CELLULAR RECOGNITION

Luis Glaser, William Frazier and David Gottlieb, Organizers

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Workshops and Poster Sessions

Cell-Cell Recognition in Development

655 CELL-CELL RECOGNITION IN DEVELOPMENT, Gerald M. Edelman, Department of Developmental and Molecular Biology, The Rockefeller University, New York, New York, 10021

Cellular recognition events have been implicated in a wide variety of biological processes. With the exception of certain viral and bacterial interactions, however, much remains to be understood concerning the soluble factors, surface receptors, ligands, and control mechanisms involved in cellular recognition. As exemplified by this meeting, many laboratories are vigorously attacking these problems.

To obtain a molecular description of cellular events in vertebrate tissue formation we have used a combination of immunological techniques and biological assays to detect and characterize cell surface components involved in adhesion between and among cells of the chick embryo. These studies have resulted in the identification of cell adhesion molecules from nerve tissue (N-CAM) and liver (L-CAM). Specific antibodies against these molecules have been used to probe their expression, distribution, and physiological function. N-CAM function has been shown to be required for nerve bundling and appearance of cell and neurite layers in retina, and anti-(L-CAM) prevents the formation of histotypic colonies of hepatocytes. Histological studies also suggest that the regulation of N-CAM expression may be a key event in the migration and aggregation of neural crest cells.

A complete description of adhesion requires analysis of modulating events mediated by cytoskeletal interactions and transmembrane control. The proposal that such modulating mechanisms are important in cell recognition has been supported by studies on the influence of cellular interactions in growth control. While a direct role of modulating events in cellular interactions remains to be demonstrated, it seems likely that the coordination of adhesion with other developmental events requires some form of modulation or control at the cellular level.

Cell Recognition in Procaryotes and Lower Eucaryotes

656 CELL-CELL INTERACTIONS BETWEEN CONJUGATING ESCHERICHIA COLI, Mark Achtman, Max-Planck-Institut fuer molekulare Genetik, Abt. Trautner, D-1000 Berlin 33, Germany.

E. coli cells carrying the F plasmid act as efficient donors of the F plasmid DNA to F^- plasmid-free recipient cells. A series of stages which together comprise the mating cycle have been defined (1, 2): a) F pilus to wall contact, b) wall to wall contact, c) in parallel: stabilization of wall to wall contacts and triggering of DNA replication, d) DNA transfer beginning from a defined origin, e) disaggregation of the mating cells. DNA transfer does not occur between any two donor cells because neither can act as a recipient. Each donor cell possesses two active barriers, one to stabilization and the other to triggering. Many of the genes and proteins encoded by the F plasmid which enable these interactions have been defined (2). The receptor in F^- cells which allows stabilization has also been defined. Three of the individual proteins have been purified and shown to have biological activity in vitro.

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657 THE ROLE OF PILI IN GONOCOCCAL ATTACHMENT TO CELLS, T.M. Buchanan, M.D. and E.R. Gubish, Jr., Ph.D., University of Washington, Seattle, WA 98195.

Neisseria gonorroheae appears as four distinct colony types when grown on artificial media (1). Two of these have sufrace appendages termed pili, two do not. Other investigators have shown that the piliated organisms are the more virulent and attach more readily to mammalian cells (2,3) suggesting that pili play a prominent role in gonococcal attachment. Pearce and Buchanan (4), using radioiodinated pili, have demonstrated attachment to a variety of cells and have defined factors which influence this attachment. Further, pili attachment has been blocked to varying degrees using purified gangliosides, the most effective being GD_{1A}, thus indicating that a pili receptor on the host cell would consist of simple sugars and their n-acetylated amino sugars. Attachment experiments using lectin resistant clones of Chinese hamster ovary cells indicate that galactose and n-acetylglucosamine are involved in pili attachment.

Cyanogen bromide cleavage of intact pili yields amino terminal and carboxy terminal peptides. Using the radiolabeled attachment assay, the amino terminal peptide was shown to contain the binding portion of the pilin molecule.

Antibody raised to gonococcal pili can also block attachment, however this blockage is strain specific. Recently monoclonal antibody raised to one strain of gonococcal pili has been shown to recognize pili from different strains and to effectively block the attachment of the homologous pili and its amino terminal fragment. The characterization of both the pili receptor and its own binding site should be facilitated by hybridoma technology.

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658 LECTIN AND ITS SACCHARIDE RECEPTORS IN THE RHIZOBIUM-CLOVER SYMBIOSIS, Frank B. Dazzo, Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan 48824 U.S.A.

Rhizobium trifolii, the nitrogen-fixing symbiont of clover, specifically adheres to clover root hairs, which are targets for subsequent infection (1). Genetic and immunochemical studies suggest that selective adhesion involves cross-bridging of similar saccharide receptors on the bacterium and the host by a multivalent, host-coded lectin called trifoliin (2,3). This specific carbohydrate-glycoprotein interaction can be reversed by the hapten 2-deoxyglucose. Quantitative adsorption and immunocytofluorimetric studies indicate that this recognition phenomenon is regulated in the plant by NO_3^- provided to the growing root (4). One form of regulation by the bacteria is accomplished through growth-phase dependent changes in surface polysaccharides (e.g., increases in quinovosamine, 2-amino, 2,6-dideoxy-hexose) which affect their affinity for trifoliin and clover root surfaces (5, Urbano, Hrabak, and Dazzo, submitted for publication). Rhizobial attachment to the clover root hair is a multiphase process involving an initial selective docking of fully encapsulated cells to root hair tips where trifoliin accumulates, followed by host-mediated modification of the bacterial symbiont surface which favors polar accumulation of lectin-binding receptors on the bacteria and their polar attachment along the sides of the root hair (Hrabak, Sherwood, Truchet, and Dazzo, manuscript in preparation).

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659 INTERCELLULAR SIGNALS IN THE DEVELOPMENT OF MYXOCOCCUS, Dale Kaiser, Robert LaRossa, Jerry Kuner and Robert Suva, Department of Biochemistry, Stanford University, Stanford, CA 94305.

Myxococcus is a bacterium that moves in multicellular swarms and that aggregates to construct multicellular fruiting bodies (1). When starved at high cell density, many thousands of cells come together, pile on top one another and build a fruiting body that has a genetically determined shape. Within the fruiting body, rod shaped vegetative cells differentiate into ovoid, environmentally resistant myxospores.

How do cells coordinate their behavior in this primitive developmental process? One possibility is the emission and reception of intercellular chemical signals. To look for such signals mutants were isolated that behaved as if defective in signal production (2). These mutants are synergetic: unable to fruit by themselves; they can fruit when mixed with wild-type or with other mutants. Presumably the wild-type cells supply the missing signal. There are four classes of synergetic mutants and many members of each class. Any pair of mutants that belong to different classes can synergize, but two mutants belonging to the same class cannot. This suggests that there are at least four different signals.

The signals behave like diffusible substances because donor and recipient strains can be separated by membrane filters having pores less than 0.2 μm in diameter. A cell-free extract of developing, but not vegetative, wild-type cells can synergize mutants of class B. If the four classes of mutants are defective in producing different signals, then mutants of different classes should map to different chromosomal regions. The transposable element, Tn5, introduced from E. coli, can insert into the Myxococcus chromosome at many different sites which then serve as landmarks for mapping chromosomal genes (3). In general mutants of the same synergism class do map to the same chromosomal region and different classes map to distinct regions.

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660 SEX-SPECIFIC MOLECULES THAT MEDIATE THE CHLAMYDOMONAS MATING REACTION, Ursula W. Goodenough, Department of Biology, Washington University, St. Louis, Missouri 63130.

An overview of the eukaryotic Chlamydomonas mating reaction will be presented, with information obtained from biochemical, genetic, and structural studies. Cell recognition between mating-type plus and minus gametes is mediated by two distinct membrane systems: flagellar membranes bear mating-type specific adhesion molecules that govern the initial agglutination reaction, while specialized sectors of the plasma membrane bear mating-type specific components that govern the fusion reaction. Flagellar membrane adhesiveness is apparently mediated, in both mating types, by a complex of 2 polypeptides, 350K and 20K. Plus flagellar adhesiveness is governed by the sag-1 and sag-2 gene loci; neither locus is linked to the mt^+ "master locus" but both require its presence for expression. Mutations abolishing minus flagellar adhesiveness, by contrast, map directly to the mt^- "master locus". Mutant imp-11 mt^- gametes activate their mating structures normally in response to adhesion, a reaction that involves actin polymerization from a dense cytoplasmic nucleating site. They lack, however, the specialized plasma-membrane differentiation required for fusion. Flagellar adhesion signals mating-structure activation in both mating types; signaling is manifested morphologically by changes in the interior of the flagellar tip and flagellar microtubule elongation. The entire mating sequence occurs within 30 sec; the cell-cell interactions involved are thus comparable in both complexity and efficiency to fertilization in higher organisms.

661 MOLECULAR BASIS OF CELL-CELL RECOGNITION: ISOLATION, CHARACTERIZATION AND FUNCTION OF AN AGGREGATION COMPLEX FROM THE SPONGE GEODIA CYDONIUM

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Dissociated sponge cell system has proved to be a useful model to study the process of cell aggregation both on cellular and subcellular level. Moscona succeeded in the isolation of an aggregation factor from the sponge Microciona prolifera; 1973 Burger solubilized from the same species a cell surface-bound aggregation receptor. These two macromolecules, which are involved in reaggregation, were 1973 and 1976 purified from the siliceous sponge Geodia cydonium (1, 2). The aggregation factor (mol. wt. 17,400) was found to be bound to a high-molecular weight particle ($S°_{20,w}$: 80; diameter: 740 - 1,260 Å) which was termed aggregation complex. Besides of the aggregation factor, the aggregation complex consists of two further functional subunits: UDP-glucuronosyltransferase and UDP-ß-D-galactosyltransferase. The aggregation receptor with a mol. wt. of appr. 17,000 was found to be a glycoprotein with D-glucuronic acid as terminal sugar moiety. Some evidence is available indicating that aggregation receptor recognition involves interaction between lysine (functional site of the aggregation factor) and glucuronic acid (aggregation receptor). From in vitro data with the Geodia system a working hypothesis is formulated (3) to explaine cell aggregation and cell separation on the level of interaction between glucuronidase and glucuronosyltransferase.
The aggregation complex of Geodia cydonium is synthesized in the "spherulous cells". The earliest stages of the complex are detected in the nuclear membrane as round-shaped particles with a diameter of 360-480 Å filled with electron dense material. After release into the perinuclear space the particles reach a diameter of 550-950 Å. Before the aggregation complex is released by the cell it associates with the cell membrane; it is egressed by exocytosis. The aggregation factor is linked to the complex during its egress from the intracellular to the extracellular space.(This work was supported by a grant from the "Stiftung Volkswagenwerk"; no. 35850, W.E.G.M.)

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662 SPONGE CELL RECOGNITION AND ORGAN FORMATION, Max M. Burger, James Jumblatt, Gradimir Misevic and Werner Burkart, Biocenter of the University of Basel, Switzerland

Species specific aggregation of sponge cells depends on Ca^{++} and an extracellular proteoglycan of about 20 x 10^6 daltons. Specificity of aggregation of Microciona cells is provided by a Ca^{++} independent binding of the protoglycan to a receptorlike baseplate on the cell surface. Aggregation force is provided by interactive forces among the proteoglycan molecules necessitating Ca^{++}. To what degree weak specific interactions among the proteoglycan molecules may contribute to the specificity of the aggregation will be discussed. Simultaneous heat, urea and EDTA treatment at elevated pH produces fragments of about 200 K daltons. Protease treatment produces pieces down to 10 K. Both the large and the small fragments contain carbohydrates and protein and both retain species specificity of binding.

The formation of the first organs during differentiation was so far thought to be due to a rearrangement of flagellated larval cells. Evidence will be provided that this concept is incorrect and that these first organs are probably formed de novo from stem cells.

Receptor Mechanisms in Fertilization

663 EGG SURFACE COMPONENTS INVOLVED IN SPERM BINDING, W.J. Lennarz, D.P. Rossignol, C. G. Glabe, and W.H. Kinsey, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205

In echinoids, sperm-egg binding prior to fertilization is a highly specific cell recognition process. This binding is preceded by induction of a secretory event in sperm which results in deposition of the protein, bindin, on the tip of the sperm. Vacquier and coworkers have shown that bindin is the species specific adhesive molecule associated with sperm that mediates binding to the egg. Our objective has been to determine if a complementary molecule, i.e., a receptor for bindin, exists on the surface of the egg.

Early studies revealed that the egg plasma membrane contains a glycoconjugate that has the properties of such a receptor. However, initial attempts to obtain a soluble, intact form of the receptor were unsuccessful. Therefore, we undertook a search for the release of a soluble receptor-like activity following protease digestion of intact eggs. In both S. purpuratus and A. punctulata eggs it was found that after extensive Pronase digestion a soluble glycopeptide fragment could be isolated that has the properties expected of a receptor, i.e., it inhibits bindin-dependent egg agglutination, sperm-egg binding and fertilization. However, in contrast to the intact receptor on the egg surface, neither isolated glycopeptide is species specific.

In view of these findings and the fact that the intact membrane associated receptor is species specific, we have recently developed methods to partially purify the intact receptor from iodinated membranes from S. purpuratus eggs. After detergent extraction and density gradient centrifugation, an ^{125}I-labeled fraction was found to bind to particulate bindin in a concentration dependent manner. This interaction with bindin was blocked by anti-bindin antibody and by the Pronase-derived glycopeptide described above. The partially purified receptor derived from S. purpuratus eggs showed saturation binding to acrosome reacted sperm from S. purpuratus, but not to unreacted sperm or to sperm from A. punctulata. Binding was dependent on both sperm and receptor concentrations. Under conditions of limiting acrosome reacted sperm, the receptor fraction inhibited fertilization of S. purpuratus eggs. At similar concentrations the receptor fraction from A. punctulata eggs showed < 10% inhibition of S. purpuratus fertilization. After extraction with detergent, 60% of the ^{125}I-label was solubilized but the majority of the receptor activity remained insoluble. The 200-fold purified receptor fraction contained approximately 4 mg protein/mg hexose. After Pronase digestion > 80% of the hexose material was in a component of mol. wt. > 2 x 10^6. This component competed with the intact receptor for binding to bindin. Present efforts are directed towards further purifying and characterizing the receptor and determining structural receptor differences between species. (Supported by an NIH grant [HD 08357] to W.J.L. and an NIH fellowship [HD 05986] to D.P.R.)

664 PERSISTENCE OF SPERM SURFACE COMPONENTS IN THE EARLY EMBRYO, Bennett M. Shapiro, C.A. Gabel, G. Gundersen, and E.M. Eddy, Department of Biochemistry and Biological Structure, University of Washington, Seattle, WA 98195.

After covalently labeling sperm with fluorescein isothiocyanate (FITC), or an iodinated derivative, diiodofluorescein isothiocyanate (^{125}IFC), we have been able to trace the fate of sperm surface components after fertilization by their fluorescence and radioactivity. Labeling is principally on the sperm midpiece surface, as demonstrated by immunoperoxidase cytochemistry, using IFC-labeled sperm and anti-IFC antisera. Greater than 95% of the sperm associated radioactivity is soluble in 3% Nonidet P40 under conditions that do not disrupt the overall morphology of the sperm. All the Nonidet P40 solubilized radioactivity is precipitable with 10% TCA, while about 25% is extractable with $CHCl_3$:methanol, suggesting that some lipid has been labeled with ^{125}IFC. A number of radioactive bands are observed on SDS-polyacrylamide gels, with all of them above 5,000 daltons insoluble in 30% ethanol.

Upon fertilization, the labeled sperm components are quantitatively transferred to the egg, where they remain as a patch throughout early development, in echinoid (sea urchin, sand dollar, starfish), mouse, and medusa embryos. In most cases, the patch remains in a single blastomere, as determined by fluorescence microscopy. With an immunoabsorbent technique, using antibodies directed against IFC, the ^{125}IFC-labeled proteins could be isolated and then identified by SDS-polyacrylamide gel electrophoresis. In sea urchins certain of the ^{125}IFC-labeled sperm proteins that are transferred to the egg at fertilization have been found to remain unmodified throughout development to the larval stage.

The patch of labeled sperm surface components is initially associated with the egg surface, but during the twenty minutes after fertilization it moves to a locus in the embryo cortex. The internalization of patch components appears to be a microfilament mediated process, as it is blocked by cytochalasins B and D, at concentrations similar to those that inhibit egg cleavage. The pathway of patch migration after fertilization can be followed by image intensification microscopy; the patch moves to its cortical location and persists in that zone throughout the multiple cell divisions leading to the blastula stage. Occasional movement of the patch is seen during cytokinesis, but the patch remains localized in an individual blastomere. The localization and persistence of the sperm patch in the early embryo suggests that it may play a role in modulating early development, a possibility that we are currently pursuing. Supported by National Science Foundation grant, PCM 7720472.

665 THE ROLE OF ZONA PELLUCIDA GLYCOPROTEINS AS REGULATORS OF SPERM-EGG INTERACTIONS IN THE MOUSE, Paul M. Wassarman and Jeffrey D. Bleil, Department of Biological Chemistry, Harvard Medical School, Boston, MA 02115

Sperm-egg interaction in mammals is initiated by binding of sperm to the zona pellucida (ZP), an acellular coat surrounding the plasma membrane of unfertilized eggs. Following fertilization, additional sperm are unable to bind to the ZP of embryos, and sperm that had partially penetrated the ZP prior to fertilization are prevented from further penetration. Thus, the ZP initially serves a recognition function permitting species-specific binding of sperm to unfertilized eggs, but subsequently serves as a block to polyspermy. The molecular bases of these biological functions of the ZP are under investigation in our laboratory.

The ZP of mouse eggs is composed of three different glycoproteins, designated ZP1, ZP2, and ZP3, having apparent MWs of 200K, 120K, and 83K, respectively. All three glycoproteins are synthesized, glycosylated, and secreted by oocytes during their growth phase. ZP1, an oligomer bonded by intermolecular disulfides, is converted to a 130K MW species, designated ZP1' (pI≈5.9), by reducing agents. ZP2 (pI≈4.3) has intramolecular disulfides and represents more than 50% of the total ZP protein. ZP3 (pI≈3.7) is the least abundant of the ZP glycoproteins. All three glycoproteins are found in ZP isolated from mouse oocytes, eggs, or embryos and analyzed by SDS-PAGE under non-reducing conditions. However, under reducing conditions, ZP2 from embryos, but not from oocytes or unfertilized eggs, migrates with an apparent MW of 90K and is designated $ZP2_f$. The evidence suggests that modification of ZP2 following fertilization involves proteolysis, but that intramolecular disulfides prevent the release of peptide fragments. The same change in ZP2 occurs in vitro following activation of unfertilized eggs with the calcium ionophore A23187. It is likely that the conversion of ZP2 to $ZP2_f$ is carried out by a cortical granule protease(s) and is responsible for certain of the biochemical and biological changes in the ZP that occur after fertilization.

Solubilized ZP from unfertilized eggs, but not from 2-cell embryos, reduce binding of sperm to eggs in vitro to as little as 10% of control values. To determine if any ZP glycoprotein has sperm receptor activity, ZP1, ZP2, and ZP3 were purified from egg and embryo ZP, and evaluated for their effect on binding of sperm to eggs in vitro. Whereas, ZP1 and ZP2 do not interfere with sperm binding, ZP3 purified from unfertilized eggs, but not from embryos, reduces sperm binding to an extent comparable to that observed with solubilized ZP. These results suggest that ZP3 possesses the receptor activity responsible for binding of sperm to unfertilized eggs. Fertilization apparently results in modification of ZP3, designated $ZP3_f$, such that it no longer serves as a sperm receptor. EM analysis has revealed that ZP3 from unfertilized eggs, but not from embryos, induces the acrosome reaction in vitro. This suggests that ZP3 is not only responsible for the attachment of sperm to eggs, but also for inducing changes in sperm that are necessary for penetration of the ZP.

The Cellular Slime Molds

666 RECEPTORS FOR CHEMOATTRACTANTS AND ENDOGENOUS LECTINS OF DICTYOSTELIUM DISCOIDEUM. William A. Frazier, James R. Bartles, Nancy J. Galvin, Nandini Kishore and Beth L. Meyers, Departments of Biological Chemistry and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110

The aggregation phase of D. discoideum is characterized by chemotaxis to the endogenous chemotransmitter cAMP and by acquisition of cellular cohesiveness, for which the endogenous lectin discoidin I is required. The developmentally regulated chemotaxis receptor binds cAMP with oscillatory kinetics on both intact cells and isolated plasma membranes. A potential regulatory system for the oscillating affinity of the receptor has been identified. The cAMP receptor has been solubilized from plasma membranes with the nonionic detergent Emulphogene BC-720, and two assays have been developed in which bound and free ^{3}H-cAMP can be separated in less than 2 s, a condition necessitated by the rapid off rate of the receptor. The affinity of the detergent solubilized receptor is about 100-fold lower than that of the membrane associated receptor and its selectivity for cAMP vs cGMP is also reduced. Both the membrane bound and detergent solubilized receptors are inactivated by sulfonyl fluorides suggesting that they are in fact the same entity.

