLUCOSE-DEPENDENT DEPHOSPHORYLATION OF GLYCOGEN SYNTHASE BY INSULIN IN RAT ADIPOCYTES. John. C. Lawrence, Jr. and Catherine James, Washington University, St. Louis, MO 63110

The control of glycogen synthase (GS) by insulin (INS) and glucose (GLC) was investigated using isolated fat cells. Adipocytes were incubated with [^{32}P]phosphate (^{32}P) to achieve steady state labeling of GS before addition of INS and/or GLC. [^{32}P]GS was immunoprecipitated and subjected to electrophoresis in the presence of SDS, where it migrated as a single species (MWt = 90,000). All of the ^{32}P could be recovered in 2 cyanogen bromide fragments. One fragment (CB-II) (MWt = 28,000) contained about 5 times more ^{32}P than the other (CB-I) (MWt = 15,000). Incubating cells with INS (500 μU/ml) alone for 10 min had little effect on the amount of ^{32}P in GS or the $K_{0.5}$ for glucose-6-P (G6P) activation of the enzyme; however, INS did increase (~60%) the activity ratio (-G6P/+G6P). GLC (5 mM) alone decreased (~25%) the ^{32}P in CB-II and increased (~20%) the activity ratio, but did not decrease the $K_{0.5}$ for G6P. When present together, INS plus GLC decreased the $K_{0.5}$ from .25 mM to .15 mM and produced an increase (3-fold) in the activity ratio much greater than the additive effects of the agents alone. The combination also decreased ^{32}P in both CB-I (↓60%) and CB-II (↓30%). 2-Deoxyglucose (5 mM), which is more effective than glucose in activating GS, decreased ^{32}P in CB-I (↓15%) and in CB-II (↓40%). These results suggest that the potentiation of INS action by GLC involves dephosphorylation of multiple sites on GS, possibly as a result of accumulation of intracellular hexose phosphate. (Supported by the Juvenile Diabetes Foundation and AM28312).

0797 REGULATION OF PHOSPHOLIPID BIOSYNTHESIS IN TETRAHYMENA, J. Donald Smith, Southeastern Massachusetts University, North Dartmouth, MA 02747.

The ciliate protozoan Tetrahymena thermophila provides a good model system for the study of regulation of phospholipid metabolism in eukaryotes. The phospholipid composition of Tetrahymena may be modified by growing the organism in the presence of various phosphonic acid analogues of the normal phospholipid bases. These compounds (2-aminoethylphosphonate, AEP and 3-aminopropylphosphonate, APP) are incorporated into the corresponding phosphonolipids with a corresponding decrease in phosphatidylethanolamine, while the level of phosphatidylcholine is unchanged. As a result, the enzymes for which phosphatidylethanolamine is a substrate have markedly decreased activity when measured in vitro. These enzymes include phosphatidylethanolamine-N-methyltransferase and phosphatidylethanolamine: serine phosphatidyltransferase. The enzymatic activity is restored to near normal levels by the addition of phosphatidylethanolamine to the assay system, indicating the constitutive nature of the enzyme proteins. In vivo the incorporation of the phospholipid bases (or precursors), serine, methionine and ethanolamine, into phosphatidylcholine in APP-grown cells compared to control cells occurs at levels comparable to those in control cells. Choline incorporation is increased about 3 fold in APP-grown cells but the activity of choline phosphotransferase is not significantly altered. Overall there appears to be some compensatory mechanism for the synthesis of phosphatidylcholine even though the amount of the normal substrate for its synthesis, phosphatidylethanolamine, is severly limited.

0798 T3 MEDIATED ACTIVATION OF T CELLS IS ACCOMPANIED BY INCREASES IN CYTOSOLIC CALCIUM, John Imboden[2], Arthur Weiss[1,2], Dolores Shoback[2], John Stobo[1,2], [1]The Howard Hughes Medical Institute and the [2]Department of Medicine, University of California, San Francisco, 94143

Recent studies have demonstrated intimate structural and functional relationships between a polymorphic T cell receptor for antigen and T3, a set of nonpolymorphic human T cell surface proteins. Several groups, including our own, have demonstrated that antibodies to T3 can substitute for antigen in T cell activation. We therefore initiated a series of studies to investigate the mechanism by which T3 participates in T cell activation. We used the T3 positive human T cell leukemia line Jurkat and a mutant of Jurkat, developed in our laboratory, which does not express T3. Maximal activation of Jurkat, as measured by production of interleukin 2, could be induced by any one of three different T3 monoclonal antibodies in combination with phorbol myristate acetate. The calcium ionophore, A23187, could substitute for T3 antibodies in this activation. As expected, T3 antibodies did not activate the T3 negative mutant, but A23187 did. Changes in cytosolic calcium($[Ca^{++}]_i$) occurring in Jurkat in response to stimulation with T3 antibodies were measured with the calcium sensitive fluorescent probe, quin 2. $[Ca^{++}]_i$ rose from basal levels of 118(+/-5)nM (mean+/-SEM) to 388(+/-25)nM. Three different control monoclonal antibodies, which bound to Jurkat but did not activate it, did not change $[Ca^{++}]_i$. These studies suggest that activation of T cells via the T3 complex is mediated by an increase in cytosolic calcium.

0799 cAMP-DEPENDENT PROTEIN KINASE AND cAMP BINDING ACTIVITIES OF DIABETIC RAT LIVER. Ashok K. Srivastava, Research Laboratory on Diabetes and Carbohydrate Metabolism. Clinical Research Institute of Montreal (Quebec) H2W 1R7, Canada.

cAMP-dependent protein kinase (cA kinase) and [^{3}H] cAMP binding activities were studied in the liver post-mitochondrial supernatants from control and streptozotocin-induced diabetic rats. Diabetic rat liver supernatants showed about 30% decrease in the cA kinase activity assayed either in the absence or presence of 1μM cAMP. A similar decrease (40-50%) was also observed in the total [^{3}H] cAMP binding activity. cAMP binding activity was further analyzed by using 8-azido-[^{32}P] cAMP, a photoaffinity probe for cAMP binding sites. In both, control and diabetic liver supernatants, the photoaffinity probe labeled one major band with an apparent molecular weight of 48,000 representing the regulatory subunit of type I cA kinase (RI), and a minor doublet with apparent molecular weights of 56,000 and 54,000 representing respectively the phospho- and dephospho- forms of regulatory subunit of type II cA kinase (RII). However, the supernatants from diabetic liver showed a selective reduction in the amount of RI labeled with the photoaffinity probe without any appreciable change in the labeling of RII. These data suggest that the decreased cA kinase activity of streptozotocin-diabetic rat liver is associated with a decrease in the cAMP binding to RI. (Supported in part by grants from Medical Research Council of Canada, Canadian Diabetes Association and Fonds de la Recherche en Santé du Québec).

0800 INDUCTION OF B CELL MEMBRANE DEPOLARIZATION AND HYPER-IA REGION ASSOCIATED ANTIGEN BY PHORBOL DIESTERS SUGGESTS A ROLE FOR PROTEIN KINASE C IN MURINE B LYMPHOCYTE ACTIVATION, John G. Monroe, Dept. Pathology, Harvard Medical School, James E. Niedel, Dept. of Medicine, Duke University and John C. Cambier, Dept. of Medicine, Natl. Jewish Hospital.

The biochemical mechanisms coupling receptor immunoglobulin crosslinking with subsequent physiologic changes indicative of B lymphocyte activation are poorly understood. We have initiated studies to define these mechanisms. We have observed that anti-receptor immunoglobulin antibodies (anti-Fab) or multivalent antigen induces murine B cells to undergo plasma membrane depolarization. This depolarization event appears critical and perhaps causal to a subsequent increase in surface Ia antigen expression. Both of these events are important early events in murine B lymphocyte activation. Interestingly, phorbol diester analogs able to bind to and activate murine B lymphocyte protein kinase C are also able to stimulate each of these physiologic changes in an identical dose dependent fashion. In contrast, inactive phorbol diester analogs, which do not bind to and activate this enzyme do not induce these events. These data are interpreted to suggest that protein kinase C activation may be an intermediary event between B cell antigen receptor crosslinking and early events in B cell activation.

0801 ACTIVATION OF PROTEASE ACTIVATED KINASE II BY PHOSPHOLIPIDS AND DIACYLGLYCEROL. Mary I. Gonzatti-Haces and Jolinda A. Traugh, University of California, Riverside, CA 92521.