We have synthesized multivalent cAMP ligands by coupling N^6-succinylcAMP to BSA, yielding both a monomeric BSA species with 10 to 15 cAMP moieties attached and a BSA polymer containing larger numbers of cAMP molecules. These derivatives have been labeled with both ^{125}I and TRITC. The derivatives are strong chemoattractants for differentiated cells, in contrast to the N^6-succinylcAMP from which they are prepared. This suggests that a multivalent interaction with receptors is a more potent stimulus than the monofunctional succinyl cAMP. The binding kinetics of the cAMP-BSA conjugates also indicate a multivalent mode of specific binding to the cAMP receptor. These labeled conjugates are being used in attempts to localize the cAMP receptors on living cells. Rapid binding assays, affinity labels and multivalent conjugates are now being developed for studies of the folate chemotactic receptor which is present on vegetative D. discoideum cells.

Using ^{125}I- and tetramethylrhodamine/ferritin-discoidin I, we have studied the interaction of this endogenous lectin with living cells under physiological conditions. Discoidin I binds to both a cell surface glycoprotein receptor and negatively charged phospholipids.

This binding leads to clustering of the bound discoidin I and its apparent internalization. Discoidin I can bind to and agglutinate negatively charged vesicles containing only purified phospholipids, and this interaction is modulated by sugars which bind to discoidin I. Preliminary data indicate that discoidin promotes vesicle fusion. These results have substantial implications for discoidin I function. [Supported by NS 13269 and PCM 78-04304. WAF is an Established Investigator of the American Heart Association.]

667 CONSTRUCTION OF STRAINS OF DICTYOSTELIUM DISCOIDEUM WITH PRIVATE CELL-CELL RECOGNITION, Richard A. Lerner, Molecular Genetics Group, Research Institute of Scripps Clinic, La Jolla, California 92037

Recent biochemical experiments have defined a lectin ligand system which is involved in cohesion and aggregation of Dictyostelium discoideum cells. The two molecules involved are a tetrameric carbohydrate binding protein which consists of 4 subunits with approximate molecular sizes of 26K and a glycoprotein receptor with an approximate molecular size of 80K. We isolated a missense mutant in which CBP is present but has lost the ability to bind to galactose. This mutant both provided definitive evidence for the role of CBP in development and gave a point of departure for further studies. If one imagines that cell-cell interaction occurs via a lock and key bond then the missense mutant can be thought of in terms of an altered key and should be suppressible by selecting for cells where an altered lock which now fits the altered key. We isolated a second site suppressor mutant which developed normally but still had a missense mutation in CBP. The prediction was that these cells had an altered 80K receptor which bound to the mutated CBP and allowed development to proceed. Two predictions follow from this hypothesis. a) Since the strain with the outside suppressor has both an altered CBP and 80K receptor, its cells should interact (synergize) with themselves but no other cells. In other words, the manipulation of mutating a cell surface binding molecule and suppressing the mutation via a second site lesion in the receptor should create a new strain with private recognitive specificity. b) In two strains which do not synergize with each other, the homologous CBPs should bind to the homologous receptors, but not visa versa. Data will be presented which suggests that both of these predictions hold. Thus, we have been able to construct in the laboratory strains of Dictyostelium discoideum with private cell-cell recognition. The general point is that where molecules involved in cellular recognition are concerned, extragenic suppression of missense mutants can lead to constraints in the abilities of the cells to recognize anybody but themselves. These strains should provide a point of departure for understanding the molecular basis of cell-cell interaction and species specific recognition.

668 CELL SURFACE LECTINS IN SLIME MOLDS AND HIGHER EUCARYOTIC CELLS, S.D. Rosen, L.B. Grabel, D.K. Drake, C.G. Glabe, M.S. Singer, and G.R. Martin, Department of Anatomy, UCSF, San Francisco, CA 94143.

Considerable evidence, accumulated over the last several years, suggests that cell surface lectins on cellular slime molds are involved in intercellular adhesion (1). It is hypothesized that the interaction between lectins and complementary, carbohydrate-containing receptors on opposing cells mediates cell-cell adhesion. Binding studies have established the existence of high-affinity receptors for the endogenous lectins on the surface of differentiated amoebae. In Polysphondylium pallidum, we have found a potent inhibitor of pallidin (the lectin purified from P. pallidum) in a soluble extract of membranes. We have purified this inhibitor and have identified it as a high molecular weight heterosaccharide, consisting predominantly of rhamnose and glucose. This potential receptor blocks agglutination of P. pallidum cells at high concentrations and augments agglutination at low concentrations. We are investigating the possibility that this substance may act as an "aggregation factor" in slime mold adhesion.

We have used an erythrocyte-rosetting assay as a probe for identifying cell-surface carbohydrate-binding proteins on mouse teratocarcinoma stem cells (embryonal carcinoma cells, EC) (2) and on various species of cultured fibroblasts. EC cells possess a cell surface lectin that binds oligomannosyl structures and to a much greater extent sulfated fucans (fucose polymers). These same carbohydrate structures inhibit the intercellular adhesion of EC cells. A lectin activity with the same carbohydrate-binding specificity as that of the cell surface component has been detected in soluble extracts of EC cells. Purification of the lectin is in progress.

Different fibroblast lines exhibit clearly distinct erythrocyte-binding specificities. We have studied the very strong interaction between BHK-21 fibroblasts and trypsinized Ox erythrocytes. Neutral sphingoglycolipids isolated from Ox erythrocytes inhibit this rosette interaction. Furthermore, incorporation of these glycolipids into nonrosetting erythrocytes

(guinea pig) causes them to bind to BHK cells. Investigations are underway to identify the carbohydrate structure of the active glycolipid. The role of the rosette-mediating factor (lectin-like substance) in the interactions between BHK fibroblasts will be investigated.

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Lymphoid Cell Interactions

669 ANALYSIS OF SPECIFIC T-CELL FUNCTIONS USING CLONED CELL LINES, Harvey Cantor, Gary Nabel, Manuel Fresno, Laila McVay-Boudrea, Department of Pathology, Harvard Medical School, Boston, MA 02115

We have produced continuously propagatable mouse lymphocyte clones. Each clone represents a cell at a progressive stage of thymus-dependent cellular differentiation. These cloned cells bear stable surface membrane glycoproteins characteristic of precursor cells and mature progeny; conditions allowing maximal cloning efficiencies for each cell type (10-85%) have been established. Mature lymphocyte clones continue to express specialized function and provide material for biochemical analysis of T-lymphocyte functions: one fully differentiated clone from the 'inducer' or 'helper' lymphocyte set synthesizes a molecule that activates other lymphocytes to secrete immunoglobulin. This activity is associated with a highly purified molecule having a M.W. 45,000 and an isoelectric point of approximately 6.0. This molecule together with clones of precursor and mature T-lymphocytes, may provide a system to further study the mechanisms of gene activation during cellular differentiation.

670 CELL SURFACE RECEPTORS IN THE DEVELOPMENT AND HOMING OF LYMPHOCYTES. I.L. Weissman, E.C. Butcher, R.V. Rouse, R.G. Scollay, F. Lepault, R.L. Coffman, L. Jerabek, E. Pillemer and M.S. McGrath. Laboratory of Experimental Oncology, Department of Pathology, Stanford Medical Center, Stanford, CA 94305.

Cells of the immune system undergo several developmentally and functionally significant stages of expression of cell surface recognition structures. These recognition structures 1) enable immature cells to migrate to central lymphoid organs containing inductive microenvironments; 2) are involved in recognition of microenvironmental signals which trigger their development along one of several pathways; 3) permit the cells to migrate from sites of maturation to peripheral lymphoid organs wherein immune responses take place; and 4) allow these antigen reactive cells to recognize antigen on appropriate accessory cells or target cells, resulting in the proliferation and differentiation of these immunocompetent lymphocytes. Lymphocyte differentiation in the T cell series involves homing of a rare but well-defined subpopulation of bone marrow cells to the thymus. Within the thymus they interact with MHC-bearing cortical epithelial cells; the result of such interactions appears to be intrathymic death for approximately 99% of the progeny of these cells. The survivors express receptors enabling them to home specifically to differentiated lymphoid tissues via interaction with receptors on subsets of differentiated endothelial cells. Lymphocyte differentiation markers on the surface of T cells (and perhaps B cells) appear to be involved in the recognition of other lymphoid cells and of specific antigens; specific recognition of antigen by these cells is necessary for the onset of proliferation via a receptor-mitogen complex. These various cell surface markers and receptor systems are expressed on malignant lymphoid cells of both the T and B cell series, and appear to be involved in the two lethal properties of malignant cells - uncontrolled proliferation, and distant metastasis.

671 ANTIBODIES: THE GENERATION OF INFORMATION IN A COMPLEX RECEPTOR SYSTEM, Leroy E. Hood and Henry V. Huang, Division of Biology, California Institute of Technology, Pasadena, CA 91125

The antibody system is an interesting model to examine genetic mechanisms for the expression and amplification of information in a complex eukaryotic membrane-receptor system. The antibody gene families evolved more than 500 million years ago with the emergence of vertebrate immunity. These gene families exhibit a "split gene" character with the individual gene segments rearranging during the differentiation of the antibody-producing (B) cell. These DNA rearrangements play two fundamental roles in B-cell development—they are correlated with the expression of antibody polypeptides and they play a fundamental role in generating antibody diversity. I will examine the general organization of antibody genes and the mechanisms for their DNA rearrangements and somatic diversification. Then I will raise the possibility that the split gene-DNA rearrangement strategy may be employed by other complex eukaryotic systems that encode membrane recognition systems. These we have denoted as area-code systems. I will discuss possible examples of area-code gene families and then consider the microchemical instrumentation that we are developing at Caltech to explore these fascinating membrane gene families.

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Cell Interactions in the Nervous System

672 SYNAPSE-SPECIFIC COMPONENTS OF THE NEUROMUSCULAR JUNCTION. Zach Hall, Laura Silberstein, Nibaldo Inestrosa, Joshua Sanes and Crispin Weinberg, Dept. Physiology, University of California, San Francisco, CA 94143.

At the adult neuromuscular junction both the postsynaptic muscle cell membrane and the extracellular basal lamina (BL) in the synaptic cleft are biochemically specialized. The postsynaptic membrane has densely clustered acetylcholine receptors (AChRs) which differ from the diffusely distributed AChRs in embryonic and denervated muscles in having a slower metabolic turnover time. Acetylcholinesterase (AChE) is also concentrated at the synapse where at least some of it is associated with the BL. In rat muscle a particular form of the enzyme (16S) appears to be specifically associated with endplates. Recent experiments have shown that the BL of the synapse can influence directly pre- and postsynaptic differentiation after damage in adult muscle (1,2). To investigate further the relation between the BL and the postsynaptic membrane, we have obtained antibodies that bind selectively to the muscle BL at the synapse. These antibodies, which define at least three different determinants (3), have been used along with the 16S AChE as markers to study differentiation of the synaptic BL in cell culture and during ectopic endplate formation in adult muscle.

We initiated endplate formation in rat soleus muscle by cutting the original nerve to a muscle in which a foreign nerve had been previously implanted. Within two days clusters of AChRs were detected by radioautography after reaction with ^{125}I-α-bungarotoxin. The initial density of AChRs in these clusters increased until at four days the adult value was achieved. In newly formed clusters, the half-time for metabolic turnover was the same as that found for unclustered receptors, about 1 day. By day 6, however, the half-time had increased to the adult value of 10 days. In contrast, neither AChR nor the BL-specific antigens could be detected by cytological methods until after about one week, when they were present at some, but not all endplates. Their appearance coincided with the rapid accumulation in the tissue of the 16S form of AChE. Maturation of the synaptic BL thus occurred after the AChRs achieved nearly their final density and had become metabolically stabilized. We conclude that differentiation of the postsynaptic membrane and the synaptic BL follow separate time courses.

We have investigated the cellular origin of synaptic components of the BL, using a mouse skeletal cell line, C2. After fusion, the cells produced the 16S form of AChE. This form of the enzyme appears to be associated with a BL as it is distributed in patches on the extracellular surface of the myotubes from which it can be released by collagenase. Each of the other three synaptic BL antigens are also produced by these cells. These experiments show that components of the synaptic BL can be made by muscle cells in the absence of nerves.

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673 CONTACT GUIDANCE OF NEURONAL MOVEMENTS DURING CORTICAL DEVELOPMENT, Pasko Rakic, Section of Neuroanatomy Yale University School of Medicine, New Haven Ct. 06510

Previous electromicroscopic analysis of cell behavior in the developing primate cerebrum indicates that the interaction between radial glial cells and migrating neurons may play a crucial role in orientation, displacement and positioning of neurons within the cortex (1). Immunocytochemical studies using antibodies to glial specific marker (GFA) show that both neuronal and glial cell classes coexist at early developmental stages (2) and that they originate from separate cell precursors situated in the proliferative zones (3). Analysis of ^{3}H-thymidine data indicates that many radial glial cells cease to divide for about two months (4) while their elongated fibers serve as guides for migration of neurons from the place of their origin near the ventricular surface to their final destinations in the distant cerebral cortex (1, 5). This arrangement of transient glial cells provides a structural framework for compartmentalization of the developing cerebral wall and insures faithful mapping of the proliferative ventricular surface upon the expanding and convoluted cerebral cortex in primates (6). Migrating neurons selectively follow the curving course of glial fibers rather than the often more regular surfaces of other cells and processes that they encounter during displacement, suggesting a strong surface affinity between these two classes of cells. I will present a model which accounts for displacement of neurons along radial glial even if the membranes of both cell classes remain fixed at any point of their interface. According to this model, the leading neuronal process acquires new membrane along the surface of elongated glial fibers while the nucleus and surrounding organelles of the migrating neuron translocate within the thus formed cytoplasmic cylinder. Although the binding affinity between neuron and glial cell surfaces may be nonspecific (e.g. all migrating neurons may have the same affinity for all radial glial cells), the spatio-temporal order of cell migration would be acheived since each postmitotic neuron becomes attached exclusively to a neighboring radial glial fiber. I will present several lines of evidence to support this model, including the pattern of cell surface growth, the speed of cell migration, and the consequences of genetic and acquired abnormalities of glial cells.

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674 THE DEVELOPMENT AND INTERACTIONS OF NEURONS AND GLIAL CELLS IN CULTURE

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We have used various conventional and monoclonal antibodies which react specifically with the major classes of cells in the rat central nervous system (CNS) and peripheral venous system (PNS) to study the development and interactions of these cells in dissociated cell cultures.

We have found that: (i) astrocytes, ependymal cells and oligodendrocytes develop on precisely the same schedule in dissociated cell cultures of 10 day old embryonic rat brain as they do in vivo; (ii) the neurites of both CNS and PNS neurons tend to associate with Schwann cells, astrocytes and oligodendrocytes, even in cases where they normally would not encounter one or other of these cells in vivo; (iii) central and peripheral neuronal cell bodies do not "sort-out" from each other in mixed CNS and PNS cultures and their neurites form mixed bundles (Fascicles) in such cultures, and (iv) oligodendrocytes (which make myelin in the CNS) continue to make myelin proteins and glycolipids for many weeks in culture in the absence of neurons, while Schwann cells (which make myelin in the PNS) stop making these molecules as soon as they are separated from their axons.

Cell Stroma Interactions

675 ORGANIZATION, ASSEMBLY AND REMODELING OF THE EPITHELIAL BASAL LAMINA. M.R. Bernfield, G. David, S.D. Banerjee, A.C. Rapraeger and J.E. Koda. Department of Pediatrics, Stanford University, Stanford, California 94305.

A basal lamina (BL), a complex of glycoproteins, non-fibrillar collagens, proteoglycans (PG) and hyaluronic acid, is closely adherent to the basal surface of parenchymal cells wherever they contact mesenchymal tissue. The BL maintains epithelial morphology, acts as a substratum upon which cells change shape and differentiate, as a permeability barrier, and as a scaffold for tissue repair. The BL is produced by the epithelium and consists of two ultrastructurally distinct layers, the lamina densa containing collagen and the lamina rara (or interna) containing laminin. Fibronectin is present in some BL and the glycosaminoglycans (GAG) present are in ordered periodic arrays. BL differ between tissue types and with development. For example, BL rich in heparan sulfate (HS) predominate on adult epithelia and on embryonic epithelial sheets and tubes, while BL rich in hyaluronate are on epithelia that bud, branch and fold (1). To study BL assembly, we have examined the PG produced by a mouse mammary epithelial cell line. When cultured on plastic, no BL forms, but when cultured on a type I collagen gel, although the PG synthesis rate is unchanged, the rate of PG degradation is markedly reduced and a BL rich in HS PGs accumulates (2). The HS PG unique to the BL appears to be produced initially as a smaller membrane-bound precursor, which is then covalently modified, either extracellularly or upon secretion, for deposition into the BL. On plastic, this PG is produced but it rapidly degrades, suggesting that the collagen stabilizes the BL. This idea is supported by studies of the mouse embryo salivary epithelium during its branching morphogenesis. At 13 days gestation, this epithelium has formed discrete lobules separated by clefts and is surrounded by the mesenchyme that is required for the morphogenesis. Type I collagen produced by the mesenchyme is within the clefts which are lined by a continuous HS PG-rich BL. Collagen fibers are nearly absent from the thin and discontinuous hyaluronate-rich BL on the distal lobules. During morphogenesis, the GAG in the BL on the lobules, the sites of growth and new branching, is degraded more rapidly than that on the interlobular clefts, the morphogenetically quiescent sites (3). This degradation is due to the mesenchyme which contains a soluble neutral hyaluronidase. The rapid remodeling of the BL on the lobules may allow more growth and folding than the more stable BL in the clefts. Regulation of BL turnover may be a general mechanism for controlling epithelial behavior. (Supported by NIH grants HD 06763 and CA 28735).

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676 FIBRONECTIN Kenneth M. Yamada, Steven K. Akiyama, and Masao Hayashi. Membrane Biochemistry Section, Laboratory of Molecular Biology, National Cancer Institute, Bethesda, MD 20205.

Fibronectin is a multifunctional adhesive glycoprotein present on the cell surface, in extracellular matrices, and in blood. Fibronectin is present antigenically in a number of invertebrate and vertebrate species. This glycoprotein has been implicated in cellular adhesion, cell-cell interactions, cell motility, and in the uptake of particles by macrophages.

Recent data suggest that the cellular and plasma forms of fibronectin are similar but structurally distinct molecules, with specific regions of polypeptide differences. Three regions of either polypeptide insertion or deletion have been identified: one is in a collagen-binding region and two are in heparin-binding regions. The identification of such internal polypeptide differences suggests the existence of more than one gene or spliced mRNA product coding for fibronectin. The two forms of fibronectin also differ in certain biological activities, although they share other activities such as chemotactic activity for fibroblasts and the ability to stimulate macrophage phagocytosis.

Dissection of fibronectin using a variety of proteolytic enzymes has provided insights into its functional domain structure. Discrete domains that interact with cell surfaces, collagen, heparin, actin, and other materials have been isolated. In addition, the identity of the cell surface receptor for fibronectin has been under investigation, and recent data suggest that it may include a ganglioside component; for example, fibronectin can function as a vertebrate lectin with specificity for the oligosaccharides of gangliosides such as G_{T1}.

The elucidation of the molecular mechanisms by which fibronectin interacts with specific ligands is providing insight into how this protein and related molecules can mediate a wide variety of cellular interactions.

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677 INTERACTIONS OF AXONS, SCHWANN CELLS AND FIBROBLASTS IN PERIPHERAL NERVE DEVELOPMENT. R. Bunge, Dept. Anatomy & Neurobiology, Washington University, St. Louis, Missouri.

Recent observations indicate that ensheathment and myelination of nerve fibers by Schwann cells involves a complex series of interactions in which fibroblasts or their products also play a role. Studies utilizing separated (and recombined) populations of Schwann cells, sensory neurons and fibroblasts in tissue culture indicate that: 1) axonal contact provides a mitogenic signal for Schwann cells, 2) axonal contact engenders Schwann cell production of certain extracellular matrix components, 3) fibroblasts appear to contribute the major portion of endoneurial collagen, 4) secretory activity by Schwann cells appears to be necessary for the expression of Schwann cell ensheathment, 5) secretory activity by Schwann cells may be instrumental in modifying the neurite mitogen to terminate Schwann cell proliferation, 6) contact of the axon by the Schwann cell will not result in normal Schwann cell differentiation unless simultaneous contact with a "third element" (present in our culture system as reconstituted tendon collagen) is provided, 7) long-term cultures containing only neurons and Schwann cells in serum-containing medium will not myelinate unless embryo extract is added in the culture medium or a population of fibroblasts is present in the culture dish and 8) axonal characteristics determine whether Schwann cells will simply ensheath axons, or manufacture a myelin sheath. Peripheral nerves of the mutant mouse dystrophic contain deficiencies of Schwann cell ensheathment which may be explained on the basis of abnormalities in certain of these interactions.

Role of Extracellular Matrix in Cell-Cell Recognition

678 TUMOR CELL INTERACTIONS WITH ENDOTHELIAL CELLS AND ENDOTHELIAL CELL EXTRACELLULAR MATRIX. Garth L. Nicolson et al., Department of Tumor Biology, University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

During blood-borne tumor metastasis circulating malignant cells must bind to the vascular endothelium and invade the underlying basement membrane. A model for this process has been developed using cultured vascular endothelial cell monolayers which synthesize a basolateral extracellular matrix (1). Metastatic tumor cells as well as some normal cells such as leukocytes bind to vascular endothelial cell monolayers and stimulate endothelial cell retraction, exposure of the underlying matrix, migration of the malignant cells to the matrix and finally destruction of the matrix (2). The movement of metastataic tumor cells to the endothelial extracellular matrix appears to be due to an adhesive difference between the endothelial cell surface and the matrix. Human and murine melanoma cells adhere much more rapidly to the extracellular matrix, and this can be mimicked by the attachment of tumor cells to polyvinyl-immobilized fibronectin (3). Anti-fibronectin inhibits slightly binding of B16 melanoma cells to matrix, but completely blocks binding to immobilized fibronectin suggesting that this molecule may mediate in part the enhanced adhesion of melanoma cells to matrix. Tunicamycin inhibits glycosylation of tumor cell glycoproteins and blocks tumor cell- endothelial cell attachment and metastasis in vivo. The specificity of organ metastasis of selected B16 lines was examined in vitro using endothelial cells from specific organs. Brain-selected B16-B sublines adhered more rapidly to brain endothelial cells than lung-selected B16-F sublines suggesting that endothelial cells posess determinants that can be specifically recognized by blood-borne metastatic cells. Suported by NCI grants ROI-CA-28867 and ROI-CA-28844 to G.L. Nicolson.

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679 CULTURE OF DIFFERENTIATED EPITHELIAL CELLS ON TISSUE-SPECIFIC SUBSTRATES OF EXTRACELLULAR MATRIX. L. Reid, Z. Gatmaitan, S. Mackensen, P. Jubinsky and B. Morrow. Albert Einstein College of Medicine, 1300 Morris Park Ave.,Bronx,N.Y.

We have proposed that critical variables controlling the differentiation of normal cells as well as partially differentiated malignant cells are found within the tissue and due to cell-cell interactions. In efforts to study controls of differentiation of adult and embryonic tissues, we have focused upon the epithelial-mesenchymal relationship, a ubiquitous cell-cell relationship in tissues, and, we believe, a primary determinant of differentiation. Our approach is to culture epithelial cells on substrates of tissue-specific extracellular matrix and in medium supplemented with cell-specific hormones and with conditioned medium from primary cultures of the appropriate mesenchymal cell derivative. The first phase of developing this new cell culture technolology was to develop methods for isolating basement membranes. We have succeeded in developing such methods by which to isolate an extract of extracellular matrix, called biomatrix, and which includes components derived from basement membrane and from ground substance. It is isolated by homogenizing tissue in water with protease inhibitors filtering through polyester cloth having an 80 micron pore diameter, sequentially treating the insoluble fibrous material with low ionic strength salt buffers, nucleases, and delipidating solutions. The procedures select for native collagen fibers and their associated proteins. Chemical characterization of biomatrix from several tissues indicates that it is approximately 50-60% collagens and the rest,non-collagenous proteins which are often rich in carbohydrate groups. All of the biomatrices examined thus far have contained fibronectin. The types and amounts of collagenous and non-collagenous proteins are specific to each type of biomatrix and correlate with the known components found in the extracellular matrix of the tissue. Methods have been developed for use of biomatrix as a substrate for cell cultures. The biomatrix is either homogenized, pulverized or prepared as frozen sections smeared onto petri dishes and is then sterilized by gamma irradiation. Use of tissue-specific biomatrix substrates has considerably enhanced the ability to culture various types of epithelial cells resulting in increased plating efficiencies, increased long-term survivals, and increased expression of various markers of differentiation. The ability of cells to grow on biomatrix in vitro is correlated with the proliferative potential of the cells in vivo. Normal cells appear to be strictly tissue-specific and to some extent species specific in preference for biomatrix types. Malignant tissues are less strict but have a tissue specificity which may be relevant for their metastatic potential in vivo. The tissue-specificity is correlated with non-collagenous proteins and can be eliminated or reduced with glycosidases or with extraction of biomatrix with guanidine hydrochloride. Studies of hepatoma cells in serum-free, defined medium indicate that hormone and growth factor requirements are altered both qualitatively and quantitatively by culturing the cells on collagenous or biomatrix substrates.

Cytoskeleton-Surface Interactions

680 MOLECULAR MORPHOGENESIS OF THE Z-DISC IN MUSCLE CELLS. Elias Lazarides, Bruce L. Granger, David L. Gard and Richard H. Gomer. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Immunofluorescent mapping of protein in isolated chicken skeletal muscle Z-disc sheets has demonstrated that the Z-disc is composed of two distinct, but interconnected, domains: A central one containing actin and the actin-binding protein α-actinin and a peripheral one containing actin, the actin-binding protein filamin and the intermediate filament proteins, desmin, vimentin and synemin. The peripheral domain surrounds each Z-disc, and may be responsible for linking and promoting the registration of adjacent myofibrils as well as for integrating associated membranous organells such as mitochondria, the transverse tubular (T) system and the sarcoplasmic reticulum. During the early stages of myogenesis in vitro desmin, vimentin and synemin exist in a filamentous form in the cytoplasm and are readily aggregated by colcemid; within a week of fusion and two to three days after the assembly of α-actinin containing Z-discs, desmin, vimentin and synemin begin to associate with the Z-discs and become resistant to rearrangement by colcemid. From these observations it becomes apparent that the Z-disc is composed of at least two distinct molecular domains which assemble sequentially. Early in myogenesis, filamin is found associated with the newly differentiating α-actinin-containing filament bundles. However within two days after fusion filamin synthesis ceases and the quantity as well as cytoplasmic fluorescence of filamin decrease rapidly. The disappearance of filamin persists for the ensuing four days of myogenesis at which time filamin synthesis resumes and filamin accumulates in the cytoplasm. The newly appearing filamin localizes now to the myofibril Z-line and is visible there shortly before vimentin, desmin and synemin become associated with the Z-line. Thus the peripheral domain assembles sequentially in at least two distinct steps. The removal of filamin from the cells suggests that either filamin may not be required or may actually interfere with a necessary process during the early stages of sarcomere morphogenesis. The reappearance and deposition of filamin to the periphery of the Z-disc indicates that this protein may be required for the transition of desmin, vimentin and synemin to the Z-disc.

681 THE T/t-COMPLEX AND EARLY DEVELOPMENT, Dorothea Bennett, Laboratory of Developmental Genetics, Sloan-Kettering Institute for Cancer Research, New York, N.Y. 10021

Mutations in the mouse T/t-complex have profound effects on differentiation in early embryos and in spermatogenesis. The impairment of differentiation appears to result from, or to be associated with, abnormalities of cell interaction caused by aberrant cell surface molecules. Recent serological and biochemical studies show that abnormal cell surface antigens are controlled by t-mutations, and that at least part of their antigenic specificity resides in specific carbohydrates (1). The t^{12}-haplotype, for example, has an immunodeterminant in terminal galactose. The specific embryonic defects associated with homozygosity for t^{12} are a failure to form normal intercellular junctions, and an inability of morulae to undergo compaction and transform into blastocysts. The dependence of these phenomena on normal galactosylation of cell surface components in normal and mutant embryos will be discussed.

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682 MICROFILAMENT FUNCTION DURING CHANGES IN CELL SHAPE AND SURFACE ARCHITECTURE, John Condeelis, AECOM, Bronx, New York 10461

In this laboratory we have studied the composition and function of actin containing structures and their association with the cell membrane. We have purified several actin binding proteins (1) from the cellular slime mold Dictyostelium discoideum which account for many actin associated functions in cell extracts and intact cells (2,3). One of these proteins, 120 K, is localized in the cell cortex immediately subjacent to the cell membrane and can account for the dense meshwork of cross-linked and branched actin filaments found there (1,4). The properties and location of 120 K indicate that it is of major importance in regulating the distribution and structure of the cortical actin cytoskeleton.

We have studied the attachment of actin filaments to the cell membrane during ligand induced receptor redistribution. Regions of the cell surface that contain Con A-receptor complexes have been purified from cells during various stages of capping (5). Analysis of these membrane fragments demonstrates that they are substantially enriched in actin and myosin as compared with the whole cell. Actin filaments are found to be attached to the cytoplasmic surface of the cell membrane opposite ligand clusters through links that are stable to ionic conditions required for disassembly of the actin cytoskeleton and contraction in cell extracts (2). Detergent extraction of these membrane fragments removes lipid without disrupting attachments between actin filaments and ligand clusters which indicates that this association involves a protein-protein interaction.

We have also investigated the role of actin filaments in receptor mediated endocytosis (6). When human B lymphoblastoid cells (Wil 2) are challenged with anti-IgM a sequence of events resulting in endocytosis of antibody into coated vesicles is activated. Further study of coated vesicles in Wil 2 and other cultured cells demonstrates that they form numerous attachments with actin filaments that have polarity away from the vesicle. The geometry of this suggests that actin is involved in production of motive force for vesicle translocation within the cytoplasm.

Hence, actin filaments with stable membrane attachments are available for interaction with myosin to produce movement at the surface of the cell regardless of or in response to disassembly of the cortical actin cytoskeleton.

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Cell Recognition in Eucaryotic Cells

683 CELL RECOGNITION IN EUKARYOTIC CELLS. L. Glaser, Department of Biological Chemistry, Washington University School of Medicine, St. Louis, MO 63110.

We will review work on cell recognition with emphasis on the functional consequences of cell adhesion. Approaches to the study of cell recognition events will focus on contact inhibition of growth in fibroblasts and the mitogenic interaction between neurites and Schwann cells. The general approach is to purify membrane components involved in these interactions and to characterize their interactions with the cell surface, and their relationship to other hormones involved in growth control.

Carbohydrate Signals on Proteins

684 VERTEBRATE LECTINS: WHAT ARE THEIR FUNCTIONS? Samuel H. Barondes, University of Calif., San Diego, La Jolla, CA 92093; and Veterans Administration Medical Center, San Diego, CA 92161.

The possible functions of three lectins that have been purified from embryonic and/or adult chicken tissues will be considered. These lectins, chicken-lactose-lectin-I (CLL-I), chicken-lactose-lectin-II (CLL-II), and chicken-heparin-lectin (CHL) are named to indicate a potent hapten inhibitor. All are composed of subunits with molecular weights ranging from 13,000 to 16,000 and all are found in both developing and adult chicken tissues. However, there are striking differences in their relative expression in different developing and adult tissues as well as in their cellular localization. For example, CLL-I is much more abundant in embryonic than in adult muscle, but much less abundant in embryonic than in adult liver. It is largely intracellular in developing muscle cells and hepatic Kupffer cells; but it is mostly extracellular in adult chicken pancreas. CLL-II is more abundant in embryonic than in adult kidney, but is far more abundant in adult than in embryonic intestine where it is localized in the secretory granules of the goblet cells and on the intestinal mucosal surface. Relatively less is presently known about chicken-heparin-lectin which has been purified from embryonic muscle and adult liver. Evidence that it and a complementary ligand are present extracellularly in muscle cultures suggests that it may interact with the cell matrix. The striking differences in distribution and developmental expression suggest that chicken lectins have a variety of functions in different tissues and at different stages of development. The role for these endogenous lectins apparently include cell-cell interactions, cell-matrix interactions, and interactions of cells with extracellular products.

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685 THE MOLECULAR BASIS FOR MEMBRANE-CYTOSKELETON ASSOCIATIONS IN HUMAN ERYTHROCYTES, Vann Bennett, Department of Cell Biology and Anatomy, Johns Hopkins School of Medicine, Baltimore, MD 21205.

Spectrin, the major cytoskeletal protein in erythrocytes, is localized on the inner membrane surface, in association with membrane-spanning glycoproteins and with intramembrane particles. The presence of a specific, high affinity protein binding site for spectrin on the cytoplasmic surface of the membrane has been established by measurement of reassociation of spectrin with spectrin-depleted inside-out vesicles[1]. A 72,000 M_r proteolytic fragment of this attachment protein has been purified[2], which bound to spectrin in solution, competed for reassociation of spectrin with vesicles, and, when incubated with erythrocyte ghosts, increased the rate of lateral mobility of integral membrane proteins[3]. A 215,000 polypeptide was identified as the precursor of the spectrin-binding fragment based on immunological cross-reactivity, ability to bind to spectrin, as well as other properties[4]. The membrane attachment protein for spectrin was named ankyrin, and has been purified and characterized[5]. Ankyrin is a peripheral membrane protein, and thus additional linkages are required to explain the observations that spectrin interacts with integral membrane proteins. Ankyrin has been demonstrated to be tightly associated in detergent extracts of vesicles with band 3 a major membrane-spanning polypeptide, and to bind directly to a proteolytic fragment derived from the cytoplasmic domain of band 3[6,7]. Ankyrin is thus an example of a protein which directly links a cytoplasmic structural protein to an integral membrane protein. The organization of the erythrocyte membrane has implications for more complex cell types, since immunoreactive forms of ankyrin distinct from myosin or filamin have been detected by radioimmunoassay in a variety of cells and tissues[8]. Indirect immunofluorescent staining of cultured epithelial cells reveals ankyrin in microvilli, ruffles and other cell surface structures in a cytoplasmic meshwork, and in a punctate distribution over nuclei. The staining changes dramatically during mitosis, with concentration of stain at the spindle poles during metaphase, and intense staining of the cleavage furrow during cytokinesis.

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686 PHOSPHOMANNOSYL-ENZYME RECEPTORS: SUBCELLULAR DISTRIBUTION AND ROLE IN INTRACELLULAR TRANSPORT OF LYSOSOMAL ENZYMES, William S. Sly, H. David Fischer and Alfonso Gonzalez-Noriega, Washington University, St. Louis, MO 63110

Cell surface lectins which recognize sugars on glycoproteins include the Galactosyl-glycoprotein receptor on hepatocytes, the Mannose/N-acetylglucosamine receptor on macrophages, a Fucosyl α-(1→3) N-acetylglucosamine receptor on hepatocytes, and the Man 6-P acid hydrolase receptor on fibroblasts (1). All of these have been recognized as pinocytosis receptors that mediate uptake of the respective ligands. The Man 6-P enzyme receptor has an even more important intracellular role, mediating transport of newly synthesized lysosomal enzymes and is not limited to fibroblasts.

Lysosomotropic amines enhance secretion of high-uptake (i.e., phosphorylated) acid hydrolases (2). β-Hexosaminidase B was purified from amine-induced fibroblast secretions and used to study enzyme receptors (3). Pinocytosis of this ligand by fibroblasts was saturable, linear with time, inhibited by Man 6-P, and abolished by treatment of the ligand with alkaline phosphatase or endoglycosidase H. Enzyme internalized over 3 hours at 37°C in 0.1 mM cycloheximide was 32 times the amount bound to cells at 4°C. The Man 6-P inhibitable component of enzyme binding to cell membranes was saturable, and specific for enzymes bearing the phosphomannosyl recognition marker. Of the Hex B receptors in human fibroblasts, only 13% were on the cell surface; 87% were on intracellular membranes. Comparable Hex B receptors were found in membranes from 9 human organs, and the corresponding rat organs. Subcellular fractions from rat liver showed the following distribution of enzyme receptors: endoplasmic reticulum (80%), Golgi apparatus (7%), lysosomes (4%), plasma membrane (9.5%), nuclei and mitochondria (less than 2%). The distribution of enzyme receptors, the gradient of receptor occupancy (from mostly occupied receptors in the endoplasmic reticulum to mostly unoccupied receptors in lysosomes), and the distribution of high-uptake endogenous rat enzymes were all compatible with a model of enzyme transport in which phosphomannosyl enzyme receptors act as vehicles for delivery of newly synthesized acid hydrolases from the endoplasmic reticulum to lysosomes. The effects of amines suggest that the intracellular receptors recycle following enzyme delivery, as do cell surface receptors following pinocytosis.

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Intercellular Membrane Recognition

687 CELLULAR MECHANISMS UNDERLYING LDL RECEPTOR ACTIVITY, Richard G.W. Anderson, Joseph L. Goldstein, and Michael L. Brown, Departments of Cell Biology and Molecular Genetics, University of Texas Health Science Center at Dallas, Dallas, Texas 75235

Extensive biochemical and genetic work has established the LDL receptor as the mediator for uptake and degradation of low density liproprotein. The receptors for this protein are primarily located in coated cell surface regions. We envision three underlying mechanisms that are involved in regulating the activity of this receptor. 1) Protein synthesis is required for LDL receptor activity. Cells that are exposed to an exogenous cholesterol source cease to synthesize LDL receptors and they disappear from the cell surface with a half time of 20 hours. A release from cholesterol supression leads to the synthesis of new receptors. Evidence suggests that these new receptors are transported to the cell surface in coated vesicles. 2) In contrast to receptor synthesis, receptor recycling is very rapid and is necessary for the sustained uptake of LDL. Recent evidence suggests that receptor recycling requires that the LDL receptor reappear on the cell surface less than 1 minute after delivering LDL to the endocytic vesicle and that the new receptors are pre-clustered. The rapid return as well as the pre-clustering of receptors are considered to be key aspects of efficient recycling. 3) Previous work with a mutant cell line that is unable to internalize LDL suggested that tight coupling must exist between receptors for LDL and coated pits for any internalization to take place. We now report that cells that have an excess number of LDL receptors are able to internalize only the LDL that is bound to those receptors that are in coated pits. This suggests that the activity of the coated membrane system can be rate limiting and affect the ability of the cell to internalize LDL.

688 REGULATION OF ORGANELLE MOVEMENT, MEMBRANE FUSION AND EXOCYTOSIS IN THE CHROMAFFIN CELL, Harvey B. Pollard, Carl E. Creutz, Christopher J. Pazoles, Janet H. Scott, and Velia M. Fowler, NIAMDD, NIH, Bethesda, Md. 20205

The process of exocytosis appears to involve a series of critical intracellular interactions between secretory vesicles and other subcellular components, necessarily involving Ca^{++} at some or all of these steps. Our approach over the last few years has been to study the chromaffin granule from adrenal medullary cells with specific interest in (a)the interaction of the organelle with cytoskeletal elements such as actin;(b)the synexin-mediated, calcium dependent interaction of granule membranes with other granules and with plasma membranes;(c) synexin dependent membrane fusion;and (d) the comparative energetics of chemiosmotic granule lysis and in situ release of catecholamines from chromaffin cells. Our aim has been to reconstitute events in the secretion process on a cell-free basis and to thereby identify proteins and other factors involved in each interaction.

We presumed that movement of chromaffin granules to the cell periphery for eventual secretion might be mediated by the specific interaction of actin with the granule membrane. In fact, we have been able to detect such interactions using a falling ball viscometer to measure cross linking of F-actin by purified granule membranes. The interactions are both pH dependent and inhibited by raising free $[Ca^{++}]$ to micromolar levels. The site on the granule membrane responsible for actin binding is itself thermolabile and protease sensitive.

In order for secretion to occur the granule membrane must associate with either the plasma membrane or with other granule membranes during compound exocytosis. Synexin, a calcium binding protein recently discovered in our laboratory, may be involved in this step. When synexin binds Ca^{++} it self-associates into rods which themselves cause granule membranes to aggregate into pentalaminar complexes. Binding sites for activated synexin have now been detected not only on chromaffin granules but also on the inner aspect of chromaffin cell plasma membranes. In all cases synexin binding, self-association and consequent membrane interaction have had identical Ca^{++} dependency.

Final release of granule contents from the cell depends on breakage and fusion of the membrane complex separating the granule core from the external medium. This poorly understood membrane remodeling process may involve osmotic stress on the granule-plasma membrane complex, perhaps by means of a chemiosmotic lysis process easily studied with isolated chromaffin granules. This membrane remodeling may also involve the action of cis-unsaturated fatty acids such as arachidonic acid which we have recently found will induce massive fusion of isolated chromaffin granules if they are first aggregated with synexin and calcium.

689 SORTING OF VESICLE CONTENTS DURING IgG TRANSPORT IN THE NEONATAL RAT INTESTINE. Richard D. Rodewald and Dale R. Abrahamson, Department of Biology, University of Virginia, Charlottesville, Virginia 22901.