Protease activated kinase II has been shown to be activated in 3T3-L1 cells in response to insulin with the consequent phosphorylation of ribosomal protein S6. The inactive form of protease activated kinase II was highly purified from rabbit reticulocytes and shown to be activated *in vitro* by limited proteolytic digestion. Protease activated kinase II phosphorylated the β subunit of initiation factor 2 (eIF-2) and histone 1 in addition to ribosomal protein S6. Treatment of the inactive enzyme with a mixture of phosphatidylinositol (10 μg/ml), phosphatidylserine (10 μg/ml) and 1,2-diolein (0.8 μg/ml) in the presence of 0.5 mM EGTA, resulted in a 2 to 5-fold activation of the enzyme as determined by phosphorylation of ribosomal protein S6, initiation factor 2 and histone 1. The activation by phospholipids and diolein was independent of Ca^{2+} and concentrations of Ca^{2+} greater than 0.5 mM inhibited the protein kinase. The degree of activation of protease activated kinase II by phospholipids and diolein was similar to that achieved by limited proteolysis. The proteolytically activated form of the enzyme could not be activated further by the phospholipid mixture. Tryptic phosphopeptides of ribosomal protein S6 and the β subunit of eIF-2 were analyzed by two-dimensional peptide mapping. Phosphopeptides obtained upon activation of protease activated kinase II by phospholipids and diolein were the same as those observed following activation by limited proteolytic digestion.

0802 INNER AND OUTER NUCLEAR MEMBRANE: DYNAMIC AND PHYSICAL DIFFERENCES, Melvin Schindler, Dept. of Biochemistry, Michigan State University, East Lansing, MI 48824

The inner and outer nuclear membrane and underlying matrix have been implicated as central components of most nuclear events, e.g., replication, hormone-growth factor stimulation, chromatin and nuclear pore assembly, viral assembly, and carcinogenesis. The outer nuclear membrane is studded with polyribosomes and closely resembles the rough endoplasmic reticulum with which it shares continuity. The inner nuclear membrane, on the other hand, has no ribosomes and maintains intimate contact with a cytoskeletal superstructure composed of nuclear matrix proteins termed nuclear lamins, chromatin, and RNA. Nuclear membrane matrix interactions may serve in analogy with plasma membrane-cytoskeletal interactions as: a) the means to signal for specific metabolic and replicative events and b) define, order and maintain specific topological sites and orientations in the nuclear inner membrane for differentiated function. Lateral mobility of nuclear membrane components has been invoked to provide a mechanism by which a two-way communication could be promoted between the inner and outer nuclear membrane, endoplasmic reticulum and other intracellular organelles. A two-dimensional diffusive pathway has also been implicated in the macroassembly of annular subunits into nuclear pore complexes in the membrane, and recent evidence suggests that it serves as a transport mechanism in nucleocytoplasmic transport of RNA and ribosomal precursors. Diffusion of lectin receptors and lipids in the outer and inner nuclear membrane is characterized and a method that selectively depletes outer nuclear membrane while retaining the inner membrane is presented. (This work was supported by NIH Grant GM 30158).

0803 ACTIONS OF INSULINOMIMETIC LECTINS ON CELLS WHICH LACK DETECTABLE PLASMA MEMBRANE INSULIN BINDING SITES AND ARE INSULIN-INSENSITIVE, Cecilia A. Hofmann and Marco Crettaz, Loyola University Medical Center, Maywood, IL 60153 and Joslin Diabetes Center, Boston, MA 02215

In Madin-Darby canine kidney (MDCK) cells, specific plasma membrane binding of ^{125}I-insulin was undetectable. Accordingly, neither insulin-stimulated incorporation of [^{14}C]glucose into glycogen nor insulin-induced uptake of radiolabeled α-aminoisobutyrate ([^{3}H]AIB) could be demonstrated, thus suggesting that MDCK cells lacked specific cell surface insulin receptors. To further probe for receptors, MDCK cultures were surface labeled using Na^{125}I and lactoperoxidase or were biologically labeled using [^{35}S]methionine. When solubilized cells were immunoprecipitated with human sera containing antibodies to the insulin receptor (AIR) and precipitates analyzed by SDS gel electrophoresis, no evidence for insulin receptor components was found. Also, when intact MDCK cells were incubated with rabbit serum containing AIR and subsequently labeled with ^{125}I-protein A, insulin receptor components were not detected. Control H4 hepatoma cells bound insulin, responded with increased glycogen synthesis and amino acid uptake, and had immunologically recognizable insulin receptor components. In MDCK cells, the insulinomimetic lectins concanavalin A (con A) and wheat germ agglutinin (WGA) stimulated [^{3}H]AIB uptake, and such stimulation was depleted by the respective lectin-specific monosaccharides D-mannose and N-acetyl-D-glucosamine. These data indicated that the insulin-like activity of Con A and WGA could be elicited even in the absence of detectable specific plamsa membrane insulin binding sites in MDCK cells.

0804 THE EFFECT OF INSULIN ON FLUID PHASE ENDOCYTOSIS AND EXOCYTOSIS IN RAT ADIPOCYTES, E. Michael Gibbs and Gustav E. Lienhard, Dartmouth Medical School, Hanover, NH 03756

In rat adipocytes, glucose transporters and insulin receptors are distributed between the plasma membrane and intracellular membranes. Insulin causes an increase in the fraction of transporters in the plasma membrane and a decrease in the fraction of receptors in the plasma membrane. If the processes that lead to these redistributions account for a significant portion of total endocytosis and/or exocytosis, then insulin should alter the rate of fluid-phase endocytosis and exocytosis in adipocytes. In order to test this possibility, we have determined the effect of insulin on the rates at 37° (a) of uptake of radiolabeled, chromatographically purified sucrose and EDTA and (b) of release of sucrose from cells previously loaded with this compound. The effect of insulin on the rates of uptake was variable in magnitude and significance: in 10 experiments, although insulin increased the rate of uptake by an average of 22% (range -24 to 67%), the effect in each experiment was not significant at P=0.05; in 6 other experiments a significant increase that averaged 47% (range 20 to 75%) was found. Basal rates of uptake were 2.0 ± 0.1 and 5.0 ± 0.4% ($\bar{x}$ ± SEM) of cell water/h for sucrose and EDTA, respectively. The increase due to insulin did not correlate with the variation in the effect of insulin on combined glucose transport and metabolism, which was also determined in each experiment. Concerning exocytosis, in 5 experiments there was no significant effect of insulin on the rate of release of sucrose. Supported by a fellowship from the Juvenile Diabetes Foundation to E.M.G. (No. 83F054) and NIH grant AM 25336.

0805 MONOCLONAL ANTIBODIES AS STRUCTURAL PROBES FOR CHARACTERIZING UNIQUE SUBCLASSES OF TYPE II cAMP-DEPENDENT PROTEIN KINASES IN BOVINE BRAIN, Sharon L. Weldon and Susan S. Taylor, University of California, San Diego, La Jolla, CA 92093.

Two unique subclasses of regulatory subunits (R) of the type II cAMP-dependent protein kinase have been distinguished in bovine brain utilizing monoclonal antibodies. The brain R has been characterized as a type II kinase on the basis of DEAE elution, autophosphorylation, and size. Monoclonal antibodies directed against heart R crossreacted 5-80 fold less efficiently with brain R than with the original antigen in competitive displacement RIA's. Brain and heart R can also be distinguished by mobility changes on SDS-PAGE following autophosphorylation. These changes in mobility along with immunoblotting data indicate that there are at least two unique subclasses of R in brain. In heart R, one of the antigenic sites has been localized to a small peptide very near the major site of autophosphorylation in the cAMP-binding domain. The corresponding region of the brain R has been sequenced and several changes in the primary structure are evident. Immunoprecipitation of total purified R from bovine brain with this antibody has indicated that less than 30% of the R is crossreactive. Similar results have been obtained with a second antibody whose antigenic site has been localized to the domain involved in dimer interaction. The differences in linear sequence establish that the brain contains unique subclasses of cAMP-dependent protein kinase II which are distinct polypeptide chains from the type II R's in heart. The expression of these different type II R gene products must result from a complex control mechanism.
Supported by USPHS Grant GM 19301.

0806 ASSEMBLY OF COMPLEX III INTO THE YEAST MITOCHONDRIAL MEMBRANE, Diana S. Beattie, Anita Sidhu and Keya Sen, Dept. of Biochem., Mt. Sinai Sch. Med., New York, N.Y. 10029

Complex III of the mitochondrial respiratory chain catalyzes electron transport coupled to ATP synthesis and ion transport from reduced coenzyme Q to cytochrome c. An enzymatically active complex II isolated from bakers' yeast in this laboratory was shown to contain 7 subunits ranging in molecular weight from 49-14 kDa. Kinetic studies using double-labeled yeast cells followed by immunoprecipitation of complex III indicated that the different subunits are assembled into the complex at very different rates. Subunits I and V are assembled very slowly, while the assembly of subunits III, IV and VII into the membrane-bound complex occurs very rapidly. The iron-sulfur protein (subunit V) is synthesized as a larger precursor, which is then cleaved into an intermediate form in the cytosol. The intermediate then undergoes further proteolytic processing, while in the inner mitochondrial membrane associated with the other proteins of the complex, to generate the mature form of the protein. In mutants of yeast lacking cytochrome b, the mitochondrial membranes contain less than 50% the amount of the iron-sulfur protein determined by the sensitive immunoblotting technique, although cytochrome c_1 is present in the same amount as in the wild-type. These results suggest that the processing and assembly of the iron-sulfur protein into the mitochondrial membrane requires the presence of cytochrome b. (Supported by NIH grant HD-04007).