The duodenum and proximal jejunum of neonatal rats efficiently transfer maternal immunoglobulin G (IgG) from the intestinal lumen to the circulation. Our past studies have defined the morphological pathway for this transport (1,2). Successful transfer depends on the specific, pH-dependent attachment of IgG to receptors on the luminal surface of absorptive cells. Receptor-bound IgG is then internalized within a specialized system of small tubules. The IgG is transferred to spherical coated vesicles which discharge at the basolateral cell surface. Although endocytosis of IgG at the luminal surface is highly selective, small amounts of other proteins can enter cells non-selectively. Unlike IgG, however, these proteins are normally not discharged functionally intact from the cells. We have sought to determine the basis for this intracellular sorting of proteins by using several tracers for electron microscopy. When we expose absorptive cells to horseradish peroxidase (HRP) as a marker for fluid phase endocytosis and IgG conjugated to ferritin (IgG-Ft) as a specific tracer for receptor-bound IgG, we find that both tracers enter cells simultaneously within the same endocytic vesicles. Although IgG-Ft is subsequently transferred across the cells in the normal manner, HRP remains within the apical cytoplasm where it is concentrated and presumably degraded within small vacuoles and lysosomes. When we use cationic ferritin (Ft^{+}) as a relatively non-specific marker for luminal membrane components, we find that this tracer also binds avidly to the membranes of endocytic vesicles and is transferred across cells. However, unlike IgG, the Ft^{+} that crosses the cells remains associated with the basolateral plasma membrane. When we present cells with a mixture of HRP and Ft^{+}, again we find internal sorting of proteins. Both tracers appear in endocytic vesicles, but only membrane-bound Ft^{+} is transported to the basolateral surface. The results of these experiments suggest that transfer of a ligand across the absorptive cell requires continued binding of the ligand to the membranes of the endocytic vesicles. This binding may be highly specific, as in the case of the interaction of IgG with its membrane receptor, or relatively non-specific, as in the case of the binding of cationic ferritin. Proteins such as HRP that enter the cells in the fluid contents of endocytic vesicles are efficiently removed from the transport pathway and are diverted to lysosomes.

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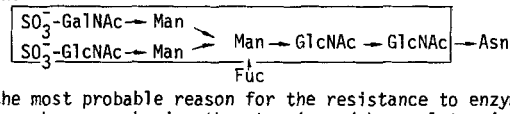
Carbohydrate Structure

690 RECOGNITION OF CARBOHYDRATE MOIETIES ON GLYCOPROTEINS BY H-2 RESTRICTED CTL. Paul L. Black, Ellen S. Vitetta, James Forman and Jonathan W. Uhr, Dept. of Microbiol. & Immunol., Temple U. Medical School, Phila., PA 19140 & Dept. of Microbiol., U. of Texas Health Sci. Ctr., Dallas, TX 75235. Mice infected with vesicular stomatitis virus (VSV) generate cytotoxic T lymphocytes (CTL) which recognize viral antigens on the surfaces of H-2 compatible VSV-infected cells. The importance of the carbohydrate moieties of target cell surface glycoproteins in recognition by VSV-immune CTL was demonstrated by using the antibiotic tunicamycin, which inhibits the addition of carbohydrates to the polypeptides of glycoproteins. Treatment of CTL with tunicamycin before and during VSV infection inhibited glycosylation of H-2 & VSV glycoprotein (G protein), & it inhibited lysis of infected cells by VSV-immune H-2 identical CTL. In contrast, tunicamycin had only a modest inhibitory effect on cytolsis by alloimmune CTL & on H-2 restricted cytolysis directed against both minor histocompatibility antigens & chemically modified (by trinitrophenylation) autologous cells. Tunicamycin treatment also did not inhibit the surface expression of either H-2 or VSV G protein as judged by several serological techniques (immunoprecipitation & SDS-PAGE, quantitative absorption, & radioimmunoassay). Furthermore, analysis of surface protein expression at the single-cell level with the fluorescence-activated cell sorter (FACS) revealed no differences in quantity or distribution of H-2 & VSV G protein after tunicamycin treatment. Therefore, glycosylation of H-2 and/or viral glycoprotein is a prerequisite for lysis of infected cells by VSV-immune H-2 identical CTL. These data support the interpretation that H-2 restricted CTL recognize antigenic determinants either consisting of or stablized by carbohydrate moieties on glycoproteins. Experiments employing nonglycosylated G & H-2 proteins incorporatedinto liposomes are in progress & will facilitate further analysis of the role of the carbohydrates of H-2 and VSV G protein in H-2 restricted cytolysis.

691 OLIGOSACCHARIDE MOIETIES OF GLYCOPROTEIN HORMONES: THE RESISTANCE TO ENZYMATIC DEGLYCOSYLATION OF BOVINE LUTROPIN BECAUSE OF O-SULFATED N-ACETYLHEXOSAMINES, Thomas F. Parsons and John G. Pierce, U.C.L.A., Los Angeles, CA 90024
The oligosaccharides of the bovine pituitary gonadotropin, lutropin, are N-linked to asparagine residues. These carbohydrates are unusual in that, while they contain the mannose, N-acetylglucosamine and fucose typical of complex type oligosaccharides, they also contain one residue of N-acetylgalactosamine but insignificant amounts of sialic acid or galactose. These oligosaccharides are completely resistant to most glycosidases. This is in contrast to the ready release of sugars from human chorionic gonadotropin, a related placental hormone which has oligosaccharides of the complex type with terminal sialic acid and galactose residues. Stability of the lutropin hexosamines to periodate oxidation, together with Smith degradation data and sulfate analysis lead to the following partial structure for the oligosaccharides of bovine lutropin:



Sulfation is the most probable reason for the resistance to enzymatic deglycosylation. The related pituitary hormones bovine thyrotropin and human lutropin also contain sulfate in contrast to the absence of sulfate in human chorionic gonadotropin, indicating the presence of sulfating enzymes in the pituitary.

692 DEVELOPMENTAL REGULATION OF GLYCOPROTEIN BIOSYNTHESIS IN DICTYOSTELIUM, Raymond Ivatt, Prem Das , Ellen Henderson and Phillips Robbins, M.I.T. Cambridge, MA 02139.
The synthesis of glycoproteins in Dictyostelium discoideum is initiated by the en bloc transfer of a precursor oligosaccharide from a carrier lipid to nascent polypeptide chains. The processing of this oligosaccharide after transfer to protein is dramatically depedent upon the stage of development. The reactions involved in this processing fall into two developmentally regulated groups. One group is expressed during early development and contributes a pathway which leaves the precursor oligosaccharide essentially intact and results in substantial (50-60%) fucosylation of the di-N-acetylchitobiose core and occassional (30%) sulfation of the glycans. This pathway demishes during aggregation. The other group of reactions is expressed during late differentiation. Together they constitute a pathway which results in extensive trimming of the precursor oligosaccharide and is characterized by the absence of sulfation and by fucosylation of peripheral rather than core sugars. This pathway makes its appearance coincident with the construction of tips on tight mounds. At this stage enzymatic activities of both pathways occur simultaneously.

Developmental regulation of the wide array of protein-linked glycans during the developmental cycle of D.discoideum is therefore the controlled modulation between a vegetative path way (similar to that in other simple eukaryotes) to a mutlicellular pathway (similar to that in higher eukaryotes). The roles played by cell-cell contact and c-AMP in regulating this transition will be presented.

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693 STRUCTURAL METHODS IN ANALYSIS OF CELL SURFACE LONG CHAIN POLYLACTOSAMINE GLYCOPEPTIDES (ERYTHROGLYCAN), ROGER A. LAINE, SALVATORE J. TURCO AND JEFFREY S. RUSH, UNIVERSITY OF KENTUCKY, LEXINGTON, KY 40536

A LARGE PROPORTION OF THE GLYCOCALYX OF THE HUMAN ERYTHROCYTE CONSISTS OF HIGH MOLECULAR WEIGHT (M_R=10,000) POLYLACTOSAMINE POLYMERS ATTACHED TO "BAND 3" AND OTHER PROTEINS (1978, J.BIOL.CHEM. 253, 8006; 1978, FEBS LETT. 89, 111; 1978, EUR. J.BIOCHEM. 90, 289). K-562 ERYTHROLEUKEMIC CELLS MAY BE USED IN BIOSYNTHETIC STUDIES OF THESE POLYMERS. STRUCTURAL ANALYSIS OF SUCH LARGE OLIGOMERS REQUIRES THE USE OF PARTIAL DEPOLYMERIZATION TECHNIQUES SUCH AS HYDRAZINOLYSIS-DEAMINATION, ENDOGLYCOSIDASE DIGESTION AND DEACETYLATION-PARTIAL HYDROLYSIS. FOLLOWING THIS, THE PRODUCTS CAN BE PURIFIED AND TREATED WITH EXOGLYCOSIDASES. THE SENSITIVITY FOR DETECTION OF THE PRODUCTS OF METHYLATION LINKAGE ANALYSIS CAN BE ENHANCED BY THE USE OF METHANE CHEMICAL IONIZATION MASS SPECTROMETRY. (RESEARCH SUPPORTED BY NIH GRANT #AM25101 TO R.A.L.)

694 THE ENZYMATIC BASIS OF THE PHOSPHORYLATION OF LYSOSOMAL ENZYME HIGH MANNOSE UNITS, Marc L. Reitman, Ajit Varki, and Stuart Kornfeld, Washington University School of Medicine, St. Louis, MO 63110.

The recognition marker for the targeting of lysosomal enzymes contains mannose 6-phosphate. The recent discovery of phosphate in diester linkage between N-acetylglucosamine (GlcNAc) and mannose led to the proposal that phosphomannosyl residues are acquired by GlcNAc-phosphate transfer from UDP-GlcNAc to mannose, followed by the removal of the GlcNAc residue (I. Tabas and S. Kornfeld (1980) JBC 255:6633). We synthesized [β-^{32}P]UDP-[^{3}H]GlcNAc and used this compound to demonstrate a UDP-GlcNAc:glycoprotein GlcNAc-1-phosphotransferase. The basis of the enzyme assay is the incorporation of ^{32}P and ^{3}H into glycopeptides with a high affinity for Concanavalin A-Sepharose. This membrane-associated transferase is neither inhibited by tunicamycin nor stimulated by dolichol-phosphate, indicating that the reaction does not proceed via a dolichylpyrophosphorylGlcNAc intermediate. Characterization of the enzyme reaction products demonstrated that α-linked GlcNAc 1-phosphate is transferred en bloc to the 6 hydroxyl of mannose in high mannose-type oligosaccharides of endogenous glycoproteins. The GlcNAcphosphotransferase will work on an exogenous glycopeptide acceptor. We have also purified (1800-fold) a highly specific α-N-acetylglucosaminyl phosphodiesterase from rat liver smooth membrane preparations. This enzyme is physically separable from the lysosomal α-N-acetylglucosaminidase and exhibits different substrate and inhibitor specificities. The purified phosphodiesterase will cleave the in vitro product of the GlcNAcphosphotransferase reaction. We propose that the combined action of these two enzymes results in the formation of 6-phosphomannosyl residues on newly synthesized lysosomal enzymes.

695 HPLC Separation of Anionic and Neutral Oligosaccharides. S.J. Mellis, M. Natowicz and J.U. Baenziger, Washington University School of Medicine, St. Louis, MO 63110

Techniques are now available for the enzymatic and chemical release of intact oligosaccharides from glycoproteins. We have developed high performance liquid chromatographic procedures for the rapid fractionation and analysis of oligosaccharides and their degradation products. Detection in the picomole to nanomole range is obtained by metabolic introduction of radiolabel or reduction with $NaB(^{3}H)_4$. Neutral and anionic oligosaccharides can be fractionated by ion exchange chromatography on MicroPak AX-10 (Varian Associates) with an aqueous mobile phase and a gradient of increasing KH_2PO_4 concentration. Oligosaccharides bearing from 0-4 sialic acid moieties or from 0-3 PO_4 moieties in mono or diester linkage can be completely resolved in less than 45 min.

Mixtures of neutral oligosaccharides containing from 1-20 residues can be fractionated into their individual components in a single 1 hr. analysis on MicroPak AX-5 using a gradient of acetonitrile:H_2O. Neutral complex, high mannose, and hybrid type oligosaccharides have been fractionated in this fashion with previously unattainable resolution. In a number of instances, high mannose type oligosaccharides have been fractionated into multiple species of identical molecular weight and composition, suggesting that these columns are sensitive to composition and to other structural features such as linkage pattern. These methods in conjunction with rapid analyses on BioGel P-4 at elevated temperatures and pressures (Anal. Biochem. 105: 159-164, 1980) have markedly improved the resolution and reduced the time required for oligosaccharide structural analyses.

696 Use of Endo-H in Clarifying the Structure and Function of Glycoproteins, Frank Maley, Robert B. Trimble, Frederick K. Chu, and Anthony L. Tarentino, Division of Laboratories and Research, New York State Department of Health, Albany, NY, 12201.

The specificity of action of endo-β-N-acetylglucosaminidase H (Endo-H) will be discussed in reference to its ability to modify polymannosyl containing glycoproteins such as invertase, thyroglobulin, IgM, ribonuclease B, deoxyribonuclease, carboxypeptidase Y and cell surfaces. Removal of oligosaccharide chains does not appear to diminish enzyme activity, although effects on stability and states of aggregation have been observed, particularly in the case of invertase, where the most stable form of the enzyme appears to be associated with an oligomer in contrast to its inactive form. It is also possible to distinguish between O- and N-type oligosaccharide on the same molecule by the ^{125}I-lectin overlay technique before and after treatment with Endo-H. The use of glycosyl-asparagine derivatives in establishing the lower limits of specificity of Endo-H will be also described, as well as a novel modification of the Smith degradation, which can be used to distinguish between different structures at the nanamole level. In this method a fluorescent dansyl group is attached to the asparagine moiety and following periodate oxidation, Dowex-50H+ is used to catalyze the removal of the modified sugars. The remaining residue is identified by thin-layer chromatography.

697 SYNTHESIS AND PROCESSING OF ASPARAGINE-LINKED OLIGOSACCHARIDES: INTERMEDIATES OF THE BRANCH GLYCOSYLATION, James R. Etchison, Univ. of California, Davis, CA. 95616. Elucidation of the complex intracellular pathway for the synthesis and processing of asparagine-linked oligosaccharide moieties of glycoproteins is important for a comprehensive understanding of the biogenesis of plasma membrane and secretory glycoproteins. Delineation of the individual reactions and their collation into a descriptive pathway provides insight into and a conceptualization of the cellular processes leading to the formation of plasma membranes. Following the en bloc transfer of a glucosylated oligomannosyl precursor oligosaccharide ($Glc_3Man_9GlcNAc_2$) from a polyisoprenyl donor to the nascent polypeptide, a series of processing reactions occur which include removal of the terminal glucose residues followed by the trimming of the oligomannosyl core to a structure containing only five mannoses ($Man_5GlcNAc_2$). It has been proposed and demonstrated in vitro that the final phase of trimming to the trimannosyl core found in numerous glycoproteins occurs after the initiation of synthesis of one of the branches found in the mature glycoprotein oligosaccharides. The results to be presented describe the isolation and characterization of intermediates formed in vivo during the branch glycosylation of the vesicular stomatitis virus glycoprotein. An intermediate containing a terminal N-acetylglucosamine attached to a pentamannosyl core has been identified after pulse-labeling of VSV-infected BHK cells. In addition, the data suggest that transfer of galactose to this initiated branch may also occur prior to the final trimming to a trimannosyl core.

Membrane Dynamics

698 BIOCHEMICAL EVIDENCE FOR THE ASSOCIATION OF SURFACE GLYCOPROTEINS WITH THE PLATELET CYTOSKELETON, R.G. Painter, M.H. Ginsberg. Res. Inst. of Scripps Clin. La Jolla, CA We have isolated cytoskeletal "shells" by centrifugation of Triton X-100 extracts of human platelets surface labeled with ^{125}I. In both ConA-stimulated and resting platelets, this sedimentable fraction is composed largely of cytoskeletal proteins including actin, myosin and filamin. Less than 10% of the two major ^{125}I-labeled surface glycoproteins, GPIIb and GPIII was associated with this fraction in resting platelets. Within 15-30 seconds after addition of ConA at concentrations >50 μg/ml, 90-95% of these two glycoproteins became associated with the Triton residue. In addition, the amount of sedimentable actin doubled. No co-sedimentation of GPIIb and III with the cytoskeleton was seen when a Triton extract of platelets was treated with ConA prior to centrifugation. This result indicates that the co-sedimentation observed in ConA-stimulated platelets was not due to precipitation of GPs by ConA or to the bridging of GPs to the cytoskeleton by ConA occurring after detergent lysis. To determine whether the ConA-induced increased sedimentability of GPIIb and III depended on an intact cytoskeleton, Triton extracts of ConA stimulated platelets were incubated with DNase I to depolymerize F actin. This caused a dose dependent decrease in ConA-mediated GPIIb and III sedimentation in association with loss of actin from the cytoskeletal shell. This result suggests that the co-sedimentation of GPIIb, GPIII and the cytoskeleton depends upon physical interactions between them. To determine whether such interactions might occur in the intact cells, platelets were incubated with Rhodaminated ConA and contraction of the cytoskeleton induced with thrombin. Under these conditions, the ConA binding sites co-distributed with a myosin-containing contractile ring.

699 INTERMEDIATE FILAMENT ORGANIZATION PROBED BY THE INTRACELLULAR INJECTION OF A MONOCLONAL ANTIBODY RECOGNIZING AN ANTIGENIC DETERMINANT COMMON TO ALL INTERMEDIATE FILAMENT CLASSES, Michael W. Klymkowsky, MRC Neuroimmunology Project, Dept. of Zoology, University College London, London WC1E 6BT U.K.

Intermediate filament networks come in a number of distinct classes with varing cellular distributions. Their functions within the cell, whether as mechanical supports or cytoplasmic organizers remain unclear. A monoclonal antibody which recognizes an antigenic determinant common to all classes of intermediate filaments (Pruss et al, in preparation) has been injected into living cells in an attempt to perturb intermediate filament organization and thus elucidate their functional role within the cell. The antibody (an IgG_1) decorates intermediate filaments in a number of different types of living cells. In 3T3 cells the injected antibody causes the rapid collapse (1-2 hours postinjection) of vimentin filaments into bundles and rings around the nucleus. This collapse occurs without obvious effects on cell morphology or microtubule or cytoplasmic myosin filament patterns. By 6 hours post-injection and increasingly thereafter these collapsed filaments then reemerge from the cluster and disperse again into the cytoplasm, still decorated with the injected antibody. Such a rapid filament collapse is not observed in glial fibrillary acidic protein containing protoplasmic or fibrous astrocytes.

Study continues on various classes of intermediate filaments using intracellular injection and other techniques.

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700 AN ACTIN CONTAINING MATRIX ASSOCIATED WITH THE PLASMA MEMBRANE OF MURINE TUMOR AND LYMPHOID CELLS, Matthew F. Mescher, Mimi J.L. Jose, Steven P. Balk and Steven H. Herrmann, Harvard Medical School, Boston, MA 02115

A detergent insoluble fraction has been isolated from purified plasma membranes of P815 tumor cells. This fraction accounts for 20-25% of the total membrane protein and appears to form a matrix at the inner face of the membrane. The major component polypeptides have molecular weights of 36k,38k,42k (actin), 69k,70k,130k and 150k. Vectorial labeling by lactoperoxidase-catalyzed iodination has provided evidence that the matrix is localized at the inner face of the plasma membrane. It consists largely of closed structures having the same size distribution as the membrane vesicles they are prepared from. No lipid bilayer is apparent in thin-section electron micrographs. A cell surface glycoprotein, 5'-nucleotidase, remains selectively associated with the detergent insoluble matrix. An insoluble fraction having major protein components of the same mobility on SDS gels has been isolated from purified plasma membranes of a number of murine tumor cell lines and normal spleen lymphocytes.

Purified cell surface H-2 antigens can be incorporated into liposomes along with the matrix. These liposomes stimulate generation of an H-2 dependent, allogeneic cytolytic T lymphocyte response much more effectively than liposomes containing just H-2 antigen and lipid. The more effective cell recognition of H-2 on matrix-containing liposomes may result from the larger size and more irregular shape of the vesicles. Alternatively, there may be an interaction between the transmembrane H-2 antigen and components of the matrix which affects recognition.

701 ACETYLCHOLINE RECEPTOR: A MEMBRANE-BOUND REGULATED IONOPHORE. J.M. Gonzalez-Ros, A. Paraschos, C. Farach, J. Mattingly and M. Martinez-Carrion. Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298

Purified acetylcholine receptor (AcChR) is isolated from Torpedo californica electroplax through the use of the easily removable detergent, β-D-octylglucopyranoside (OG). The entire purified AcChR preparation can be reconstituted into functional AcChR-membrane vesicles at the proper lipid/protein ratio. Physicochemical properties of OG purified AcChR are investigated in an attempt to clarify the reasons for the differences between OG and other detergents with regard to feasibility of reconstitution of AcChR membranes. AcChR-enriched and AcChR-depleted electroplax membrane fractions are used to provide information on the nature of those lipids which topographically or, perhaps, functionally relate to the receptor protein in situ. Both quantitative and qualitative differences in the chemical composition of membrane lipid components are found. In these receptor-rich membranes, several fluorescent pyrene derivatives are used as spectroscopic tools to assess: 1) the effects of either agonists, antagonists or neurotoxins on receptor-lipid interactions, and 2) the cation translocation events induced by agonist binding to the receptor protein through the perturbation of the quenching effect of externally added thallous cations. The potential of these techniques to probe molecular events of ion translocation on the millisecond time scale is discussed. (Supported by grants from the National Science Foundation and the Muscular Dystrophy Association.)

702 IDENTIFICATION OF SPECTRIN IN NONERYTHROID CELLS, Steven R. Goodman and Robert R. Kulikowski, The Milton S. Hershey Medical Center, Hershey, PA 17033

Spectrin is the major protein of the human erythrocyte cytoskeleton, which functions in controlling cell shape (review, Lux, 1978) and mobility of cell surface proteins (review, Goodman and Branton, 1978). Indirect immunofluorescence with monospecific anti-spectrin IgG has indicated the presence of proteins with common antigenic determinents to spectrin in embryonic chick cardiac myocytes (ECCM), mouse fibroblast lines (3T3, SV-40 transformed 3T3) and rat hepatoma lines (HTC, HMOA). Size and sequence analogy for ECCM and human erythrocyte spectrin has been demonstrated by the immunoprecipitation of 240,000 and 220,000 dalton peptides from octyl glucoside solubilized ECCM which (1) comigrate on $NaDodSO_4$ gel electrophoresis and (2) yield similar one dimensional tryptic peptide maps with human erythrocyte spectrin bands 1 and 2. Cytoskeletal protein interactions remain intact in the presence of octyl glucoside as demonstrated by immunoprecipitation of the established cytoskeletal proteins [syndeins (Bands 2.1-2.6), 3, 4.1, actin (5), 7] along with spectrin from the human erythrocyte. The spectrin associated cytoskeleton from ECCM includes actin, myosin, α-actinin, 10 nm filament protein, tropomyosin and other peptides of 150,000, 26,000, 19,500 and 15,000 daltons. We propose that by analogy to the erythrocyte, spectrin in nonerythroid cells may function in binding actin, and hence the cytoskeletal microfilament cables to the protoplasmic surface of the cell membrane. This is the first demonstration of the existence of spectrin in nonerythroid cells. (NIH Grant #HL26059 awarded to S.R.G.)

703 CHANGES IN CYTOSKELETAL PROTEINS UPON ENDODERM DIFFERENTIATION FROM TERATOCARCINOMA STEM CELLS, James L. Grainger and Gail R. Martin, Dept. of Anatomy, UCSF, San Francisco California 94143

Certain clonal cell lines of teratocracinoma stem cells (for example PSA1 cells) retain the capacity to mimic the differentation of the fetus-forming inner cell mass of the early mouse embryo and subsequently, to differentiate into many cell types in culture. When aggregates of PSA1 cells are placed in suspension, the first detectable morphological change is the appearance of endoderm on the outer surface of the cell clumps. A comparison by two-dimensional gel electrophoresis, of the cytoskeleton associated proteins from pure populations of PSA1 stem cells and of PSA1-derived endoderm has shown that this morphological change is accompanied by the synthesis of a new set of cytoskeletal proteins with molecular weights, 56,000, 45,000, and 38,000 daltons. At least one of these cytoskeletal markers, the 56K endodermal protein, appears to be associated with intermediate filaments and not with microfilaments or microtubules. Although this protein does not comigrate on two-dimensional gels with any of the known intermmediate filament proteins (vimentin, desmin or keratins isolated from mouse skin), a partial peptide analysis has shown that there are significant homologies between the peptide maps of the 56K protein and vimentin. The 45K protein appears to be unique to endoderm cells as there was no homology found between the peptide map of this protein and any of the cytoskeletal proteins examined.

704 SPECIFIC LIPOSOME-CELL INTERACTIONS. Lee D. Leserman, Jacques Barbet, Patrick Machy, Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille Cedex 2, France, & John N. Weinstein, National Cancer Institute, NIH, Bethesda, MD 20205 USA.

Small sonicated liposomes composed of phospholipid and cholesterol and containing the water-soluble fluorophore carboxyfluorescein do not bind to cells in vitro in the absence of specific ligands. Three kinds of ligands have been introduced into liposomes to facilitate specific interaction. 1) Phosphatidylethanolamine with DNP modification of the available NH_2 group became bound to cells of the murine myeloma MOPC 315 which express surface immunoglobulin with affinity for DNP; but not to myeloma cells of different SIg specificity (J. Immunol. 122, 585, 1979), 2) When these DNP-bearing liposomes were incubated with IgG Fc-receptor bearing tumor cells in the presence of IgG antibody to DNP they bound to and were endocytosed by the cells. $F(ab')_2$ anti-DNP did not mediate binding or uptake. When liposomes contained methotrexate the drug effect was greater than the same amount of unencapsulated methotrexate (Proc. Natl. Acad. Sci. USA. 77, 4089, 1980), 3) We have recently developed a method to covalently couple monoclonal antibodies and other hydrophilic proteins to preformed liposomes (Nature, in press). This technique is being extended to studies of hormone receptor-mediated endocytosis and specific deletion of receptor-bearing cells by coupling of insulin, EGF, and NGF to liposomes containing fluorescent markers or toxins.

705 ASSOCIATION OF ACTIN WITH RECEPTOR BOUND IgG, James L. Lessard and Ann Akeson, Children's Hospital Research Foundation, Cincinnati, OH 45229

Koch and Smith (Nature 273: 274-281, 1978) have shown an increased association between actin and surface IgG in patched myeloma cells using a myosin affinity procedure to bind actin and its associated proteins. We have used a similar approach to follow binding of ^{125}I labeled mouse IgG to P3X63 Ag8 cells. About 12±6% of the bound IgG was isolated in the myosin pellet in non-patched cells while 32±19% of the bound IgG was associated with myosin in cells patched with rabbit antibody versus mouse IgG (n=20). When the myosin was pretreated with actin, only 7±7% of the IgG was bound to patched cells. Thus, there is about a three fold increase in immunoglobulin binding to the myosin pellet during patching. Moreover, this interaction appears to take place via actin and both receptor bound IgG and surface IgG behave similarly. ^{3}H-leucine labeled myeloma cells were also carried through the myosin affinity procedure before and after patching. Fluorographs from SDS patterns of myosin pellets showed that a large number of proteins bind to myosin and can be competed by presaturating the myosins with actin. Two proteins with apparent molecular weights of about 50,000 and 45,000 daltons showed a small increase (20-25%) in patched compared to non-patched cells. These components may represent endogenously synthesized IgG heavy chain and actin. (Supported by NIH grant HD12285 and a Basic Research Grant from the National March of Dimes for Birth Defects Foundation).

706 FILAMENTOUS ACTIN STRUCTURES IN DEVELOPING DICTYOSTELIUM DISCOIDEUM Federico Goodsaid-Zalduondo and William A. Frazier, Washington Univ. School of Medicine, St. Louis, MO 63110

We have examined the filamentous actin structures in the unicellular, chemotactic, tight aggregate, slug and fruiting body stages of D. discoideum development by labeling with NBD-phallacidin (1). This fluorescent-labeled (λ_{ex}=488 nm) circular heptapeptide has a high affinity for filamentous (F) actin. Live cells incorporate enough NBD-phallacidin from an agar support containing 100 ng /ml label to yield actin patterns easily observed with fluorescence microscopy. Both NBD-phallacidin and the original nonfluorescent phalloidin have no detectable morphological effects on development when present in the agar substrata below concentrations of 1 ug/ml. Optimal resolution of morphological detail in the actin patterns was obtained by "staining" fixed cells. As little as 5 ng/ml NBD-phallacidin is sufficient for visualization of actin in the podia of motile cells and labeling is abolished by a 50-fold excess of unlabeled phalloidin. In chemotaxing cells F actin is highly concentrated in podial extensions, while a diffuse distribution is seen in early aggregates. In tight aggregates, F actin appears concentrated under the plasma membrane, particularly at points of cell-cell contact. At lower resolution we observe a uniform distribution of label in mature slugs which becomes polarized during culmination resulting in discrete, ring-like concentrations of F actin in the basal and apical regions of the spore cap, while the stalk is unlabeled. (Supported by NS-13269 and PCM 78-04304, WAF is an Established Investigator of the American Heart Association.)

(1) Barak, L., et al (1980), Proc. Nat. Acad. Sci. USA, 77, 980-984.

707 PRECISION ESTIMATION FOR LATERAL SURFACE MOBILITY MEASUREMENTS. Stephen Felder and Nils O. Petersen, Washington University School of Medicine, St. Louis, MO 63110

The fluorescence photobleaching recovery (FPR) experiment involves the determination of three parameters - the diffusion constant, the percent of bleaching, and the fraction of fluorophore that is mobile. According to statistical theory for the propagation of errors, the precision (more precisely the expected variance) of a parameter can be directly related to the partial second derivative of the sum of the squares of the residuals with respect to that parameter. A computer method has been developed to calculate the precision in this way. Comparison is made between estimated precision and the precision found for the parameters by the analysis of multiple, computer-generated data curves with added, random gaussian noise.

The resulting method for precision estimation has the following advantages: (1) The method is generally applicable to any type of data analyzed by minimization of residuals. (2) The accuracy of the precision estimate is directly tested by simulation - which need be done only once for a given experimental design. (3) The precision estimation for a parameter can be calculated from one typical data curve by simple computer subroutines. (4) Comparison of the estimated precision for the parameter, with the actual precision found for a given experimental condition can be used to determine whether variability is the result of an inherent imprecision in the method due to random noise (predicted variance is the same as actual variance), or is the result of non-random variation within the experimental system. (5) An analysis of this type can be used in choosing experimental design so as to maximize sensitivity to a particular parameter of interest. Examples of these advantages will be presented. [Supported by NIH Training Grant GM 07067-06]

708 EFFECT ON DIFFUSION MEASUREMENTS OF OUT-OF-FOCUS FLUORESCENTLY LABELLED CELL MEMBRANES, Nils O. Petersen+, William B. McConnaughey* and Elliot L. Elson*, Univ. of Western Ontario+, London, Ontario, and Washington Univ. Sch. of Med., St. Louis, MO 63110.

Fluorescence Photobleaching Recovery (FPR) experiments provide measurements of dynamic processes (diffusion, flow) on surfaces of cells or in small volumes. Measurements of diffusion of labelled plasma membrane components of tissue culture cells, generally, are made by focusing on one of the cell surfaces. Nevertheless, there may be a significant contribution to the detected fluorescence from the "off-focus" membrane. The bleaching area, and hence the amount of bleaching and the rate of recovery, as well as the detection efficiency depends on the separation of this membrane from the focus, and on the shapes of the illumination and detection volumes. We have employed a new sample translation device (N.O. Petersen, W.B. McConnaughey and E.L. Elson, Biophysical J. 1981 submitted) to measure accurately both the illumination volume and the detection volume for several focusing conditions. With this information we have estimated the effects on diffusion measurements of the out-of-focus membrane as a function of cell thickness (membrane separation). The sample translator also permits rapid characterization of beam sizes directly on the biological samples used in a given experiment. The precision and advantages offered by this approach will be presented. [Supported by grants GM21661 and GM27160 from the NIH.]

709 PHOTOBLEACHING RECOVERY STUDIES OF ANTIGEN-SPECIFIC MOUSE LYMPHOCYTE STIMULATION BY DNP-CONJUGATED POLYMERIZED FLAGELLIN. B.G. Barisas and J.S. Peacock, St. Louis University School of Medicine, St. Louis, MO 63104.

We have used fluorescence photobleaching recovery to study diffusion of antigen-receptor complexes during stimulation of DNP-specific mouse lymphocytes with the T-independent antigens DNP-polymerized flagellin (DNP-pol). Depending on epitope density and dose, these materials can behave either as immunogens or tolerogens. Lymphocyte DNP receptors binding $DNP_{0.5}$ flagellin monomer show $D = 2.2 \times 10^{-10}$ cm^2 sec^{-1} and ca 60% fluorescence recovery after bleaching. For DNP-pol binding to DNP-specific lymphocytes, the observed diffusion constants decrease monotonically with increased antigen dose and epitope density. Under optimally immunogenic conditions of DNP_{2-3}-pol at 1µg/ml, $D = 1.5 \times 10^{-11}$ cm^2 sec^{-1} implying that ~14 receptors have been crosslinked. Under tolerogenic conditions lower diffusion constants approaching 0.8×10^{-11} cm^2 sec^{-1} are observed implying somewhat larger receptor aggregates. The fraction of aggregates mobile on the time scale of the experiment remains constant at 50-60% in all immunogenic situations but falls abruptly to 25-33% in precisely those situations where the antigen/dose combination is tolerogenic. This might support hypotheses that there exist critical epitope densities above which antigens and receptors form rigidly crosslinked aggregates which are tolerogenic. Receptor aggregate diffusion is unaffected by colchicine or cytochalasin B and by the presence or absence of T cells in culture. This research supported in part by NSF grant PCM-78-13708 and by NIH RCDA AI 00291.

710 LATERAL DIFFUSION OF A LIPID ANALOG IN THE SURFACE MEMBRANE OF CELLS AND IN MULTIBILAYERS FORMED FROM PLASMA MEMBRANE LIPIDS. K. Jacobson[1], Y. Hou[2], Z. Derzko[1], J. Wojcieszyn[1] and D. Organisciak[3], [1]Lab. for Cell Biology, Department of Anatomy, Univ. of North Carolina, Chapel Hill, NC 27514; [2]Roswell Park Memorial Institute, Buffalo, NY 14263 and [3]School of Medicine, Wright State Univ., Dayton, OH 45401.

The diffusion, as measured by fluorescence recovery after photobleaching, of a fluorescent lipid analog, dihexadecylindocarbocyanine [diI-C_{16}(3)], was compared in several systems: the cell surface membrane of living human fibroblasts, the bottom surface membrane remaining after the top surface, nucleus and cytoplasm were removed, multibilayers reconstituted from the total cell lipids, and multibilayers reconstituted from plasma membrane (PM) lipids. Diffusion coefficients (D) for diI-C_{16}(3) inserted into the living cell's surface membrane or bottom surface ghosts were similar--ranging from about 3.5×10^{-9}cm^2/sec at 5° to about 2×10^{-8}cm^2/sec at 37° with a change in slope at about 25°. For diI-C_{16}(3) incorporated into extracted PM lipid multibilayers, D ranged from about 5×10^{-9}cm^2/sec at 5° to over 6×10^{-8}cm^2/sec at 37° with a discontinuity at 10° and slope change at 25°. Above 25°, the temperature dependence of D was similar in all systems with activation energies (E_a) in the range of 5-7 kcal/mole. Below 25°, E_a ranged from 9-11 kcal/mole for the intact cells and bottom surface ghosts to 17 kcal/mole for the PM lipid multibilayer. Although at 5° diffusion in the PM was nearly matched by that in the PM lipid multibilayer, at 37°, diI-C_{16}(3) diffusion was four times faster in the multibilayer composed of PM lipids than in the PM itself. The role of PM proteins in producing this difference will be discussed.

711 LATERAL MOBILITY OF BAND 3 IN THE HUMAN ERYTHROCYTE GHOST MEMBRANE STUDIED BY FLUORESCENCE PHOTOBLEACHING RECOVERY: EVIDENCE FOR CONTROL BY CYTOSKELETAL INTERACTIONS, William R. Veatch and David E. Golan, Harvard Medical School, Boston, Massachusetts 02115. Band 3, the major intrinsic protein of the human erythrocyte membrane, was specifically and covalently labeled with eosin-5-isothiocyanate, and its lateral mobility was examined using the fluorescence photobleaching recovery technique. We reasoned that the same parameters important for the complete dissociation of the erythrocyte cytoskeleton from the membrane--low ionic strength, high temperature, and time--might progressively and reversibly lift the mobility restrictions on band 3. Low temperature (21°C) and high ionic strength (46 mM $NaPO_4$) favored immobilization of band 3 (10% mobile) and slow diffusion of the mobile fraction (diffusion coefficient, $D = 4 \times 10^{-11} cm^2 sec^{-1}$). Upon incubation of ghost membranes at moderate ionic strength (13 mM $NaPO_4$) at 37°C, the diffusion coefficient of the mobile fraction increased 50-fold ($D = 200 \times 10^{-11} cm^2 sec^{-1}$) and the mobile fraction increased to 90%. This rapid lateral diffusion is almost as fast as that of rhodopsin in the photoreceptor rod outer segment disk membrane ($D = 400 \times 10^{-11} cm^2 sec^{-1}$ at 23°C) and approaches the expected diffusion rate of band 3 limited only by the viscosity of the lipid bilayer. Increases in the fraction of mobile band 3 were markedly dissociated from increases in the diffusion rate of the mobile fraction, under both steady-state and time-dependent conditions. Further, changes in diffusion rate with temperature were totally reversible while increases in the mobile fraction were only partially reversible. This very fast lateral diffusion of band 3 occurred under conditions where spectrin is still attached to the membrane, probably in a reversibly loosened state which precedes complete spectrin dissociation and concomitant ghost vesiculation.

712 DIFFUSIBILITY MODULATION MECHANISMS-INFERENCES FROM RECENT EXPERIMENTS, Watt W. Webb, Larry S. Barak, David W. Tank and En-Shinn Wu, Cornell University, Ithaca, N.Y. 14853. *Measurement of protein diffusion in membranes of vertebrate cells in tissue culture* have generally yielded diffusion coefficients (D) less than $10^{-10} cm^2/sec$ while for lipid analogs $D \sim 10^{-8} cm^2/sec$. In model lipid bilayer membranes small reconstituted integral proteins are now found to diffuse nearly as fast as lipids, $D \sim 10^{-8} cm^2/sec$, as expected on the basis of the fluid mechanics of viscous fluid membranes. Inhibition of protein diffusion on cell membranes has been attributed to various intermolecular interactions including those with cytoskeleton and exoskeleton and amongst membrane molecules. Recently we have found that diffusion of membrane receptors on living cell membranes is enhanced by orders of magnitude by detaching the actin cytoskeleton through swelling or blebbing the cell. Receptor diffusion coefficients $D \gtrsim 10^{-9} cm^2/sec$, comparable with rhodopsin on visual disc membranes have been observed in several systems. Experiments include the acetylcholine receptor on muscle, LDL receptor on fibroblasts and Con A receptor on lymphocytes.

Surface Receptors and Hormone Action

713 MODULATION OF FIBROBLASTOID MURINE BONE MARROW CELL LINES TO ADIPOSE CELLS BY SERUM AND INSULIN, R. W. Anderson, S. L. Mann, D. A. Crouse and J. G. Sharp, University of Nebraska Medical Center, Omaha, Nebraska 68105.

Adipose cells are an integral stromal component of bone marrow *in vivo*. It has also been suggested that fat cells are essential for the maintenance of hematopoietic stem cells *in vitro*. Four stromal cell populations (designated MC_1 through MC_4) obtained from the adherent cells of murine bone marrow cultures have been enriched for and purified by multiple trypsinizations. Although some specific characteristics vary, morphologically these cell lines are all fibroblastoid when grown as monolayers or organ cultures with RPMI 1640 medium and 10% fetal calf serum. When such organ cultures are transplanted under the kidney capsules of syngeneic mice, one line, MC_1 establishes a graft composed primarily of fat cells, whereas the others form aggregates of fibroblastic cells. When these cell lines are transferred from 10% fetal calf serum to 10% horse serum, MC_4 cells accumulate large quantities of lipid. This lipid is lost when the line growing in horse serum is transferred to fetal calf serum. We have investigated the role of insulin in this transformation. These cell lines were cultured in medium containing fetal calf serum supplemented with insulin (10 µg/ml). All of the cell lines accumulated lipid at this insulin concentration although it was most evident in cell line MC_4. Thus it appears that insulin at high concentrations stimulates murine bone marrow cell lines to accumulate lipid. One cell line appears much more responsive to this stimulus than the others tested. To date, none of these cell lines have demonstrated any evidence of hematopoiesis when grafted *in vivo*, and their abilities to support hematopoiesis *in vitro* are still under investigation. (Supported by NIH grant AM 26636).

714 PHOSPHORYLATION OF THE RECEPTOR FOR IMMUNOGLOBULIN E ON BASOPHILS, Clare Fewtrell, Andrew Goetze and Henry Metzger, NIAMDD, NIH, Bethesda, Md. 20205.