0807 INSULIN RECEPTOR-LINKED SIGNALING MECHANISMS REGULATING CELL GROWTH, Jonathan Seals, Carol McDonald and Catherine Chang, U. of Massachusetts Med. Sch., Worcester, MA 01605

The signaling and effector mechanisms linking the insulin-receptor interaction to acute regulation of target cell enzyme activities have been extensively studied. The potential roles of receptor autophosphorylation, putative insulin mediators, and target enzyme phosphorylation changes have been emphasized, though no conclusive model has coalesced from the pieces of data now available. In contrast, the mechanism by which insulin regulates cell growth has been studied sparingly. Most consideration has been given to the role of receptor tyrosine kinase activity (similar to other growth factors) or to the internalization and binding of insulin to the nucleus. We have investigated the possible existence of endogenous cellular regulatory components that act directly on nuclear processes and are altered by insulin, as potential elements of the effector system. Pariculate-free extracts (150,000xg supernatants) were made from cultures of H-35 hepatoma cells grown in the presence or absence of insulin for 24 hr after serum-deprivation for 48 hr. The extracts were added to nuclei isolated from rat liver, and the incorporation of ^{3}H-TTP into DNA was measured. The extracts from insulin-treated cells stimulated thymidine incorporation by 2-3 times more than control extracts. The active component(s) was destroyed by proteases and appeared to be of M_r<3000 by exclusion HPLC. Purification by a factor of ~5000 has been achieved using anion exchange and reversed phase HPLC. The purified component will be identified and compared to reported mediators of insulin action on metabolic enzymes. These results will contribute to our concept of the insulin effector system and to the understanding of growth regulation at the molecular level.

0808 L. PNEUMOPHILA TOXIN SELECTIVELY INFLUENCES THE ACTIVATION OF MEMBRANE BOUND NADPH OXIDASE, Janis E. Lochner and Barbara H. Iglewski, Lewis and Clark College, Oregon Health Sciences University, Portland; OR 97201

Activation of the oxidative metabolism of polymorphonuclear cells (PMN) graphically illustrates the influence of surface interactions on cell function. Upon interaction with appropriate stimuli, a membrane bound pyridine nucleotide oxidase is activated with resultant production of superoxide ($O_2^{\cdot}$). Further reactions of $O_2^{\cdot}$ produce other toxic oxygen metabolites including the hydroxyl radical ($OH^{\cdot}$), singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2). These oxygen metabolites are critical to the PMN's physiological function of eliminating invading microorganisms. Legionella pneumophila, manages to evade the microbicidal action of PMN and survive intracellularly. Critical to the organisms intracellular survival is the production of a low molecular weight toxin, L. pneumophila toxin. This toxin selectively influences the activation capability of PMN. Phagocytic stimuli fail to activate the oxidative metabolism of toxin treated PMN. The membrane bound oxidase of toxin treated PMN is, however, triggered by a variety of soluble stimulators including the phorbol diester 12 o-tetradecanoyl phorbol-13-acetate. The ability of L. pneumophila toxin to selectively influence oxidase activation appears to be related to the effect toxin has on inhibiting the uptake of extracellular calcium. Studies using $^{45}Ca^{++}$ indicate that the uptake of this ion in response to stimulatory agents by toxin treated PMN is severely depressed as compared to control PMN.

0809 ROLE OF PHOSPHATIDYLINOSITOL HYDROLYSIS AND PROTEIN KINASE C ACTIVATION IN EARLY B CELL ACTIVATION EVENTS, Kenneth M. Coggeshall and John C. Cambier, National Jewish Hospital, Denver, Co 80206. Murine B cells treated with specific antigen or rabbit anti-mouse immunoglobulin (RAMIG) undergo a rapid loss of membrane potential and a subsequent increase in surface I-A antigen expression. We have proposed that these events are important consequences of "first signals" in thymus-dependent B cell activation. In view of this we have begun investigations of the mechanism by which B cell surface Ig crosslinking and depolarization are coupled. We have observed that certain phorbol esters are able to induce B cell depolarization suggesting that PKC activation may be an important coupling event. Nishizuka et al. have suggested that PKC activation is linked to phosphatidylinositol (PI) hydrolysis and the generation of diacylglycerol (DG). Maino et al. have demonstrated activation of PI hydrolysis in RaMIg-treated B cells. We hypothesize that this PI hydrolysis results in sequential PKC activation and alterations in ion flux manifest by depolarization. Here we describe our efforts to test this hypothesis by examining the effects of artificially inducing or inhibiting the production of DG. We have observed that exogenously added phospholipase C or DG is able to induce depolarization in B cells in a dose-dependent manner. We have further observed that RaMIg-induced but not phorbol-induced depolarization is blocked by raising intracellular cyclic AMP, a condition known to inhibit DG production. We suggest this is because receptor-mediated activation of PK-C requires DG production and thus is susceptible to cyclic AMP inhibition while direct activation of PK-C is not. Results are consistent with the notion that DG production and PK-C activation are important biochemical events in receptor Ig mediated transmembrane signalling in B cells.

0810 PHOSPHORYLATION OF THE 17β-ESTRADIOL RECEPTOR, Ferdinando Auricchio and Antimo Migliaccio, Istituto di Patologia Generale, Università Napoli, 80138 Napoli, Italy

Recent work from our laboratory has shown that the hormone binding of the 17β-estradiol receptor from calf uterus is controlled by a cytosol receptor-kinase that activates this binding and by a nuclear receptor-phosphatase that inactivates it (Biochem. Biophys. Res. Commun. 1982, 109 , 1002). In a crude system it has been observed that Ca^{2+} stimulates the receptor-kinase. Using a purified system it is now shown that calmodulin mediates Ca^{2+}-stimulation of kinase and that Ca^{2+} and calmodulin are effective at physiological concentrations. These experiments as well as the results of phosphoaminoacid analysis of the receptor are presented together with the classical model of mechanism of action of estradiol modified according to our more recent findings.

0811 ACTIVATION OF RIBOSOMAL PROTEIN S6 PHOSPHORYLATION AND ITS POSSIBLE IMPLICATION IN THE REGULATION OF PROTEIN SYNTHESIS, Jorge Martin-Perez, Michel Siegmann and George Thomas, Friedrich Miescher-Institut, P.O. Box 2543, CH-4002 Basel, Switzerland

Stimulation of 3T3 cells to proliferate by mitogens leads to phosphorylation of ribosomal protein S6 and a parallel increase in protein synthesis. Results with inhibitors of both processes in intact cells argue that phosphorylation is a prerequisite for activation of protein synthesis and not a consequence of it. Indeed recent findings demonstrating that the requirement for S6 phosphorylation cannot be separated from protein synthesis by titrating with increasing concentrations of serum or epidermal growth factor (EGF) and that the most highly phosphorylated forms of S6 have a selective advantage in entering polysomes (Cell, 30, 235, 1982). Amino acid analysis of S6 reveals that phosphate is mostly in serine and a small amount in threonine. Analysis of typtic peptides demonstrate that there are 11 major phosphopeptides and that following activation by serum these phosphopeptides are phosphorylated in a highly specific order (PNAS, 80, 926, 1983). Agents such as EGF, prostaglandin $F_{2\alpha}$ and insulin when added alone have only partial effects on S6 phosphorylation and protein synthesis but act synergistically on both processes when added together. Separately they phosphorylate the same peptides suggesting that synergism is expressed through a common kinase(s). Experiment in vitro and, in intact cells show that cAMP affect the first two sites of phosphorylation induce by insulin, but cannot beyond that, whereas insulin operating through and independent kinase(s) can phosphorylate the rest of the sites.