Mast cells and basophils have high affinity receptors for immunoglobulin E (IgE) on their surface membranes. Aggregation of these receptors to form dimers or larger receptor clusters results in Ca^{2+}-dependent exocytosis. We have studied the state of phosphorylation of the receptor for IgE in order to learn more about its biochemistry and the molecular mechanism by which it triggers secretion. These studies employed a rat basophilic leukaemia (RBL) cell line in which the receptor for IgE has been well characterized. The receptor is composed of two sub-units, a 55K α chain which binds IgE and a β chain with a M_r in the region of 35K. The two chains interact non-covalently in a 1:1 stoichiometry and this association can be stabilized using covalent cross-linking reagents. Short-term (~ 2hr) exposure of RBL cells to $^{32}P_i$ results in the labelling of a protein which is indistinguishable from the β chain of the receptor for IgE. Thus, specific immune precipitation of IgE:receptor complexes using anti-IgE antibodies yields a single phosphorylated protein M_r ~ 35K. This species disappears after exposure to the cleavable cross-linking reagent dimethyl dithiobispropionimidate. Instead a phosphorylated species with a M_r of about 90K is seen which is consistent with the 55K + 35K (α+ β) complex of the receptor for IgE. Subsequent reduction leads to the recovery of the 35K phosphoprotein which we conclude is the β chain of the receptor for IgE. The αchain of the receptor does not appear to be phosphorylated. Incorporation into the β chain was observed even when the cells were exposed to $^{32}P_i$ after the receptors had bound IgE. This suggests that phosphorylation occurs after the receptor has been inserted into the plasma membrane. We have so far been unable to observe any changes in the state of phosphorylation of either chain of the receptor during IgE-mediated triggering of RBL cells.

715 AN ACTIN AND MYOSIN BASED MEMBRANE-CYTOSKELETON FROM DICTYOSTELIUM DISCOIDEUM. Elizabeth J. Luna, Velia M. Fowler, Joel Swanson, Daniel Branton and D. Lansing Taylor, Harvard University, Cambridge, MA. 02138

SDS-gel electrophoresis and comparative two-dimensional peptide mapping identify actin and myosin as components of isolated Dictyostelium discoideum plasma membranes. These membranes bind exogenous rabbit muscle F-actin if, and only if, the endogenous actin and myosin are previously removed. The binding between actin and D. discoideum plasma membranes is examined both by qualitative sedimentation studies and with a new application of falling ball viscometry in which the membranes are treated as multivalent actin-binding particles analogous to divalent actin-gelation factors. Actin-membrane interactions are monitored before and after extraction of peripheral membrane proteins, after heat- or pronase-treatment of the membranes, and in the presence and absence of Triton X-100. Our results suggest that integral membrane proteins are responsible for at least some of the actin binding to membranes. In the absence of ATP or MgATP, Triton X-100 extraction of isolated membranes results in a detergent-insoluble residue composed of actin, myosin, and apparently-associated membrane proteins. The inclusion of ATP or MgATP before and during Triton extraction greatly diminishes the amount of protein in the Triton-insoluble residue without appreciably altering its composition. Our results suggest the existence of a protein complex stabilized by actin and/or myosin (membrane-cytoskeleton) associated with the D. discoideum plasma membrane.

716 REGULATION OF INSULIN RECEPTORS IN FROG ERYTHROCYTES BY INSULIN AND CONCANAVALIN A Ronald D. Vale, Andre De Lean, Robert J. Lefkowitz and Jeffrey M. Stadel, Duke University, Durham, NC 27705

Preincubation of frog erythrocytes with insulin for 4 hr at 30°C induces a 50% decrease in the ability of these cells to bind ^{125}I-insulin. Under identical conditions, turkey erythrocytes exhibited no insulin-mediated insulin receptor down regulation. Down regulation of the insulin receptor in frog erythrocytes was dependent upon the concentration of insulin and the time of exposure to the hormone but was largely independent of temperatures ranging from 4-30° C. Detailed competition binding data from control and insulin down regulated frog erythrocytes were analyzed by computerized non-linear least squares curve fitting methods. Control competition curves could be best fitted with a model for two insulin binding sites: Site I being of high affinity, low capacity (K_d = 575 pM; R_o = 425 sites per cell) and Site II being of low affinity, high capacity (K_d = 100 nM; R_o = 4300 sites per cell). The analysis revealed a 50% reduction in the number of high affinity sites after insulin promoted down regulation. No significant change in the K_d of Site I or the K_d and R_o of Site II could be detected. Exposure of frog erythrocytes to concanavalin A also produced a 50% decrease in the ^{125}I-insulin binding to these cells. Computer analysis revealed a two-fold decrease in the affinity of Site I in con A treated as compared with untreated cells. No significant effect of con A was observed on the R_o of Site I or the K_d and R_o of Site II. Thus, both insulin and con A are able to regulate the high affinity insulin binding sites without affecting the low affinity binding component. These experiments support the notion of independent classes of binding sites in the frog erythrocyte.

717 PURIFICATION AND CHARACTERIZATION OF THE INSULIN RECEPTOR, Gönül Veliçelebi, The Biological Laboratories, Harvard University, Cambridge, MA. 02138

We have approached the problem of purification of the insulin receptor by preparing an insulin affinity column in which insulin is linked to agarose through a chemically cleavable bond. Such a scheme enables us to elute the insulin-receptor complex off the affinity column by means of a specific chemical reaction instead of the use of high concentrations of denaturing agents such as urea, GuHCl, etc. To this end, we have prepared a sulfhyryl containing derivative of insulin by using a bifunctional reagent and reacted it with organomercurial agarose. Receptor binding properties of this derivative have been investigated, indicating a K_d of about 200 nM. Triton X-100 solubilized extract of rat liver membranes was reacted with the agarose-bound-modified-insulin to facilitate the binding of the solubilized receptor to the immobilized insulin, and the remaining proteins were extensively washed away. The insulin-receptor complex was subsequently eluted by treatment with a reducing agent, following which excess reducing agent was removed by gel filtration. The insulin receptor thus obtained is first detected through binding of native ^{125}I-insulin and is further characterized by standard biochemical methods such as SDS polyacrylamide gel electrophoresis and autoradiography of ^{125}I-iodinated receptor protein.

718 COVALENT LABELING OF THE NERVE GROWTH FACTOR RECEPTOR OF RABBIT SYMPATHETIC GANGLIA, Claudia J. Morgan, and Ralph A. Bradshaw, Wash. U. School of Med. St. Louis MO 63110 Joan Massague, Barbara J. Guillette and Michael P. Czech, Brown U. Providence RI 02912

Nerve growth factor (NGF) is an insulin-related protein which regulates the growth and viability of sympathetic and certain sensory neurons. The interaction of NGF with these target tissues involves initial complexation with specific plasma membrane receptors followed by receptor-mediated internalization. The cell surface NGF receptor of both sensory and sympathetic neurons has been characterized primarily in terms of kinetic and equilibrium measurements of the binding of ^{125}I-NGF to whole cell and plasma membrane preparations of a variety of responsive tissues. Physical studies (Costrini et al JBC 254: 11242, 1979) of the detergent extracted NGF receptor from rabbit sympathetic ganglia have provided a molecular weight estimated on the basis of hydrodynamic measurements of 135,000. The ^{125}I-NGF-receptor complex of crude microsomes and Triton X-100 solubilized microsomes has been covalently crosslinked using the heterobifunctional reagent N-hydroxysuccinimidyl-4-azidobenzoate. SDS-polyacrylamide gel electrophoresis of the crosslinked microsomes revealed two labeled bands of 115000-120000 and 150000, specifically competed by cold NGF. These bands were also present in detergent extracted microsomes but with a greater amount of nonspecific background. Efforts to covalently label the nuclear NGF receptor from rabbit sympathetic ganglia are currently in progress. Determination and comparison of the structures of cell surface and intracellular NGF receptors should provide a better basis for elucidating the mechanism of action of nerve growth factor in molecular terms. Supported by USPHS Research Grants NS10229 and AM17893.

719 BINDING, SEQUESTRATION, AND DEGRADATION OF NERVE GROWTH FACTOR (NGF) AND EPIDERMAL GROWTH FACTOR (EGF) BY PC-12 PHEOCHROMOCYTOMA CELLS, Charles E. Chandler*, Leonard H. Rome°, and Harvey R. Herschman°, *UMC, Stanford, CA 94305 and °UCLA, Los Angeles, CA 90024

The rat pheochromocytoma cell line, PC-12, specifically binds both NGF and EGF. The time when maximal binding is reached at 37°C is 40 minutes for NGF (100 ng/ml) and 20 minutes for EGF (60 ng/ml). In the continued presence of ^{125}I-labeled growth factor, PC-12 cells show a decrease in total cell-associated growth factor: NGF decreases by 20% and EGF decreases by 50% after 160 minutes. These decreases are blocked by the inclusion of 20 mM NH_4Cl. When growth factor binding is done at 0.5°C, most of the cell-associated growth factor can be removed by the addition of 100-fold excess of unlabeled growth factor. In contrast, when binding is done at 37°C, 65 - 70% of the cell associated NGF and only 10 - 20% of the cell associated EGF are removed by 0.5°C incubation with an excess of the respective unlabeled growth factor. The sequestration of NGF at 37°C is 50% complete by 4 minutes and is maximal by 12 minutes while EGF sequestration at 37°C is 50% complete by 1 - 1.5 minutes and is maximal by 10 minutes. Upon further incubation at 37°C, 50% of the sequestered NGF is degraded and 50% is released as intact NGF into the incubation medium, while virtually all of the sequestered EGF is degraded. The sequestration of both growth factors is temperature dependent: the half maximal rate being achieved at approximately 15°C. The intracellular localization of both growth factors is followed by using sub-cellular fractionation on silica/PVP density gradients. The differences in processing may be important in the differential actions of these two growth factors.

720 CONFORMATIONAL CHANGE OF ADENYLATE CYCLASE REGULATORY PROTEINS MEDIATED BY GUANINE NUCLEOTIDES. Thomas H. Hudson and Gary L. Johnson, Brown University, Providence, Rhode Island 02912 .

Cholera toxin, in the presence of (^{32}P)NAD^{+}, catalyzes the specific radiolabeling of a M_r=42,000 subunit of a protein (G-protein) which mediates guanine nucleotide regulation of hormone-sensitive adenylate cyclase in pigeon erythrocytes membranes. In the absence of added guanine nucleotide, treatment of labeled membranes with trypsin results in the partial digestion of the M_r= 42,000 radiolabeled peptide and production of several minor trypsin specific fragments which remain associated with the membrane. Incubation of membranes with guanosine-5'-O-(3-thiotriphosphate) (GTPγS) alters the partial tryptic digestion of the M_r=42,000 radiolabled peptide, resulting in a loss of the minor fragments and the quantitative generation of a M_r=41,000 peptide. GTPγS exposes a new site to tryptic cleavage which releases from the membrane an inactive fragment of the G-protein containing the M_r=41,000 labled peptide. Guanosine-5'-O-(2-thiodiphosphate (GDPβS) fails to activate adenylate cyclase and does not cause the generation of the M_r=41,000 tryptic fragment. Incubation of membranes with GDPβS does, however, decrease tryptic digestion of the 42,000 labeled peptide.

Generation of the GTPγS specific M_r=41,000 tryptic fragment from the M_r=42,000 toxin labeled peptide exhibits a time dependent increase upon incubation of membranes with GTPγS which is similar to that for cyclase activation. Addition of isoproterenol to the GPTγS incubation diminishes the lag time for cyclase activation and the generation of the M_r=41,000 fragment. Similar findings are observed in human erythrocyte membranes.

721 Endocytic Redistribution of the High Affinity Receptor for Nerve Growth Factor. Bruce A. Yankner and Eric M. Shooter, Dept. Neurobio., Stanford University School of Medicine, Stanford, CA 94305.

Incubation of cells of a continuous line of rat pheochromocytoma (PC12) with ^{125}I-βNGF resulted in the downregulation of 45-50% of the cell surface binding sites after 6 hr. A ligand-induced conversion of receptors to a higher affinity form preceded internalization. High affinity receptors depleted from the cell surface by internalization were replenished by the additional conversion of low affinity receptors. Association of internalized NGF with lysosomes and nuclei has been previously demonstrated (Yankner and Shooter 1979 PNAS 76, 1269). Cytochalasins B and D inhibited the lysosomal degradation of NGF without preventing surface binding or downregulation suggesting the involvement of microfilaments in the intracellular lysosomal pathway. Colchicine was not inhibitory. These cytoskeletal disrupting agents enhanced the translocation of NGF to the nucleus presumably by redirecting vesicles originally destined for lysosomes.

A spontaneously arising mutant of PC12 (NR2) which is not responsive to NGF has been cloned and found to be completely lacking in cell surface receptors for NGF. Binding of NGF to nuclei isolated from PC12 and NR2 revealed the same number of lower affinity nuclear receptors (Kd≈9X10^{-9}M) on both. However, high affinity receptors (Kd≈8X10^{-11}M) present in PC12 were absent from both the cell surface and nucleus of NR2. These results suggest that NGF and its high affinity receptor may be internalized as a complex which is subsequently deposited on the nuclear membrane. The appearance of NGF in the nucleus correlated with the commitment of the cell to subsequent neurite outgrowth.

722 MECHANISMS LEADING TO THE RETENTION OF PEPTIDE HORMONES BY TARGET CELLS, David B. Donner, Robert E. Corin, Michael L. McCaleb and Patrice Ferriola, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

The dissociation of ^{125}I-insulin from rat hepatocytes was biphasic and was resolved into rapidly and slowly dissociating components. As association time between insulin and hepatocytes increased the proportion of slowly dissociable radioactive label increased. This process was receptor mediated. Rat livers were perfused *in situ* with physiologic concentrations of insulin at 15°C. Insulin bound to membranes during perfusion dissociated at the slow rate *in vitro*. Together with parallel studies with isolated liver plasma membranes, these data suggested a role for the cellular periphery in the retention of insulin. Livers perfused with insulin were further subfractionated to yield a crude phagosome-lysosome fraction. At 15°C intact insulin was in this fraction whereas at 37°C mainly products of insulin degradation were found. This observation suggests the operation of a second mechanism leading to insulin retention by cells. Subsequent to binding, a fraction of receptor associated hormone may be internalized into the cytoplasmic compartment where it is degraded. Although the change in hormone binding at the plasma membrane and insulin sequestration within subcellular compartments may be related, one process did not necessarily require the other. Qualitatively similar results obtained with human growth hormone and glucagon suggest multiple pathways resulting in the retention of peptide hormones by target tissues. Supported by grants AM 19846, AM 22121, CA 08748 and T32 AM 07313 from NIH.

723 CYTOPLASMIC MICROTUBULES AFFECT THROMBIN BINDING AND INITIATION OF DNA SYNTHESIS.
K.L. Crossin and D.H. Carney, Univ. of Texas Medical Branch, Galveston, Texas 77550
Several reports have indicated that cytoplasmic microtubules (MTs) modulate the response of cells to mitogens or growth factors. To elaborate the mechanism and extent of this modulation, we have examined the effects of MT modifying drugs on thrombin binding to its receptors and its initiation of DNA synthesis in primary cultures of mouse embryo cells. A two hour treatment of these cells with colchicine (10^{-6} M) depolymizes MTs. This treatment has no effect on ^{125}I-thrombin binding at low concentrations, but inhibits binding of mitogenic concentrations of thrombin. A one hour treatment with Taxol (10 µg/ml) prior to colchicine addition prevents gross MT depolymerization and the inhibition of specific binding indicating that this inhibition is directly related to MT depolymerization. That this inhibition of receptor binding only occurs at mitogenic concentrations suggested that the concentration dependent thrombin-receptor event leading to cell division might involve receptor-MT interactions. We recently found that MT depolymerization itself is sufficient to initiate DNA synthesis and cell division (Crossin and Carney, Cell, in press), therefore, it was important to determine whether MT stabilization could inhibit initiation of DNA synthesis by thrombin. Taxol pretreatment inhibited up to 50% of the thrombin-stimulated DNA synthesis without affecting nucleotide transport or thrombin binding. Addition of taxol within eight hours of thrombin addition also decreased thrombin stimulation. These results suggest that MT integrity is important for normal interaction between thrombin and its receptor and that MT depolymerization may be necessary for thrombin to initiate DNA synthesis and cell division.
(Supported by AM-25807)

724 IDENTIFICATION OF A STEROID RECEPTOR ON THE SURFACE OF AMPHIBIAN OOCYTES,
Susan E. Sadler and James L. Maller, Department of Pharmacology, University of Colorado School of Medicine, Denver, Colorado 80262.
Ripe Xenopus oocytes in first meiotic prophase when incubated with progesterone in vitro progress synchronously in 3-5 hr without interphase to 2nd meiotic metaphase, where they remain until fertilization or activation. Previous studies in several laboratories have provided evidence that progesterone induces this cell division by interacting with the oocyte surface rather a conventional cytoplasmic steroid receptor. To determine directly whether surface steroid receptors exist in oocytes, manually dissected plasma membranes were exposed to ^{3}H-promegesterone (R5020) under photoaffinity labelling conditions. Labelled membranes were analyzed by SDS gel electrophoresis, followed by slicing and counting of the gel. A single peak of covalently labelled protein was found, corresponding to a molecular weight of 110,000. The binding component was of low capacity as indicated by competitive elimination of 85% of the labelling in the presence of a 100-fold excess of cold R5020. Time course studies of covalent labelling reach a plateau in 10 min and the concentration dependence of labelling is also saturable, providing further evidence that the binding site is of low capacity, as expected for a receptor. Kinetic studies have shown a direct correlation between the initial rate of covalent labelling of the protein and the R5020 concentration. Other studies have shown that the photoaffinity ligand can induce cell division in the oocyte, although the EC_{50} is approximately 10-fold higher than with progesterone. These results indicate that steroids induce cell division in the oocyte by interaction with a surface receptor. (Supported by NIH grant GM-27643 to JM).

725 THE EFFECT OF EGF AND INSULIN ON S6 PHOSPHORYLATION AND PROTEIN SYNTHESIS DURING THE TRANSITION OF QUIESCENT 3T3 CELLS INTO G_1.
George Thomas, Jorge Martin-Pérez and Michel Siegmann,
Friedrich Miescher-Institut, POB 273, CH-4002 Basel, Switzerland.
Previously, we have shown that within minutes of the addition of serum to quiescent cells there is a rapid multiple phosphorylation of ribosomal protein S6, as measured by its electrophoretic shift on 2-dimensional-PAG (PNAS 76, 3952, 1979). Results of subsequent studies with inhibitors of both protein synthesis and S6 phosphorylation suggest that serum stimulation of S6 phosphorylation is a necessary pre-requisite for the activation of protein synthesis and not a consequence of it (Cell 19, 1015, 1980). Present studies with EGF (epidermal growth factor) and insulin further support this view. The addition of either mitogen at saturating concentrations (nM range), to quiescent 3T3 cells in each case leads to a partial activation of protein synthesis and a limited increase in the extent of S6 phosphorylation. When both mitogens are added together, the increase in protein synthesis and S6 phosphorylation are found to closely approach those values obtained with 10% serum. Whether both mitogens are acting through a separate or common pathway is presently under investigation.

726 INTERACTIONS OF EGF AND ANTI-RECEPTOR ANTIBODIES WITH THE MEMBRANE RECEPTOR FOR EGF, Graham Carpenter and Christa Stoscheck, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

The membrane receptor for epidermal growth factor (EGF), purified by affinity chromatography, was used as an antigen to produce rabbit antisera containing antibodies to the receptor. High titer antisera was assayed by the ability to block ^{125}I-EGF binding to cultured cells. IgG was purified from the immune and preimmune sera and F(ab $)_2$ and Fab produced from the IgG. All factions from the immune sera retained a capacity to block ^{125}I-EGF binding and to inhibit the stimulation of DNA synthesis by EGF. Antireceptor IgG also immunoprecipitated the EGF receptor from detergent solubilized cell extracts. The EGF receptor is tightly associated with a protein kinase activity in the cell membrane and the activity of the kinase is increased by the binding of EGF to the receptor. This kinase activity copurifies with the EGF receptor and was present in the purified material used as the antigen. The immune sera, however, did not contain inactivating antibodies to the EGF-sensitive protein kinase, but did block the EGF activation of kinase activity. Further characteristics of the anti-receptor antibody will be reported.

727 FERROMAGNETIC-DEXTRAN PARTICLES: NEW REAGENTS FOR LABELING OF CELL SURFACE ANTIGENS AND RECEPTORS AND MAGNETIC CELL SEPARATIONS. R.S. Molday and D.J. Mackenzie, Department of Biochemistry, University of British Columbia, Vancouver, B.C. V6T 1W5 Canada.

Small, magnetic, iron oxide colloidal particles have been prepared and coated with dextran and other polysaccharides. These particles were (i) in the size range of 300-400 Å, (ii) stable against degradation and aggregation, (iii) ferromagnetic and electron dense and (iv) nonadherent and nontoxic to cells. Various proteins including Protein A, anti-immunoglobulin antibodies and lectins have been covalently coupled to these iron-dextran particles using the sodium periodate-sodium borohydride reaction. These reagents have been used to label antigenic sites and lectin receptors on the surface of normal and tumor cells for visualization by scanning and transmission electron microscopy. Ferromagnetic-dextran-protein A conjugates have also been used in the magnetic separation of specifically labeled cells. The application of these novel reagents in the separation and analysis of membranes and receptors is described.

728 A UNIQUE PROTEOLYTIC CLEAVAGE SITE ON THE β SUBUNIT OF THE INSULIN RECEPTOR, Michael P. Czech, Joan Massague and Paul F. Pilch, Brown University, Providence, RI 02912

The affinity-labeled insulin receptor of intact adipocytes is a M_r 350,000 disulfide-linked complex which is split into two M_r 210,000 receptor halves under mild reduction conditions. A second step of dissociation occurs whereby two fully reduced species that we denote as α (M_r 125,000) and β (M_r 90,000) insulin receptor subunits are generated from each M_r 210,000 fragment. Thus, the subunit stoichiometry of the M_r 350,000 insulin receptor form present in intact adipocytes is $(\alpha\beta)_2$, all the subunits being linked in the receptor complex by disulfide bonds. In contrast, affinity labeling of isolated plasma membrane preparations shows a heterogeneous population of insulin receptor structures of M_r 350,000, M_r 320,000, and M_r 290,000. The two lower M_r receptor species are shown to evolve from the M_r 350,000 species by a sequential single proteolytic cleavage of each of the β subunits to a M_r 49,000 fragment (β_1) that remains disulfide-linked to the receptor complex. While homogeneous plasma membrane fractions contain the three forms of insulin receptor in approximately equivalent proportions, insulin receptors in a plasma membrane fraction associated with lysosomes are found almost exclusively in the M_r 290,000 $(\alpha\beta_1)_2$ form. Conversion of the β subunit in the M_r 350,000 receptor to the β_1 subunit is achieved by incubation of plasma membrane receptors with a lysosome-rich membrane fraction or incubation of receptors on intact adipocytes with elastase. We conclude that 1) the native insulin receptor in intact cell surfaces is minimally composed of two α and two β subunits, all disulfide-linked and 2) susceptibility of the β subunit to specific proteolytic cleavage to the β_1 form, mediated by lysosomal protease, is characteristic of the insulin receptor in all tissues studied.

729 POLY(LYSINE) INTERACTION WITH THE CELL SURFACE: ITS ENDOCYTOTIC UPTAKE AND CARRIER FUNCTIONS. H. J.-P. Ryser, I. Drummond, W.-C. Shen and R. Mandel, Boston University School of Medicine, Boston, MA 02118.
Poly(lysine) (PLL) is taken up efficiently by cultured cells and can serve as carrier in the membrane transport of enzymes (Proc. Natl. Acad. Sci. US 75, 1872, 1978) and drugs (Proc. Natl. Acad. Sci. US 75, 3867, 1978). Conjugation of methotrexate to PLL increases drug uptake enough to overcome drug resistance in a transport-deficient CHO mutant. The use of horseradish peroxidase-PLL conjugate shows that PLL is taken up by endocytosis through both coated and uncoated vesicles. The strong binding of PLL to the cell surface, and its non-saturable transport kinetics indicate that endocytosis of PLL is adsorptive and not receptor-mediated. The decrease of PLL transport in Ca^{++}-free medium can be related to decreased surface binding. To test whether PLL causes a general stimulation of endocytosis, ^{3}H-inulin uptake was measured in the presence of various concentrations of PLL of different molecular weights. PLL does not at all influence the uptake of inulin, which indicates that its strong uptake at the cell surface cannot be ascribed to an increased surface vesiculation, but rather to its strong surface binding with the subsequent internalization of bound PLL by a vesiculation process occuring at a steady rate. When labeled PLL of different MW are compared, their uptake increases with their MW, even when tested at constant weight per ml. Increase in molecular size appears to increase membrane transport not only in terms of mass per cell, but in terms of number of molecules per cell. At equimolar concentrations of PLL of MW 20,000 and 280,000, the ratio of molar uptake per mg cell protein is 1 to 3. (Supported by NCI grant CA 14551 and the Community Technology Foundation)

730 ASSOCIATION OF THE P-GLYCOPROTEIN WITH MULTIPLE DRUG RESISTANCE: IMMUNOLOGICAL DETECTION AND DNA-MEDIATED TRANSFER. N. Kartner*, P.G. Debenham+, J.R. Riordan+, L. Siminovitch+ and V. Ling*. Ontario Cancer Inst.*, Hosp. for Sick Children+ and University of Toronto*+, Toronto, Canada.
A 170K dalton cell surface glycoprotein (P-glycoprotein) is associated with pleiotropic drug resistance in colchicine resistant Chinese hamster ovary ($CH^{R}C5$) cells (Bech-Hansen et al. 1976, J. Cell. Physiol., 88: 23; Riordan and Ling 1979, J. Biol. Chem., 254: 12701). Similar plasma membrane alterations are observed in a number of mammalian cell lines possessing the multiple drug resistance phenotype. Rabbit antibodies raised against $CH^{R}C5$ plasma membrane vesicles bind the $CH^{R}C5$ P-glycoprotein on SDS-PAGE Western blots. These antibodies also bind a 170K dalton cell surface glycoprotein expressed in different mammalian cell lines bearing the pleiotropic drug resistance phenotype. Hence, P-glycoprotein expression appears to be a phenomenon universal to pleiotropic drug resistance in mammalian cells. This drug resistance phenotype has been transferred to mouse LTK recipient cells, using genomic DNA from $CH^{R}C5$ cells. Under the same conditions no drug resistant transformants were detected using DNA from drug sensitive donors. Rabbit antibodies against $CH^{R}C5$ P-glycoprotein are able to detect a 170K dalton component in the drug resistant transformants, suggesting that the mouse recipient cells may be expressing the P-glycoprotein of hamster origin. Work is in progress to further corroborate these results. These findings are consistent with the functional involvement of the P-glycoprotein in pleiotropic drug resistance.
(Supported by the Medical Research Council and the National Cancer Institute of Canada)

Lectins

731 PURIFICATION OF AGGREGATION INHIBITORY PROTEIN FROM NON-ADHESIVE MOUSE TERATOMA CELLS, Steven B. Oppenheimer, Christopher B. Capelle, Steven E. Sorensen and James T. Meyer, California State University, Northridge, CA 91330
A protein was extracted from intact 129/J ascites mouse teratoma cells (subline OTT6050) which do not aggregate. This protein inhibited aggregation of cultured teratoma cells of the same subline that normally can aggregate in rotary culture. Extraction of the protein from ascites cells was accomplished without significant loss of viability (determined by electron microscopy and dye exclusion) by a technique involving the application of an electric field to 5 to 6 x 10^{8} cells suspended in a hypertonic electrode buffer. The isolated protein consistently and immediately (after 5, 10, 15 and 30 minutes of rotation) inhibited the aggregation of cultured teratoma cells by nearly 50% using 145 µg purified protein/ml and 1 x 10^{6} cells/vial in glucose-free Earl's balanced salts solution. The inhibitory effect directly varied with protein and cell concentration. Characterization of the protein on polyacrylamide gels suggests that it is pure and composed of 4 subunits, each $\leq 2 \times 10^{5}$ daltons. Preliminary results suggest that the protein is carbohydrate-free. Ascites teratoma cells (that possess the inhibitory protein) do not aggregate in culture while the cultured teratoma cells derived from the same subline as the ascites cells do aggregate. Continued work with this protein may help explain the reasons for the defective adhesiveness of the ascites cells and the reasons for the adhesiveness characteristic of the cultured teratoma cells.
(Supported by USPHS Grant CA 12920 to S. Oppenheimer from the National Cancer Institute)

732 ANTIBODY-INDUCED DYSHESION OF ADHERENT HUMAN MELANOMA CELLS IN MONOLAYER CULTURE
G.M. Stuhlmiller, K.H. Singer, K. Hashimoto, N.J.Sawka, G.S. Lazarus; Duke University Medical Center Durham, North Carolina 27710

Alteration of adhesive properties of malignant cells might greatly influence the metastatic potential of the tumor. We have demonstrated that antibody induces dyshesion of non-malignant (normal epidermal) cells by a proteolytic mechanism. Using similar techniques, we have studied the effects of antibody binding on the adhesive properties of melanoma cells in culture. IgG fractions were prepared from a multispecific monkey anti-melanoma antiserum (α-mel) and from normal monkey (NM) serum by affinity chromatography on Protein-A Sepharose. Monolayer cultures were exposed for 6-24 hr. @37° to 1-10 mg/ml IgG. Cells were then subjected to repeated washing with the overlying medium using a Pasteur pipette. Prelabeling of melanoma cells with 51-Cr permitted enumeration of cells which detached from the dish and allowed the monitoring of cytotoxicity. Dyshesion of cells following treatment with NM IgG varied in experiments from 15-30% and did not differ from dyshesion resulting from exposure to medium devoid of IgG. In contrast, 50-80% of cells were released from the dish following treatment with α-mel IgG. No cytotoxicity was demonstrated. Using a caseinolytic assay to detect proteolysis, we have demonstrated a substantial increase in proteolytic activity of medium collected from cells treated for 6 hr. with 1 mg/ml α-mel IgG compared to medium from cells treated with NM IgG or with no IgG. Studies are underway to characterize the enzyme(s) and to establish the role of this proteinase in IgG-induced cellular dyshesion.
Supported by USPHS grants AM21848 and CA20364

733 STUDIES ON ERYTHROID DEVELOPMENTAL AGGLUTININ SUGGEST THAT SMALL, β-GALACTOSIDE-SPECIFIC, ANIMAL, PROTEIN LECTINS ('GALAPTINS') HAVE A CELL-CELL BRIDGING FUNCTION.
C. James Chesterton and F. Lynne Harrison, Dept. Biochemistry, King's College, London, U.K.

During their development in the adult rabbit bone marrow, erythroblasts are clustered tightly together forming erythroblastic islands. When such tissue is extracted with lactose media, a lectin termed erythroid developmental agglutinin (EDA) is released. This has a Mol. Wt. of 13,000, a pI of 5.6, β-galactoside specificity and contains no carbohydrate (Harrison & Chesterton, Nature 286, 502-504, 1980). Sheep anti-EDA IgG used in conjunction with an indirect immunofluorescence technique has revealed that EDA is located only on the surface of erythroblasts and reticulocytes in fresh bone marrow. Cells at all stages of the erythroid lineage are labelled but the fluorescence diminishes when the reticulocyte is released from the island. The lectin is thus tissue specific, a finding confirmed by in vitro agglutination studies, and its presence is concomitant with the requirement for adhesion during development. EDA induces the agglutination of inert glutaraldehyde-fixed trypsinized erythrocytes. In addition, the lectin-mediated clustering of erythroblasts can be blocked by incubation of the EDA-laden cells with anti-EDA Fab fragments. Both these findings point to a cell-cell bridging mechanism for the lectin.

Small, β-galactoside-specific, animal, protein lectins have been isolated from a wide variety of higher animal tissues and species. Recently, we have suggested the name 'galaptin' for this class of molecule (Greek: Hapto, to touch or join). The species and tissue specificity of EDA and other rabbit galaptins will be discussed.

734 IMMUNOLOGICAL CHARACTERIZATION OF A GLYCOPROTEIN (CONTACT SITES A) INVOLVED IN INTERCELLULAR COHESION OF DICTYOSTELIUM DISCOIDEUM, Ben A. Murray and William F. Loomis, University of California San Diego, San Diego, CA 92093

We have prepared antisera in rabbits to the "contact sites A" glycoprotein (gp 80) purified from Dictyostelium discoideum by the method of Muller et al. (Eur. J. Biochem 99:419-426, 1979). IgG isolated from these antisera reacts with many different proteins in D. discoideum lysates, as analyzed by immune precipitation and by antibody staining of gel electropherograms transferred to nitrocellulose. Blocking experiments indicate that this cross-reactivity reflects the presence of common antigenic determinants on gp 80 and other cellular proteins, rather than the presence of extraneous antibodies in the antisera. The spectrum of reactive proteins is different at different stages of development. In particular, gp 80 itself is synthesized only for a restricted period during the cell aggregation phase. The protein persists throughout development and can be detected in spores. Anti-gp 80 IgG binds to the surface of developing D. discoideum cells and specifically blocks their developmentally-regulated cohesion. Most, but not all, of this cohesion-blocking activity can be removed by repeated absorption of the IgG with vegetative cells. The absorbed IgG stains only gp 80 and (to a lesser extent) one other band in lysates of aggregation-competent cells. Thus antibodies of several different specificities are capable of blocking cohesion, but they all may act through their ability to bind gp 80. However, the possibility that other molecular species reactive with these antisera may be functionally-significant targets of the cohesion-blocking activity cannot be ruled out.

735 BIFUNCTIONALITY OF DISCOIDIN I-MEMBRANE INTERACTION, James R. Bartles and William A. Frazier, Washington University School of Medicine, St. Louis, MO 63110

The endogenous slime mold lectin discoidin I is required for the acquisition of cellular cohesiveness during the differentiation of Dictyostelium discoideum. Indirect evidence suggests that discoidin I on the cell surface mechanically crosslinks cells via carbohydrate-containing receptors. ^{125}I-discoidin I binding studies have detected two types of receptors for discoidin I on the surface of fixed and living cells. Binding to one type (the C site) is inhibited by hapten sugars, and thus represents a carbohydrate recognition mechanism. The C site appears to be a developmentally regulated glycoprotein. Binding to the other type of discoidin I receptor (the I site) is not inhibited by hapten sugars, but is inhibited by high ionic strength or polyelectrolytes. This electrostatic interaction is detected only at physiological ionic strength. The I site receptor is extracted from fixed cells with $CHCl_3/CH_3OH$, suggesting it is a lipid. Discoidin I binds to and agglutinates negatively charged liposomes containing only purified phospholipids. Thus discoidin I has the ability to peripherally associate with and crosslink membranous structures by two distinct mechanisms. [Supported by NS-13269 and PCM 78-04304. WAF is an Established Investigator of the American Heart Association.]

736 ASIALOGLYCOPROTEIN RECEPTOR MEDIATES THE TOXICITY OF AN ASIALOFETUIN-DIPHTHERIA TOXIN FRAGMENT A CONJUGATE ON CULTURED RAT HEPATOCYTES, David L. Simpson, Daniel B. Cawley and Harvey R. Herschman, Dept. of Biological Chemistry and Lab of Nuc. Med. and Rad. Biology, University of California School of Medicine, Los Angeles, California 90024.

We have designed and constructed a toxic hybrid protein which is recognized by asialoglycoprotein receptors of cultured rat hepatocytes. The asialoglycoprotein receptor recognizes galactose-terminated glycoproteins. The conjugate consists of fragment A (DTA) of diphtheria toxin linked by a disulfide bond to asialofetuin (ASF). This conjugate is more toxic than any conjugate of DTA previously reported, inhibiting protein synthesis in primary rat hepatocytes at concentrations as low as 10^{-11} M. The ASF-DTA conjugate was 600 and 1800 times as toxic as diphtheria toxin and DTA, respectively, on primary hepatocytes. We tested a series of glycoproteins for their ability to block the action of the conjugate. Fetuin and orosomucoid, two glycoproteins with terminal sialic acid on their oligosaccharide chains, did not block the action of the conjugate. The N-acetyl-glucosaminyl-terminated derivative (asialo-agalacto-orosomucoid) had no appreciable effect on the activity of the conjugate. The galactose-terminated asialoderivatives, ASF and asialo-orosomucoid, as expected, did block the action of the conjugate. We tested the ASF-DTA conjugate on six cell types; except for primary rat hepatocytes, none were affected by a high concentration (10^{-8}M) of ASF-DTA conjugate. A fetuin-DTA conjugate was 300-fold less toxic than the ASF-DTA conjugate and apparently exerted its effects primarily through non-receptor mediated uptake systems. The ASF-DTA conjugate is the first DTA conjugate which is highly toxic, cell-type specific and whose action is mediated by a well-defined receptor associated with a specific biochemical activity and function.

737 THE DEMONSTRATION OF A CELL SURFACE UDP-GALACTOSYLTRANSFERASE ON MAMMALIAN CELLS BY INDIRECT IMMUNOFLUORESCENCE, Joel H. Shaper and Paul L. Mann, Johns Hopkins University School of Medicine, Baltimore, MD 21205

A cell surface UDP-galactose:N-acetylglucosamine galactosyltransferase (GT) has been directly demonstrated on bovine kidney epithelial and bovine turbinate fibroblast cells in tissue culture by immunohistochemical techniques. A conventional rabbit heteroantiserum was prepared against a soluble form of GT from bovine milk after purification to apparent homogeneity by sequential affinity chromatography on UDP-Sepharose 4B and α-lactalbumin-Sepharose 4B. Using an IgG fraction isolated from this rabbit antiserum, GT appears as a uniformly stippled pattern over the exposed membrane surface of intact cells as visualized by indirect immunofluorescence. Exposure of living cells to the anti-GT-antiserum in serum supplemented or serum-free culture medium induced a time and temperature dependent aggregation of the GT in the plane of the membrane. GT can be rapidly released from the membrane surface by trypsin digestion under conditions in which the cells remain firmly attached to their substrates. Reappearance of the cell surface GT requires protein synthesis; cycloheximide (5 μg/ml) effectively prevents repopulation of the cell surface. Lastly, cell surface GT is not the result of spurious adsorption of the soluble enzyme from the growth medium. Exogenously added affinity-purified GT to serum supplemented or serum-free culture medium is not adsorbed to the cell surface of trypsin-treated cells in detectable quantities as monitored by indirect immunofluorescence.

738 THE INTERACTION OF DISCOIDIN I WITH LIVING D. DISCOIDEUM CELLS ASSESSED BY FLUORESCENCE AND ELECTRON MICROSCOPY. Nancy J. Galvin and William A. Frazier, Washington University School of Medicine, St. Louis, MO 63110.

The interaction of the endogenous lectin discoidin I with live D. discoideum cells has been examined in this laboratory using ^{125}I-discoidin I. These binding studies performed under physiological conditions, indicate that the lectin binds reversibly to a carbohydrate-containing receptor (the C site) and negatively charged phospholipids (the I site). A fraction of the bound discoidin (20-40%) becomes irreversibly cell-associated. We have prepared a ferritin-discoidin conjugate which is also labeled with TRITC to yield a derivative which can be visualized by both fluorescence and electron microscopy. Binding of this TR-FE-discoidin I to live cells is inhibited by GalNAc (50 mM), a C site inhibitor and by I site inhibitors such as heparin and BSA, but not by GlcNAc. This pattern of inhibition is found with ^{125}I-discoidin I as well. Observed at 30 min (22°, steady state binding) TR-FE-discoidin I fluorescence is patchy. At 60 min fluorescence is punctate and appears associated with intracellular vesicular structures where it persists as long as 2 hr. This pattern of association is seen with both the pinocytic A-3 strain as well as the phagocytic wild type NC-4, and is seen with both vegetative and differentiated cells consistent with the presence of the I site at both stages. Extension of these low resolution observations by electron microscopy of TR-FE-discoidin I treated cells is now in progress. [Supported by NS-13269 and PCM-78 04304; WAF is an Established Investigator of the American Heart Association.]

739 RAT LUNG AND CHICKEN LIVER CONTAIN SIMILAR HEPARIN-BINDING LECTINS, Marie Roberson, Howard Ceri, Paula Shadle and Samuel H. Barondes, University of Calif., San Diego, La Jolla, CA 92093; and Veterans Administration Medical Center, San Diego, CA 92161.

A lectin with heparin-sensitive hemagglutination activity has recently been purified from adult chicken liver (Ceri et al., J. Biol. Chem., in press). Young rat lung also contains a similar lectin. Both lectins are solubilized by the addition of either 0.1M N-acetyl-D-galactosamine or 1M NaCl to the homogenization medium and are inhibited by heparin and N-acetyl-D-galactosamine. The rat and chicken lectins can be purified by gel filtration on Sepharose CL-2B followed by affinity chromatography on heparin-Sepharose. Both lectins behave as high molecular weight aggregates. Electrophoresis of both the rat and chicken lectins in SDS results in two peptides of apparent molecular weights of 13,000 and 16,000. Mouse antisera raised against the chicken lectin will effectively block the hemagglutination activity of both the chicken lectin and the rat lectin. Spectrophotometric readings of the purified rat lectin indicate a high degree (≥20%) of contamination with nucleic acid. Both the size of the aggregates and the hemagglutination activity of the rat lectin can be greatly reduced by treatment with micrococcal nuclease. However, heparin-binding activity is retained by the lectin preparations after nuclease digestion. These results suggest that the nucleic acid may function to increase hemagglutination activity by the formation of large multivalent protein-nucleic acid aggregates. Our results do not discriminate between a functional or a spurious association of the heparin-binding peptides with the nucleic acid. The function of these lectins remains to be determined. (Supported by the McKnight Foundation and the USPHS)

740 ELICITATION OF CELL SURFACE LECTIN IN CELLULAR SLIME MOLDS, Wayne R. Springer and Samuel H. Barondes, Veterans Administration Medical Center, San Diego, CA 92161; and University of Calif., San Diego, La Jolla, CA 92093.