0812 IONIC CONTROL OF PARATHYROID ADENYLATE CYCLASE ACTIVITY, Susan B. Oldham and Loren G. Lipson, USC School of Medicine, Los Angeles, CA 90033

The parathyroid gland is unusual among secretory tissues in that calcium inhibits rather than stimulates secretion of the cellular biosynthetic products, in this case, parathyroid hormone (PTH). Pharmacologic agents which stimulate parathyroid adenylate cyclase (AC) activity stimulate PTH secretion, and AC antagonists suppress PTH secretion. The role of AC in the physiological regulation of PTH secretion by calcium is less clear. Using an EGTA buffer system to attain low ambiant calcium concentrations, we have studied the effects of calcium on AC activity in partially purified porcine parathyroid membranes. Calcium produced a biphasic inhibition of AC activity which can be mathematically described by two independent calcium inhibition sites with an apparent K_I of 1 μM and 200 μM, respectively. The high affinity calcium inhibition site does not appear to be mediated by calmodulin since extensive washing of the membranes with EGTA failed to abolish this site; and neither of the calmodulin inhibitors, trifluoperazine and W_7, altered the affinity of this site or produced a calcium-dependent alteration of AC activity. Kinetic analysis of the calcium inhibition at the high affinity site revealed calcium to be a competative inhibitor of magnesium, which is a requisite activator of the enzyme. Our findings suggest that the AC of parathyroid tissue has an intrinsic high sensitivity to calcium, a characteristic which is not generally shared by the AC present in most tissues.

0813 EFFECTS OF POLYMYXIN ON THE MEMBRANE COMPOSITION OF PM2 GROWN IN ALTEROMONAS ESPEJIANA, Arun K. Misra, Dept. of Biology, University of Mississippi,University 38677 PM2, a bacterial virus, lyses the marine bacterium A. espejiana and is enveloped by a membrane which is assembled inside the host. The ratio of phosphatidylglycerol(PG) and phosphatidyl-ethanolamine(PE) in the virus membrane(1.7) is different from that of the host membrane (0.37). Polymyxin was utilized to alter the phospholipid composition of the host membrane and the virus, PM2, was grown in these mutant cells. As determined by ^{32}P autoradiography, the lipid composition i.e. the PG/PE ratio, of the host was changed from 0.37 to 1.45. It was assumed that the higher proportion of PG in mutant cells might facilitate enhanced assembly of PM2 because the PG/PE ratio more closely approximated that of viral membrane. However, the production of PM2 was reduced 20-fold when grown in the mutant cells, and PG/PE ratio of the virus changed from 1.7 to 0.7. It was concluded that either replication or adsorption(to bacterial cells) of the virus was inhibited by altered membrane composition of the host, and that there may be a direct role for host membrane in the formation of viral envelope.This work was conducted in the laboratory of Gregory J. Brewer,Dept. of Microbiology and Immunology Southern Illinois School of Medicine, Spring field,PO Box 3926, Illinois,62708; under a grant from NIH(AI17697) and NSF(PCM-77-02733).

Regulation of Gene Expression

0814 PROCESSING, SECRETION and FUNCTION OF THE YEAST KILLER TOXIN. Donald J. Tipper and Keith A. Bostian, U. Mass. Medical School, Worcester, MA 01605, and Brown University, Providence, R.I. 02912.

Yeast killer toxin consists of two disulfide-linked 9.5 Kb subunits, α and β. Toxin binds to a β-1,6D-Glucan cell wall receptor and then, in an energy-dependent step, inserts into the cytoplasmic membrane, producing proton-permeable spores. Sequence analysis shows a probable membrane-inserting active site in α. This may be masked in the disulfide-bonded dimer and released on binding to the receptor.

Toxin is processed in secretion vesicles from a preprotoxin by removal of a 44 amino acid N-terminal leader and excision of a central 103 amino acid γ peptide that separates α and β. γ is co-translationally glycosylated and may protect producing cells against toxin activation, both during processing and subsequently, as the specific toxin immunity determinant. Investigations of expression of the natural dsRNA killer plasmid and of a cDNA copy of the preprotoxin gene suggest that the secretion signal peptide is not co-translationally processed. We are attempting, by manipulation and in vivo mutagenesis of the cDNA copy, to confirm the role of γ in immunity, localize the determinant in vivo, and determine the requirements for secretion and processing of toxin.

0815 REGULATED EXPRESSION OF PORCINE MAJOR HISTOCOMPATIBILITY DNA SEQUENCES INTRODUCED INTO MOUSE L CELLS. M. Leonardo Satz and Dinah S. Singer, Immunology Branch, NCI, NIH, Bethesda, MD 20205

Mouse L cells have been transformed with a swine genomic clone, which encodes a major histocompatibility (MHC) antigen SLA^d. The regulation of expression of this heterologous genomic DNA segment and its chromatin structure in mouse L cells have been investigated. The transformed L cells, which contain about 2 copies of the 17.8 Kb pig DNA insert per haploid genome, stably and uniformly express SLA antigen on their surface. This expression is the result of differential transcription of the 3.4 Kb long SLA gene; the other 14 Kb of pig DNA sequences flanking the coding suquence are not transcribed. Although the entire pig DNA segment is packaged into nucleosomes, only the transcriptionally active DNA sequences are packaged in a DNase I sensitive conformation. Treatment of the transformed cells with mouse interferon (IFN) markedly enhances the expression of the heterologous SLA^d as well an endogenous $H\text{-}2^k$ antigens, but does not affect the expression of an unrelated surface antigen, gp 70. Flanking swine DNA sequences which are not transcribed in the transformant in the absence of IFN are also not transcribed in the presence of IFN. The elevated expression of SLA^d antigens results from an increased rate of transcription of SLA coding sequences in IFN-treated cells. Taken together, these results suggest that the expression of the foreign DNA segment is actively regulated in L cells.

0816 EXPRESSION OF IL2 RECEPTOR ON A T HELPER CELL CLONE, Alice Dautry-Varsat, George Bismuth, Maryse Duphot, Jean-Louis Moreau and J. Thèze

A T cell helper clone specific for the synthetic random polymer poly(Glu^{60}, Ala^{30}, Tyr^{10}) (GAT) has been derived from long term cultured cells. This clone, 52.3, has 2 states of differentiation in vitro. Unstimulated cells do not express Interleukin 2 (IL2) receptors and are morphologically close to small resting lymphocytes. The same cells can be activated after culture with the antigen GAT and antigen presenting cells. These cells now express IL2 receptors and are morphologically close to blast cells. These two states, activated and inactivated are fully reversible.

0817 SYNTHESIS AND OLIGOSACCHARIDE PROCESSING OF NORMAL AND ALTERED MOUSE IMMUNOGLOBULIN M DURING B-CELL DIFFERENTIATION, M. Patricia Beckmann, Luz M. Vazquez, and William J. Grimes, Department of Biochemistry, University of Arizona, Tucson, AZ 85721.

In order to study glycoprotein biosynthesis and processing, we have chosen the murine IgM system as a model. This system presents cells which synthesize intracellular, membrane-bound, and secreted IgM in a developmental manner. Using 35S-methionine pulse-chase labeled cells of each developmental stage, we have examined the kinetics of IgM synthesis and processing. This work was done with immunoprecipitated IgM which was treated with or without endoglycosidase H, analyzed on SDS-PAGE and autoradiographed. Our results suggest that the timing of synthesis and processing is dependent on the final subcellular destination of this glycoprotein, and that characteristic rates for membrane versus soluble IgM may be dependent on the extent of oligosaccharide processing.

In addition, we are determining how protein structural requirements affect final glycosylation patterns on the glycoprotein. Using mutant cells which secrete a larger or smaller than normal heavy chain, we have analyzed for gene rearrangements with restriction enzyme mapping and Southern blots, and have characterized timing of processing. We are currently evaluating the major oligosaccharide structures at individual glycosylation sites on the mutants. One of the mutants is characterized as hyperglycosylated, and the other - a deletion mutant - is missing portions of $C\mu 3$ and $C\mu 4$ in the protein.

0818 CLONING AND EXPRESSION IN E.COLI OF A GENE FOR HUMAN INSULIN-LIKE GROWTH FACTOR I (IGF-I), Mary Peters+, Ed Lau+, David Snitman+, Margery Nicolson*, Judson Van Wky#, Louis Underwood#, +Amgen Development Inc., Boulder CO 80301, *Amgen Inc., Thousand Oaks CA 91320, #University of North Carolina, Chapel Hill NC 27514

IGF-I, also called somatomedin C, is a 70 amino acid polypeptide that exhibits not only insulin-like properties, but also growth promoting properties in response to human growth hormone. Receptors for IGF-I have been found on adipocytes, chondrocytes, liver membrane and placental membrane. Although levels of IGF-I have been correlated with several factors including nutritional state, human growth hormone levels and insulin levels, its mode of action has not been determined. Lack of sufficient purified IGF-I has hindered research in this area. We therefore undertook the synthesis, cloning and expression of a synthetic gene for IGF-I in E.coli. Synthetic genes coding for IGF-I and as well as analogs of IGF-I have been manufactured, cloned, and expressed at high levels in E.coli. The HPLC purified material is active in all in vitro assays to date for IGF-I. These assays include a radioimmunoassay for human derived IGF-I, stimulation of ^{3}H-thymidine uptake in human foreskin fibroblasts and binding to IGF-I receptors in human placental membrane.

0819 HORMONAL REGULATION OF PHOSPHOENOLPYRUVATE CARBOXYKINASE GENE TRANSCRIPTION, Elmus G. Beale, Kazuyuki Sasaki, Tim Cripe, Stephen Koch and Daryl Granner, University of Iowa, Iowa City, IA 52240

The mechanisms by which cAMP and insulin regulate phosphoenolpyruvate carboxykinase messenger RNA ($mRNA^{PEPCK}$) were investigated in H4IIE hepatoma cells. A nuclear "runoff" assay was used to show that 8-CPT-cAMP and insulin affect transcription of the gene coding for $mRNA^{PEPCK}$. When 0.1 mM 8-(4-chlorophenylthio)cAMP (8-CPT-cAMP) was added to H4IIE cells, there was a 10-fold increase in transcription. Subsequent addition of 1 nM insulin caused a rapid decrease of transcription to the level seen in uninduced cells. Approximately 40 μM 8-CPT-cAMP was required for 50% stimulation of transcription, whereas 2-5 pM insulin or 200 pmol proinsulin was required for 50% inhibition of transcription. These effects were specific since neither 8-CPT-cAMP nor insulin had an effect on the synthesis of total RNA. In addition, the effects of both agents were rapid as 50% changes in transcription occurred within 15 min after the addition of either 8-CPT-cAMP or insulin. The insulin mediated inhibition of $mRNA^{PEPCK}$ transcription was noted in untreated cells or in cells treated with 8-CPT-cAMP, forskolin or dexamethasone. Thus, among these compounds, insulin is the dominant regulatory molecule. The magnitude of the effects and the kinetics of the responses for both 8-CPT-cAMP and insulin indicate that the primary action of both compounds in the acute regulation of phosphoenolpyruvate carboxykinase synthesis is exerted at the level of gene transcription.

0820 SEPARATE MEMBRANE RECEPTORS MEDIATE IMMUNOGLOBULIN MESSENGER RNA INDUCTION AND SECRETION OF TRANSLATED PRODUCT,E.H.Lipford,J.B.Trepel,J.Cossman,L.M.Neckers,NIH,Beth.MD

The time-course of induction of immunoglobulin messenger RNA (mRNA) for u heavy chain, and of appearance of cytoplasmic and secreted IgM, were investigated in normal peripheral blood B cells. We report those studies, plus the unexpected result that the signals regulating immunoglobulin (Ig) mRNA induction and translation are apparently separate from those regulating Ig secretion. B cells were stimulated with Cowan Staph A and culture supernatant (sup) of PHA activated T cells. Mu heavy chain mRNA was maximally induced (2-3 fold) by 18-24 hours. Blastogenesis was seen by 24 hours, but cell proliferation was not detected until 72 hours. Cytoplasmic Ig was detectable by 24 hours, but Ig secretion was not seen until 120 hours. Our results led us to investigate whether these events are independently or jointly regulated. Staph A alone induced Ig mRNA, Ig synthesis and blastogenesis, but had no ability to stimulate secretion.In contrast, T cell sup stimulated Ig secretion, but had no effect on Ig mRNA levels or blastogenesis.We also found that following induction of B cell Ig synthesis by culture with Staph A, the addition of phospholipase C on day 3 or 4 stimulated secretion. Phospholipase C, like T cell sup, did not stimulate Ig mRNA or blastogenesis. These data support a model of B cell activation in which crosslinking of membrane-bound Ig by mitogen or antigen induces an elevation in specific Ig message as well as its translation. This process precedes and is independent of proliferation. Secretion, however,depends on activation of a unique B cell surface receptor by a T cell product. Since phospholipase C can also stimulate secretion, this receptor may regulate an endogenous membrane phospholipase, whose activation may be required for Ig secretion to occur.

0821 REGULATION OF APOLIPOPROTEIN E GENE EXPRESSION BY CHOLESTEROL, Karen Reue, Tripathi Rajavashisth, John Kaptein, and Aldons Lusis, Departments of Microbiology and Medicine, University of California, Los Angeles, CA 90024.

Apolipoprotein E (apo E), a major component of several lipoprotein classes, has previously been shown to occur at elevated levels in plasma of hyperlipidemic humans and animals, and is secreted by cholesterol-loaded mouse macrophages and human monocytes. A nearly full length cDNA clone for mouse apo E has been isolated, sequenced, and utilized to study the regulation of apo E in mouse liver, intestine, and macrophages in response to cholesterol uptake. Apo E mRNA levels in mouse liver and intestine, the major tissues involved in lipoprotein synthesis, remain unchanged (1.0% and 0.01% of total mRNA, respectively) in mice maintained on a high lipid, high cholesterol diet as compared to those on a normal diet. This indicates that the observed increase in circulating apo E protein in cholesterol-fed animals does not result from an induction of apo E mRNA in these tissues. In contrast to liver and intestine, when macrophages are loaded with cholesterol by incubation in vitro with acetylated-low density lipoprotein, levels of apo E mRNA are found to increase at least 3-fold as determined by quantitation of Northern blots, and increases in levels of apo E mRNA can be seen directly with in situ hybridization studies. Interestingly, the apo E mRNA species detected in macrophages appears to be about a hundred nucleotides larger than that seen in other tissues, a feature which may be related to observed differences in regulation of apo E mRNA by cholesterol in distinct cell types.

0822 RETINOIC ACID INDUCED EXPRESSION OF TISSUE TRANSGLUTAMINASE IN MOUSE PERITONEAL MACROPHAGES, Peter J.A. Davies, Michael P. Murtaugh and William T. Moore, University of Texas Medical School at Houston, Houston, TX 77025.

Tissue transglutaminase is an intracellular enzyme that catalyzes the covalent crosslinking of proteins. The enzyme can be found in most cells but is particularly abundant in inflammatory macrophages. Culture of mouse resident peritoneal macrophages in serum-containing media induces a 150-fold increase in the rate of enzyme synthesis and large accumulations (1-2% of cellular protein) of the enzyme. De-lipidization of serum abolishes its inducing activity and re-addition of trans-retinoic acid fully restores the effect. Retinoic acid is a very potent inducer of transglutaminase, the ED50 (10 nM) is comparable to the plasma levels of this retinoid. The effect is very specific, trans-retinoic acid is 50 times more potent than either cis-retinoic acid or retinol. The induction is rapid, increased enzyme synthesis is detectable within 60 min of exposure of the cells to the retinoid. The increased rate of enzyme synthesis is also rapidly reversed by removal of the retinoid. Although delipidized serum potentiates the induction of the enzyme by retinoic acid (likely due to plasma retinol binding protein), retinoic acid also induces tissue transglutaminase in human promyelocytic leukemia cells (HL-60) grown in a chemically defined media. We believe that retinoic acid directly regulates transglutaminase gene expression and that this induction is a component in the functional maturation of myelocytic cells.

0823 α1(III) and α2(I) COLLAGEN mRNA ARE NOT COORDINATELY REGULATED BY TRANSFORMATION IN MOUSE FIBROBLASTS, Gene Liau and Benoit de Crombrugghe, M.D., National Institutes of Health, National Cancer Institute, Bethesda, MD. 20205

A mouse genomic clone was isolated by cross-hybridization using a DNA fragment which codes for the N-propeptide of chicken α1(III) collagen. The region of cross-hybridization within the mouse clone was localized, sequenced and an exon coding for the N-propeptide of mouse α1(III) collagen was identified. This DNA fragment hybridized to a single RNA band of approximately 5,300 nucleotide, this is slightly larger then the α2(I) collagen RNA. The mouse type III collagen probe was used to examine the effect of transformation on α1(III) collagen RNA levels in mouse cells. We find the level of type III collagen RNA, unlike type I collagen RNA, does not decrease extensively after oncogenic transformation. The most striking result was found in RSV transformed cells where the level of α1(III) RNA increased 2-3 fold whereas the level of α2(I) RNA decreased 15-20 fold. Balb 3T3 cells transformed by Kirsten sarcoma virus exhibited decrease type I collagen RNA levels with little change in type III collagen RNA. However, NIH-3T3 cells transformed by various human RAS oncogenes showed little if any effect on type III as well as type I collagen RNA levels. Our data suggests that the type III collagen gene does not respond to the same transformation mediated regulatory mechanism that strongly inhibits the expression of type I collagen gene, as well as the fibronectin genes.

0824 MOLECULAR CLONING OF cDNA FOR THE MOUSE TRANSFERRIN RECEPTOR, Jane R. Parnes, Linda K. Clayton, Thomas P. St. John, Irving L. Weissman and Amy C. Vollmer, Stanford University Medical Center, Stanford, CA 94305

The transferrin receptor is a 190,000 dalton cell surface glycoprotein consisting of two apparently identical chains. In addition to its role in transport of transferrin-bound iron into cells, this protein is notable for the tight linkage of its expression to cellular proliferation. We have attempted to isolate cDNA clones encoding the mouse transferrin receptor to study the relationship of its expression to cell proliferation. We have used monoclonal antibodies directed against the mouse transferrin receptor to screen two cDNA libraries constructed from mouse T cell tumor lines using the expression vector λgt11. This vector allows expression of the cDNA inserts as fusion proteins with β-galactosidase. We have isolated about 20 clones which reproducibly synthesize a fusion protein containing the epitopes recognized by the monoclonal antibodies against the mouse transferrin receptor. We are using a variety of methods to further characterize these clones.

0825 GLUCOCORTICOID REGULATION OF rGH GENE EXPRESSION IN MOUSE L CELLS, M. J. Birnbaum and J. D. Baxter, Met. Res. Unit, Univ. of Calif. San Francisco, CA 94143

Glucocorticoids and thyroid hormone increase rat growth hormone (rGH) gene expression in cultured rat pituitary tumor cells. In order to elucidate the mechanism of hormone regulation, the cloned rGH gene was stably transferred into mouse L cells using the Herpes Simplex Virus Thymidine Kinase gene for selection of transformants. L cells that took up the rGH gene expressed a mature transcript approximately 0.75 kb in length, in contrast to the 1.1 kb transcript found in pituitary cells. In addition, glucocorticoids increased rGH message levels in L cells 3- to 5-fold. When a mutant rGH gene lacking all sequences upstream from the physiological start site was transferred into L cells, two predominant mature transcripts of 0.75 and about 1.1 kb were found. Both were regulated by glucocorticoids and, as ascertained by single-stranded nuclease protection experiments, terminate at the physiological polyadenylation site. The smaller transcript appears to start early in the second intron, while the larger transcript initiates in the region of, but not at, the start site used in pituitary cells. Thus, we have shown that: 1) 5'-flanking sequences of rGH gene are not required for regulation by glucocorticoids; and 2) glucocorticoids regulate levels of mRNA initiated at non-physiological sites.

0826 HETEROGENEITY OF SB2-SPECIFIC HUMAN CYTOTOXIC T CELL (CTL) CLONES. Stephen Shaw, Timothy A. Springer and William E. Biddison, NIH, Bethesda, MD 20205

To explore the mechanisms which contribute to T cell diversity, we have analyzed differences between Ia-specific human CTL clones of similar antigen-specificity. Ten CTL clones, derived from 2 donor pairs, were all "SB2-specific" (i.e. they proliferated to cells expressing SB2 but generally not to SB2-negative cells). However, numerous differences between clones were observed: a) Fine specificity: 3 clones proliferated in response to a subset of SB2-negative donors; b) Phenotype: 9 of the clones were OKT4+8- but one was OKT4-8+. c) anti-Ia inhibition: the 18 monoclonal antibodies (of 70 tested) which were able to inhibit CTL activity were those which bound SB region products. Differences in inhibition of the clones provided no evidence for recognition of different Ia epitopes, but suggested that some clones were more easily inhibitable than others. d) anti-effector antibody inhibition: differences were observed between antibodies to T3, T4, LFA-1 and LFA-2 with respect to extent of inhibition, and consistency of inhibition on different clones. For example, the inhibition by LFA-2 was similar between clones (70-90% inhibition of lytic activity) while inhibition by OKT3 ranged between less than 30% to greater than 95% for different clones. Variability was also seen with anti-T4 antibody blocking. Although clonal differences in antigen-specific receptor presumably contribute to such heterogeneity, these data indicate that there may be differences between clones in the organization of the ensemble of other molecules CTL can use in recognition/activation.

0827 IDENTIFICATION OF SEQUENCES IMPORTANT FOR INTRACELLULAR TRANSPORT AND MEMBRANE INTEGRATION OF A TRANSMEMBRANE GLYCOPROTEIN BY IN VITRO MUTAGENESIS, M. C. Zuniga, S. J. Horvath and L. E. Hood, California Institute of Technology, Pasadena, CA 91125

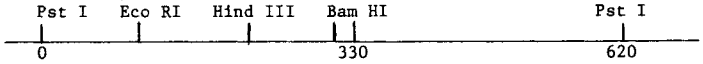
Class I histocompatibility antigens are integral membrane glycoproteins found on virtually all nucleated cells of a mammal. The class I molecule has a 24-residue hydrophobic transmembrane (TM) segment flanked by charged residues. The basic residues on the cytoplasmic side of the TM segment have been postulated to interact with the negatively-charged phospholipid groups on the inner face of the membrane, thereby fixing the protein in the membrane. Alternatively, these residues might serve as signals for intracellular transport of the protein. We employed in vitro mutagenesis of a cloned class I histocompatiblity antigen gene, the L^d gene of the BALB/c mouse. We used BAL31 nuclease to delete sequences which encode the basic amino acids following the TM segment, and subsequently replaced the deleted sequences with stop codons or codons resulting in acidic residues followed by a stop codon. The replacement was achieved by ligation of the BAL31 digestion products to a synthetic oligonucleotide linker containing stop codons in every reading frame. Using this strategy we generated a variety of mutant L^d genes whose products range from those having no basic residues at the end of the transmembrane segment to those having one or more basic residues and from those having no acidic residues at the end of this region to those having acidic residues either alone or in combination with remaining basic residues. The expression of the mutant L^d genes was examined after introduction into mouse L cells and the cellular distribution of the mutant L^d gene products was determined with anti-L^d monoclonal antibodies.

0828 Analysis of the Glucocorticoid Receptor Binding Site in the LTR of Mouse Mammary Tumor Proviral DNA. Magnus Pfahl, The Salk Institute for Biological Studies, Regulatory Biology Laboratory, P.O. Box 85800, San Diego, CA. 92138.

Knowledge of the exact DNA sequence or sequences recognized by a steroid-receptor complex is necessary for a better understanding of the regulatory mechanism by which steroid hormones act. We have therefore undertaken a detailed analysis of the DNA sequences to which glucocorticoid receptor when complexed with a steroid (agonist) binds on the LTR of MMTV and have also investigated the possibility of altered DNA structure in or near those sites. A 500 bp LTR fragment previously shown to contain a specific receptor binding site or sites was cloned into the PsTI site of PBR322 and deletions were constructed extending from the EcoRI site on PBR322 to various degrees into the cloned LTR fragment. Binding characteristics of the 500 bp fragment and deletions extending to -139, -100 and +26 with respect to the RNA cap site were analyzed in the DNA-cellulose competition assay. DNA footprinting studies using purified glucocorticoid receptor and cloned DNA fragments were also carried out. Results of these and previous studies suggest that the glucocorticoid receptor recognizes DNA sequences 200 to 70 bp 5' of the RNA cap site and that this binding region is composed out of subsites. To investigate the possibility that an altered DNA structure, sensitive to S1 nuclease is present in or near the receptor binding site, a supercoiled plasmid carrying a LTR insert was digested with S1 nuclease. An S1 sensitive site in the receptor binding region could be detected by blot hybridization.

0829 PARTIAL STRUCTURE OF A HORMONALLY-INDUCIBLE ENZYME, TYROSINE AMINOTRANSFERASE, AND ITS mRNA. James L. Hargrove and Barbara Baumstark, Emory University, Atlanta,Ga. 30322.

No information exists concerning the primary structure of tyrosine aminotransferase, an hepatic enzyme whose synthesis is regulated by several hormones and that is subject to co- or post-translational acetylation, phosphorylation and limited peoteolytic cleavage. We are approaching this problem by seeking to purify and sequence specific peptide fragments from the enzyme and by determining the sequence of cDNA's that have been cloned into pBR322 and pUC8 by Scherer et al. {Proc. Nat'l. Acad. Sci. USA 79, 7205-7208 (1982)}. One of the four plasmids (pcTAT2) appears not to overlap the translated coding sequence, whereas the other three share some restriction fragments and appear to include as much as 700 bases within the coding sequence. Restriction analysis provides the following map of one of these, pcTAT3:



An open reading frame found in this DNA begins on the left of the Eco RI site and continues through all fragments sequenced to the right-hand Pst I site. The predicted C terminal amino acids agree with results derived from timed hydrolysis with carboxypeptidase B (Gly. Ser...). We have isolated a peptide containing the active site lysine to which the cofactor, pyridoxal phosphate, binds, and have obtained a partial amino acid sequence. Details concerning these data will be presented.

0830 LIVER TYROSINE KINASE ACTIVATION DURING EARLY STAGES OF CHEMICAL HEPATOCARCINOGENESIS, Jack W. Olson, College of Pharmacy, University of Kentucky, Lexington, Ky. 40506.

Protein phosphorylation at tyrosine residues is believed to be involved in several important cellular processes since tyrosine-specific protein kinase activation is associated with stimulation of cellular proliferation by hormones and growth factors, embryogenesis and retroviral cell transformation. Because cell proliferation is thought to be an essential component of chemical carcinogenesis, liver tyrosine-specific protein kinase activity was examined during the early stages of the Solt and Farber chemical hepatocarcinogenesis model. Rats were given diethylnitrosamine (DEN) in one dose (200 mg/kg, i.p.) followed by 2 weeks of dietary 0.02% 2-acetylaminofluorene (2-AAF) starting at day 14 after DEN, followed by partial hepatectomy (PH) on day 21. By day 28 this regimen produces a relatively synchronized population of hyperplastic liver nodules about 1 mm in diameter. Rats were sacrificed, their livers perfused with cold buffer, homogenized and centrifuged at 1,000 g for 10 min. The resulting supernatant was centrifuged at 30,000 g for 30 min and the pellet was assayed for tyrosine kinase activity using the synthetic peptide, Val^5Angiotensin II, as substrate. At day 32, rats which received the complete regimen had a 2.6 fold increase in their liver tyrosine kinase activity as compared to sham controls (2.4 pmoles/min/mg protein vs. 6.4 pmoles/min/mg protein, $p<.05$). In contrast, rats which received a partial regimen (i.e. PH only, or 2-AAF + PH, or 2-AAF + DEN) did not have elevated tyrosine kinase activity nor did they have hyperplastic nodules. This preliminary data suggests that prolonged activation of liver tyrosine kinase is associated with the very early stages of chemical hepatocarcinogenesis. Supported in part by PHS grant number CA31099, awarded by the National Cancer Institute, DHHS.

0831 REGULATION OF EXPRESSION OF T CELL GROWTH FACTOR IN A HUMAN T CELL TUMOR LINE Vernon C. Maino and Doran Pace, Becton Dickinson Monclonal Ctr., Mt. View, CA 94043

Requirements for induction of T cell growth factor (IL-2) in the human T cell line, JM, were found to be negative for constitutive production of IL-2. However these cells were stimulated to produce up to 100 units/ml when treated with anti-Leu-4 antibody in the presence of a mouse B cell line, 2PK3. IL-2 was not produced in the absence of either 2PK3 cells or anti-Leu-4. Less optimal expression of IL-2 was also observed when other mouse and human B cell and monocyte lines provided accessory cell function. Furthermore other anti-T cell monoclonal antibodies could be substituted for anti-Leu-4 to induce 50% less IL-2 than was possible with anti-Leu-4. In a serum-free medium containing fatty acids, JM cells constitutively produced up to 50 units/ml of IL-2; the addition of anti-Leu-4 and 2PK3 cells enhanced production of IL-2 by at least two-fold. Subcellular fractionation of induced JM cells indicated that in addition to activity in the culture medium, IL-2 was also expressed as a membrane-associated protein. The relationship of membrane and soluble IL-2 synthesis and the cell surface requirements for induction is currently being evaluated.

0832 ACTIVATION OF A TYROSINE SPECIFIC KINASE AT FERTILIZATION. Dr. William H. Kinsey. Univ. of Miami School of Medicine. Miami, Florida 33101.

The sea urchin egg contains a protein kinase which phosphorylates tyrosine residues of endogenous membrane proteins as well as synthetic peptide substrates. Fertilization results in an increase in tyrosine kinase activity which first becomes apparent 20-30 minutes post insemination and continues throughout the early cleavage stages. To evaluate the role of the sperm in this process, eggs were pretreated with cytochalisin B, then fertilized and assayed for kinase activity. This treatment allows sperm to bind to the egg and induce the cortical reaction, but prevents incorporation of the sperm into the egg cytoplasm. Eggs inseminated under these conditions exhibited the normal increase in kinase activity at 30min post insemination. Fertilization normally results in the onset of protein synthesis beginning 15-20 min post insemination. However, when protein synthesis was inhibited by pretreating eggs with emetine (>99% inhibition) the normal increase in kinase activity occured 15-20 minutes post fertilization although some inhibition became apparent after two hours of development. Similarly, NH_4Cl, a parthenogenic agent which induces a normal burst of protein synthesis, failed to illicit an increase in tyrosine specific kinase activity within 30 min.
These results demonstrate that the initial cell surface interaction(s) between sperm and egg are sufficient to trigger the early (20-30 min) rise in tyrosine kinase activity, and suggest that protein synthesis is not involved in this early response to fertilization.

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0833 THE MULTIPLE LEVELS OF REGULATION OF TYPE 1 HUMAN INTERFERON GENES, P.M. Pitha-Rowe, J.D. Mosca, M. Kellum, and N.B.K. Raj, The Johns Hopkins University Oncology Center, Baltimore, MD 21205

The regulation of α and β_1 interferon genes was studied in cells induced to synthesize interferon. In Namalva cells, there is a correlation between the synthesis of both α and β interferons and their mRNAs. The rate of α and β_1 interferon genes transcription in these cells was examined in nuclei isolated from induced cells at different times after induction, in vitro labeled RNA transcripts of α and β_1 interferon genes were separated from total transcripts by hybridization to immobilized probes. No detectable transcription of the interferon genes was observed in uninduced cells. The transcription of both α and β_1 interferon genes starts at the cap site and no initiations of the transcription in 5' flanking region of these genes were observed. The transcription of the β_1 interferon gene does not terminate at the poly(A) site, but continues into the 3' flanking region. Accordingly, larger molecular weight poly(A) containing transcripts representing primary transcripts were identified which hybridized both with β_1 cDNA and the cloned 3' flanking region. Such longer transcripts were not identified in α_2 interferon gene suggesting proper termination. However, about 250 nucleotides downstream from the α_2 interferon gene, another gene (a unique DNA sequence) was identified which is constitutively expressed in human cells. In the mouse Ltk- cells transfected with α_2 and β_1 interferon genes, only the β_1 interferon gene can be induced by viral infection or dsRNA, while α_2 gene although expressed constitutively is very poorly induced. Thus both in human fibroblast and transfected mouse cells, viral infection and dsRNA induces only β_1 and no α_2 interferon genes.

0834 REGULATORY BASIS FOR THE DECREASE IN CELL SURFACE EXPRESSION OF IgD AFTER B CELL ACTIVATION, Dorothy Yuan and Philip W. Tucker, University of Texas Health Science Center, Dallas, Texas 75235

The majority of adult B lymphocytes express high densities of IgD and lower densities of IgM on the cell surface. After B cell activation, the expression of cell surface IgD decreases. We have examined the regulatory basis for this decrease by examining the nuclear transcriptional rate, steady state mRNA concentration and biosynthetic rate of δ chains after B cell activation by lipopolysaccharide. Our findings allow us to conclude that the decrease in surface expression is not due to a change in transcriptional rate of the δ gene but can be attributed to a change in processing rate and/or half life which result in a lower steady state concentration of the δ mRNA after B cell activation.

0835 ASSOCIATION OF TRANSLOCATION OF CHROMOSOME 7 WITH OVERPRODUCTION OF EPIDERMAL GROWTH FACTOR RECEPTOR, D.M. Thompson, N. Shimizu, W. Weber and G.N. Gill, University of California, San Diego and University of Arizona, La Jolla, CA 92093

Human epidermoid A431 carcinoma cells contain large numbers of epidermal growth factor (EGF) receptors which appear responsible for their unusual growth inhibitory response to EGF. This study was designed to determine the cause of the high number of receptors. In initial experiments we noted that A431 cells contained a translocation of the p13-q22 region of chromosome 7, previously shown to contain the EGF receptor gene. When the frequency of occurrence of this translocation (7:11) of the EGF receptor gene was compared with the amount of EGF receptor protein present in a series of stable variant A431 clones, a close association was found, whereas the number of copies of normal chromosome 7 failed to show this association. This correlation was further tested by constructing hybrids from human A431 cells and mouse A9 cells which lack EGF receptors. The expression of high EGF binding activity was correlated with the presence of the translocation but not with normal chromosome 7. Tryptic phosphopeptide mapping showed that the EGF receptor protein was structurally similar in clones with or without translocation of chromosome 7. These results suggest that translocation of chromosome 7 results in over-expression of EGF receptor protein. By analogy with translocations involving cellular genes corresponding to retroviral onc genes, translocation of the EGF receptor gene may be involved in genesis or maintenance of tumors.

0836 CATECHOLAMINE REGULATION OF LACTATE DEHYDROGENASE A-SUBUNIT GENE EXPRESSION, Richard A. Jungmann, Dorothy C. Kelley, Michael F. Miles and Deborah Milkowski, Department of Molecular Biology, Northwestern Univ. Med. School, Chicago, IL 60611

Our laboratory has investigated the mechanism of isoproterenol and cyclic AMP-mediated induction of lactate dehydrogenase A subunit mRNA (LDH mRNA) in rat C6 glioma cells. During the induction phase the concentration of nuclear LDH mRNA sequences increased about 2.5-fold 4 hours after the addition of isoproterenol or cyclic AMP. Analysis of nuclear ^{32}P-labeled LDH mRNA sequences showed that isoproterenol or cyclic AMP increased the basal rate of in vitro LDH mRNA transcription about 3.6-fold within 4 hours. The relative rates of in vivo LDH mRNA synthesis were additionally measured by pulse-labeling of glioma cells for 15 min with ^{3}H-uridine. The induction of LDH mRNA by isoproterenol and cyclic AMP was quantitatively comparable to that observed in isolated nuclei and the relative rate of ^{3}H-uridine incorporation into LDH mRNA was maximal 4 to 5 hours after the initial induction stimulus. Increased synthesis of LDH mRNA in vivo as well as in isolated nuclei occurred only at isoproterenol concentrations that caused elevated levels of glioma cell cyclic AMP.

Analysis of the kinetics of decay of ^{3}H-uridine-labeled LDH mRNA showed a linear rate of decay of noninduced LDH mRNA with a $t_{\frac{1}{2}}$ of 45 min. After isoproterenol stimulation LDH mRNA decayed as two populations with a $t_{\frac{1}{2}}$ of 50 min and with a $t_{\frac{1}{2}}$ of 2.5 h.

Tyrosine Kinases and Phosphatases (continued)

0837 PURIFICATION AND PROPERTIES OF A HIGH AFFINITY 94 kDa SUBSTRATE FOR EPIDERMAL GROWTH FACTOR RECEPTOR KINASE, P. Ghosh-Dastidar, D.D.L. Woo and C.F. Fox, University of California, Los Angeles, California 90024

In the presence of epidermal growth factor (EGF), purified EGF receptor kinase phosphorylated 125, 94, 92 and 34 kDa proteins in human placental extracts. The 92 and 94 kDa proteins were purified by chromatography on hydroxyapatite and DEAE cellulose. The 94 and 92 kDa proteins were resolved on DEAE cellulose and the 94 kDa form purified to apparent homogeneity by DNA cellulose chromatography. With purified EGF receptor kinase as catalyst, 94 kDa phosphate acceptor exhibited a Km of 10^{-7} M, and a Vmax of 12 mol per min at 0°C per mol of EGF receptor EGF binding activity. Phosphotyrosine was the sole phosphoamino acid in 92 and 94 kDa proteins radiophosphorylated by EGF receptor kinase. Two dimensional phosphopeptide mapping of fragments derived from 92 or 94 kDa proteins digested with trypsin yielded identical maps displaying one major and several minor radiolabeled phosphopeptides, demonstrating homology between phosphate acceptor sites on 92 and 94 kDa proteins. Phosphate acceptor capacity for DNA cellulose purified 94 kDa protein was 0.14 mol of Pi per mol of protein. Preliminary studies on intracellular localization revealed 92 and 94 kDa phosphate acceptor proteins in both the nuclear fraction and cytosol. Supported by grants from NIH (AM 25826), ACS (BC-370) and MDAA.

0838 PROGESTERONE RECEPTOR SUBUNITS ARE HIGH AFFINITY SUBSTRATES FOR PHOSPHORYLATION BY EPIDERMAL GROWTH FACTOR RECEPTOR, P. Ghosh-Dastidar, W.A. Coty, R.E. Griest, D.D.L. Woo and C.F. Fox, University of California, Los Angeles, California 90024
Purified preparations of epidermal growth factor (EGF) receptor were used to test hen oviduct progesterone receptor subunits as substrates for EGF receptor catalyzed phosphorylation. Both the 80 kDa (A) and 105 kDa (b) progesterone receptor subunits were phosphorylated in a reaction which required EGF and EGF receptors. No phosphorylation of progesterone receptor subunits was observed in the absence of EGF receptor, even when Ca^{2+} was substituted for Mg^{2+} and Mn^{2+}. Phosphoamino acid analysis revealed phosphorylation at tyrosine residues with no phosphorylation detectable at serine or threonine residues. Two dimensional maps of phosphopeptides generated from phosphorylated 80 or 105 kDa subunits by tryptic digestion revealed similar patterns with resolution of two major, several minor and a number of very minor phosphopeptides. The Km of progesterone receptor for phosphorylation by EGF-activated EGF receptor was 100 nM with a Vmax of 2.5 nmol/min/mg of EGF receptor protein at 0°C. The stoichiometry of phosphorylation/hormone binding for progesterone receptor subunits was 0.31 at ice bath temperature and approximately 1.0 at 22°C. Supported by grants from NIH (AM 26826, AM 30869, CA 09056 and GM 7185), ACS (BC-370) and MDAA.

0839 EPIDERMAL GROWTH FACTOR AND POTENT PHORBOL TUMOR PROMOTERS INDUCE EPIDERMAL GROWTH FACTOR RECEPTOR PHOSPHORYLATION, S. Iwashita and C.F. Fox, University of California, Los Angeles, California 90024
When human epidermoid carcinoma A431 cells labeled with ^{32}Pi to steady state specific activity were treated either with epidermal growth factor (EGF) or with active phorbol ester tumor promoters such as 12-O-tetradecanoyl-13-acetate (TPA) or phorbol 12,13-dibutyrate, labeling of 160 kDa EGF receptors isolated by immunoprecipitation with monoclonal anti-EGF receptor IgG was increased 2- to 3-fold. These treatments produced no significant increase in ^{32}Pi labeling of acid precipitable material present in detergent extracts of the cells. Phosphoamino acid analysis of radiolabeled EGF receptors isolated from these cells revealed several differences: the relative abundance of phosphotyrosine in EGF receptors was increased in cells treated with EGF, but decreased in cells treated with TPA; the overall relative abundance of phosphothreonine in EGF receptors was decreased in cells treated with EGF, but remained constant within the limits of experimental detection in cells treated with TPA. Two-dimensional mapping of the radiolabeled phosphopeptides produced from EGF receptors isolated by immunoprecipitation and treated with trypsin revealed 9 independent labeled regions, two of which contained phosphothreonine and were present only in EGF- or TPA-treated cells. These two phosphopeptide regions were more highly labeled in cells treated with TPA than with EGF. Supported by grants from NIH (AM 25826), ACS (BC-370) and MDAA.

0840 LOW DENSITY LIPOPROTEIN AND EPIDERMAL GROWTH FACTOR BINDING INTERNALIZATION AND DEGRADATION IN HUMAN A431 CELLS, AND IN VARIANT A431 CELLS SELECTED FOR RESISTANCE TO EPIDERMAL GROWTH FACTOR, C. Warden and C.F. Fox, University of California, Los Angeles, California 90024
Low density lipoprotein (LDL) binding, internalization, and degradation are altered in A431 cells resistant to epidermal growth factor (EGF). EGF inhibits the growth of human epidermoid carcinoma A431 cells. As a consequence, EGF receptor levels are often reduced in A431 cell variants selected for resistance to EGF-induced cytostasis. EGF resistant clone 18 has 10- to 15-fold decreased levels of EGF and LDL binding, uptake and degradation. Both EGF and LDL internal pools and rates of degradation were less in clone 24 than in A431 cells. Scatchard analysis revealed 2 classes of surface bound (heparin dissociable) LDL with A431 and clone 24 cells at 37°C. Clone 18 cells had 10-fold higher affinities for both EGF and LDL than A431 cells when binding was performed at 4°C. Quantitative comparisons of LDL and EGF binding revealed several differences in uptake and degradation. A431 cells had approximately the same number of cell surface receptors for both EGF and LDL at 37°C. However, maximum saturable internal LDL pools were 5 times larger than saturable intracellular EGF pools. Furthermore, the maximum rate for saturable LDL degradation was 50 times higher than the rate for EGF. These results suggest that processes catalyzing uptake and degradation show preference for LDL. Supported by grants from NIH (AM 25826, HL-07386).