The lectins of cellular slime molds are extremely abundant in differentiating cells. Of the approximately 5×10^6 molecules per aggregating cell, only about 2% is present on the cell surface. However, much more can appear on the surface in response to crosslinking of the lectin already there. We have shown that crosslinking purpurin, the lectin of Dictyostelium purpureum by addition of divalent immunoglobulin, leads to a striking increase of cell surface purpurin. Univalent antibody fragments had no effect (Springer, et al., J. Cell Biol., December 1980). Recent studies indicate that multivalent glycoproteins which react with the carbohydrate-binding site of purpurin also elicit more cell surface purpurin, although they are less potent than divalent antibody. When the majority of the purpurin is eluted from the cell surface with lactose, prior to addition of these glycoproteins, little or no elicitation occurs. Crosslinking of other surface molecules with lectins such as concanavalin A (con A) also elicits the appearance of cell surface purpurin which, in this case, occurs even if the purpurin initially present had been eluted from the surface with lactose. This elicitation requires crosslinking since succinyl-con A, which does not itself cause crosslinking, has no effect unless followed by reaction with antibody to con A which forms a crosslinked complex. Exogenous purpurin also elicits the appearance of endogenous purpurin on to the cell surface. These results raise the possibility that the large intracellular pool of purpurin may become available for cell surface reactions under appropriate conditions.
(Supported by the Veterans Administration Hospital, USPHS, and the McKnight Foundation)

741 SEVEN ISOLECTINS FROM DICTYOSTELIUM PURPUREUM, Douglas N. Cooper and Samuel H. Barondes, University of Calif., San Diego, La Jolla, CA 92093; and Veterans Administration Medical Center, San Diego, CA 92161

Cellular slime molds synthesize large quantities of lectins as they differentiate. Considerable evidence indicates that these lectins and complementary cell surface receptors are involved in cell-cell adhesion. All slime mold lectins studied to date have more than one subunit raising the possibility of multiple forms. We have examined the composition of purpurin, the lectin from Dictyostelium purpureum and demonstrate seven tetrameric isolectins by PAGE at pH 8.9. The isolectins are assembled from four distinct subunits resolved by SDS-PAGE and tryptic peptide mapping. Two of the subunits (III and IV) form only homotetramers. The other two subunits combine randomly to form mixed tetramers (I_4, I_3II_1, I_2II_2, I_1II_3, II_4) in binomial proportions. The isolectins can be functionally discriminated and separated on the basis of their relative affinity for columns derivatized with complementary saccharides. Neither III_4 nor IV_4 are bound by a GalNAc-conjugated column, while isolectins of subunits I and II bind and are sequentially eluted by a galactose step gradient. Isolectin IV_4 binds to a lactose-conjugated column, but III_4 does not. The hemagglutination profiles for III_4 and IV_4 are distinct from each other and from isolectins composed of I and II, which have very similar profiles. None of the subunits is glycosylated, and all form tetramers with molecular weights of approximately 88,000. Forms III_4 and IV_4 appear earlier in development than isolectins of subunits I and II. The existence of multiple functionally and developmentally distinct lectins suggests that lectin function in cellular slime molds may be more complex than presently envisioned. (Supported by USPHS and the McKnight Foundation)

742 ENDOGENOUS LECTIN IN CHICKEN INTESTINE: POSSIBLE ROLE IN INTESTINAL MUCIN SECRETION, Eric C. Beyer and Samuel H. Barondes, University of Calif., San Diego, La Jolla, CA 92093; and Veterans Administration Medical Center, San Diego, CA 92161

The major lectin in adult chicken intestine, chicken-lactose-lectin-II (CLL-II) has been purified to homogeneity and specific antibodies (unreactive with chicken-lactose-lectin-I) have been raised and employed for immunohistochemistry and radioimmunoassay. Immunofluorescence studies show localization of CLL-II in the mucous secretory granules of goblet cells. Purified chicken intestinal mucin, which is also concentrated in these granules can interact with CLL-II, since it is a potent inhibitor of its hemagglutination activity. Localization of the lectin and mucin at the same site, have raised the possibility that they may interact in the secretory granule and/or that they may interact after secretion onto the intestinal mucosal surface. CLL-II was indeed detected on the intestinal mucosal surface and could be eluted by washing intestinal segments with lactose. Secretion of CLL-II was demonstrated by incubation of intestinal segments with cholinergic compounds known to stimulate mucin secretion. These results suggest that CLL-II may have a function in secretion of mucin and/or its association with the intestinal mucosal surface.
(Supported by USPHS and the McKnight Foundation)

743 FORMATION AND ISOLATION OF CELL SURFACE ASIALOGLYCOPROTEIN RECEPTOR PATCHES FROM RAT HEPATOCYTES BOUND TO SYNTHETIC GALACTOSIDE SUBSTRATA. Paul H. Weigel, Division of Biochemistry, The University of Texas Medical Branch, Galveston, Texas 77550

Using a recently developed model system to study cell interactions with synthetic carbohydrate culture surfaces we have shown that asialoglycoprotein receptors can mediate the binding of rat hepatocytes to galactoside surfaces. Binding is sugar specific, requires Ca^{+2} and occurs as a threshold response only above a critical concentration of galactose (Weigel et. al., J. Biol. Chem. 254, 10,830; 1979). The distribution of cell surface receptors (monitored with fluorescein-asialo-orosomucoid; fl-ASOR) appears diffuse on non-bound cells whereas on bound cells, which are released from the substratum at 0° C with EGTA, there is a single large ($4\mu^2$) patch. When cells released at 0° C are then incubated at 37° C, the receptor patches disappear with first order kinetics ($t_{1/2}$=2.6 min). To estimate the number of receptors in a patch region we have used an antiserum (a gift from G. Ashwell), which blocks the binding of [^{3}H]asialo-orosomucoid by the receptor. Inhibition of only 20% of the cell surface receptors with antibody completely prevents cell binding to the substratum, indicating that an average of up to 150,000 receptors/cell may be necessary for cell binding. The receptor patches were isolated by gently shearing bound cells off the galactoside surface. The patch left on the substratum cannot be seen by phase contrast microscopy, but can be visualized by fluorescence microscopy using fl-ASOR or antiserum to the receptor. These membrane regions are not observed on control or galactoside surfaces on which cells have been incubated but to which they do not bind. Experiments are in progress to quantitate the number of receptors that can be solubilized from isolated patches. (Supported by NIH grant GM 26228.)

744 INTERACTIONS OF THE ISOLECTINS OF WGA WITH SIALYLOLIGOSACCHARIDES, K. Anne Kronis and Jeremy P. Carver, University of Toronto, Toronto, Ontario, Canada M5S 1A8.
Lectins such as wheat germ agglutinin (WGA) have proven useful in the study of protein-carbohydrate interactions, similar to those which have been implicated in differentiation, cancer and metastasis. The binding of WGA to sugars which inhibit its interactions with cell surface carbohydrates have been studied extensively. However, these inhibitory sugars may not accurately reflect the carbohydrate moieties at the cell membrane to which WGA binds. For instance, N-acetyl-D-neuraminic acid (NeuNAc) is not a potent inhibitor of WGA yet neuraminidase treatment of a wide variety of cell types greatly reduces WGA binding. Recently, crystallographic studies have demonstrated that WGA crystals bind NeuNAc and a sialyloligosaccharide, 1*. We have undertaken a solution binding study of the isolectins WGA I and II to a series of sialyloligosaccharides, 1,2 and 3*, isolated in our laboratory from bovine colostrum. Dissociation constants were measured by 360 MHz proton magnetic resonance spectroscopy. A comparison of the affinities of 1 and 2 reflects the effect of the NeuNAc linkage, (α2,3 versusα2,6). Ligand 3 differs from 2 in the presence of an N-acetyl group on the glucose residue and is identical to the distal portions of many complex Asn-linked glycopeptides. This, in addition to the importance of GlcNAc residues in WGA binding makes 3 an ideal cell surface analogue. The affinities we have measured are still orders of magnitude weaker than those found for WGA binding to cell surfaces. We postulate an additional interaction exists when WGA binds to oligosaccharides of the cell membrane.

*1, NeuNAcα2,3Galβ1,4Glc; 2, NeuNAcα2,6Galβ1,4Glc; 3, NeuNAcα2,6Galβ1,4GlcNAc.

745 COMPARISON OF CELLULAR LOCALIZATION AND CARBOHYDRATE BINDING PROPERTIES OF DOLICHOS BIFLORUS SEED LECTIN AND CRM FROM STEMS AND LEAVES. Marilynn E. Etzler and Carl Borrebaeck, University of California, Davis, CA 95616.
The stems and leaves of the Dolichos biflorus plant contain a lectin-like glycoprotein (CRM) that cross reacts with antibodies to the seed lectin. This CRM is a dimer composed of a subunit with identical electrophoretic mobility to subunit I of the seed lectin and a subunit heavier than the seed lectin subunits (Talbot & Etzler, 1978, Biochemistry 17, 1474). Both the CRM and the seed lectin subunits have similar NH_2-terminal amino acid sequences.
Affinity electrophoretic and affinity chromatographic studies show that the CRM and seed lectin have different carbohydrate binding properties. Fluorescence microscopy and cell fractionation studies indicate that a substantial portion of the CRM is localized in the cell walls of cells of the stems and leaves whereas the seed lectin is found in the protein bodies of the cells of the cotyledons. The implications of these findings with respect to the relationships of these two glycoproteins and lectin function will be considered. Supported by NIH Grant GM 21882 and USDA Grant SEA 5901-0-0242.

746 COMPARISON OF MITOGENIC PROPERTIES AND METAL ION CONTENT OF DOLICHOS BIFLORUS SEED LECTIN AND CRM FROM THE STEMS AND LEAVES. Carl Borrebaeck, Bo Lönnerdahl and Marilynn Etzler, University of California, Davis, CA 95616.
The stems and leaves of the Dolichos biflorus plant contain a glycoprotein (CRM) that cross reacts with antibodies against the lectin from the seeds of this plant. CRM and the seed lectin have many structural properties in common (Talbot & Etzler, 1978, Biochemistry 17, 1474) including amino acid and carbohydrate composition and NH_2-terminal amino acid sequence. CRM has also recently been found to bind to carbohydrate structures under certain conditions. In spite of the structural similarities we have found distinct differences in mitogenic properties and metal ion requirements between the two plant proteins. At 1-300 µg/ml the seed lectin is a non-mitogen whereas CRM has weak mitogenic properties towards neuraminidase treated human peripheral lymphocytes. The dose-response maximum is at 70 µg/ml.
The native seed lectin from Dolichos biflorus contains the divalent cations Ca, Mg, Mn, Zn and Cu but requires only Ca to retain full carbohydrate binding activity as tested by an affinity electrophoretic system. On the other hand CRM contains Ca, Mg, Zn and Mn, in quite different ratios as compared to the seed lectin, and seems to require Ca and Mg to retain carbohydrate binding activity.
We have also recently been able to show, using a monoclonal antibody, that CRM and the seed lectin have immunological differences. Supported by NIH Grant GM 21882 and USDA Grant SEA 5901-0-0242.

747 DISCOIDINS I AND II: HOMOLOGOUS AND UNIQUE STRUCTURAL RELATIONSHIPS BETWEEN TWO FAMILIES OF ENDOGENOUS LECTINS IN DICTYOSTELIUM DISCOIDEUM, Edward A. Berger, D. Randall Armant, and Lauren Jensen, Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, Ma. 01545.

As D. discoideum amoebae differentiate from the non-cohesive to the cohesive state, they synthesize two lectin-like proteins, Discoidins I and II, which have been implicated as primary components of the morphogenetic cell cohesion system. We have performed extensive immunochemical and biochemical analyses in order to assess the degree of structural relatedness between the two molecules, and to ultimately identify structural and functional domains. Antisera raised against highly purified preparations of either Discoidin I or II were tested for their reactivity against each protein by both immunoprecipitation and double diffusion analyses. The pattern of cross-reactivity suggested the presence of both shared and unique antigenic determinants. This interpretation was supported by thin-layer peptide maps of the radio-iodinated Discoidins I and II, which revealed a substantial number of common peptides. The finding of homologous as well as unique regions may have important functional implications for these cell recognition proteins.

Upon SDS gel analysis of affinity purified Discoidin preparations, we have consistently observed not only the prominent Discoidin I and II bands (subunit M_r's 26,000 and 24,000 respectively), but also three distinct minor species, all in the Discoidin region of the gel. Peptide maps of the two slowest migrating minor species are identical to that of Discoidin I, whereas the map of the fastest migrating minor species is indistinguishable from that of Discoidin II. In view of the recent discovery of multiple Discoidin I genes, it is intriguing to speculate that the major and minor species reflect the activity of multigene families for both Discoidins I and II.

748 THE CARBOHYDRATE SPECIFICITY AND PROPOSED FUNCTION OF THE TERATOCARCINOMA STEM CELL SURFACE LECTIN, L.B. Grabel, C.G. Glabe, M.S. Singer, G.R. Martin, and S.D. Rosen, Department of Anatomy, UCSF, San Francisco, CA 94143.

As previously reported, teratocarcinoma stem cells have a cell surface carbohydrate-binding component (rosette mediating factor, RMF) that recognizes and binds specific carbohydrate moieties on the surface of glutaraldehyde-fixed, trypsinized rabbit erythrocytes (GTRs). We have now shown that soluble cell extracts of stem cells contain a hemagglutinin of GTRs. The RMF and the hemagglutinin, which are both sensitive to trypsin, are inhibitable by the same specific glycoconjugates (mannans and fucans), indicating identity or close similarity of the two: fucoidan, a sulfated fucan from Fucus vesiculosus, is 2-3 orders of magnitude more effective than mnn 2 mannan as an inhibitor of rosette formation and hemagglutination. Desulfated fucoidan, hydrolyzed fucoidan, and a sulfated fucan from sea urchin egg jelly are also effective whereas chondroitin sulfate, heparin and glycogen are not.

The role of the RMF/hemagglutinin in cell adhesion is demonstrated by studies showing that mannans and fucans partially inhibit the reassociation of disaggregated stem cells. Again, fucoidan is a more potent inhibitor than mnn 2 mannan. Partial inhibition of reaggregation can also be obtained by removal of divalent cations with EDTA. In the presence of both fucoidan and EDTA, complete inhibition of reaggregation occurs. These results suggest a two component system of intercellular adhesion, one requiring divalent cations, and the other dependent upon a cell surface lectin.

749 ASIALOGLYCOPROTEIN RECEPTOR MEDIATES THE TOXICITY OF AN ASIALOFETUIN-DIPHTHERIA TOXIN FRAGMENT A CONJUGATE ON CULTURED RAT HEPATOCYTES. David L. Simpson, Daniel B. Cawley, and Harvey R. Herschman, Dept. of Biological Chemistry and Lab of Nuc. Med. and Rad. Biology, University of California School of Medicine, Los Angeles, California 90024.

We have constructed a toxic hybrid protein which is recognized by asialoglycoprotein receptors of cultured rat hepatocytes. The asialoglycoprotein receptor recognizes galactose-terminated glycoproteins. The conjugate consists of fragment A (DTA) of diphtheria toxin linked by a disulfide bond to asialofetuin (ASF). This conjugate is more toxic than any conjugate of DTA previously reported, inhibiting protein synthesis in primary rat hepatocytes at concentrations as low as 10^{-11}M. The ASF-DTA conjugate was 600 and 1800 times as toxic as diphtheria toxin and DTA, respectively, on primary hepatocytes. We tested a series of glycoproteins for their ability to block the action of the conjugate. Fetuin and orosomucoid, two glycoproteins with terminal sialic acid on their oligosaccharide chains, did not block the action of the conjugate. The N-acetyl-glucosaminyl-terminated derivative (asialo-agalacto-orosomucoid) had no appreciable effect on the activity of the conjugate. The galactose-terminated derivatives, ASF and asialo-orosomucoid, as expected, did block the action of the conjugate. We tested the ASF-DTA conjugate on six cell types; except for primary rat hepatocytes, none of were affected by a high concentration (10^{-8}M) of ASF-DTA conjugate. A fetuin-DTA conjugate was 300-fold less toxic than the ASF-DTA conjugate and apparently exerted its effects through non-receptor mediated uptake systems. The ASF-DTA conjugate is the first DTA conjugate which is highly toxic, cell-type specific and whose action is mediated by a well-defined receptor.

750 IMMUNOCHEMICAL ANALYSIS OF DISCOIDINS I AND II AT THE CELL SURFACE OF DICTYOSTELIUM DISCOIDEUM, D. Randall Armant and Edward A. Berger, Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

The endogenous lectins Discoidins I and II are believed to be primary components of the morphogenetic cell cohesion system of D. discoideum. We have developed immunochemical methods to analyze the association of the Discoidins with the cell surface. One method is a two-stage antibody binding assay in which intact cells are incubated on ice with rabbit serum (either normal serum or anti-Discoidin I + II), washed, then incubated with ^{125}I-Protein A. Reaction mixtures are spun through sucrose cushions, and radioactivity in the cell pellets and supernatants is determined. Specific antibody binding is defined as the difference between cell-bound radioactivity with anti-Discoidin versus normal serum during the first stage. Substantial specific antibody binding is observed with differentiated cells, but not with vegetative cells or with differentiated cells of an aggregation-defective mutant displaying negligible Discoidin in cell extracts. As a complementary method, quantitative immunoadsorption analyses demonstrated that intact differentiated cells are capable of nearly complete adsorption of immunoreactivity, as judged by assaying adsorbed supernatants for both specific antibody binding activity against fresh intact cells and immunoprecipitation activity against purified ^{125}I-Discoidin. By contrast, vegetative cells have no effect. Most importantly, in experiments where increasing numbers of differentiated cells are added to fixed dilutions of antisera, there is a quantitative reciprocal relationship between initial specific antibody binding and residual immunoreactivity in the adsorbed supernatants. We thus conclude that the specific antibody binding assay is a reliable measure of the relative quantities of surface-localized Discoidin. It was also possible to show by adsorption analyses that both anti-Discoidin I and anti-Discoidin II antibodies bind to differentiated cells. This is the first demonstration that both lectins are present at the cell surface.

751 Neutral glycosphingolipids are implicated in the specific adhesion of ox erythrocytes to BHK fibroblasts. Charles Glabe, M.S. Singer, L.R. Grabel and S.D. Rosen Dept. of Anatomy University of California, San Francisco 94143

BHK fibroblasts form rosettes specifically with trypsinized ox (tOx) erythrocytes. BHK cells interact to a much lesser extent with sheep and rat and not at all with rabbit, guinea pig and hamster erythrocytes. This same specificity is observed in the adhesion of different erythrocytes to monolayers of BHK fibroblasts. We have investigated the nature of the components responsible for the interaction between BHK and tOx cells. The rosette mediating factor (RMF) of the fibroblasts is destroyed by prolonged proteolysis, suggesting that it may be proteinaceous. Neutral glycosphingolipids extracted from ox erythrocytes were found to competitively inhibit rosetting whereas neutral glycolipids from rabbit erythrocytes had no effect on rosetting at similar concentrations. Incorporation of ox glycolipids into guinea pig erythrocytes caused these non-rosetting cells to adhere to BHK fibroblasts. The highest rosette inhibitory activity was found in fractions containing the major neutral glycolipid galactosyl paragloboside (Galα1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Ceramide). The terminal α galactose residues may be recognized by the fibroblast RMF. In support of this possibility is the finding that neoglycoproteins containing α galactose residues inhibit the adhesion of tOx cells to BHK monolayers while β galactose-BSA has no effect. Furthermore, treatment of tOx cells with α galactosidase results in a decreased ability of tOx cells to adhere to BHK cells. The role of the BHK RMF in the social behavior of the fibroblasts is being studied.

752 PURIFICATION OF A CANDIDATE FOR THE RECEPTOR TO PALLIDIN, THE LECTIN OF THE CELLULAR SLIME MOLD POLYSPHONDYLIUM PALLIDUM. Debra K. Drake and Steven D. Rosen, UCSF, San Francisco, California 94143.

We have purified a large molecular weight, carbohydrate-containing molecule, a candidate for the receptor for pallidin, the cell surface lectin in the cellular slime mold, Polysphondylium pallidum. Previous studies have both implicated pallidin in mediating intercellular adhesion and provided evidence for the existence of a high-affinity, cell-surface receptor for pallidin on differentiated amoebae. A potent antagonist of pallidin hemagglutination activity is found in a soluble extract of crude membranes from aggregation-competent cells. This inhibitory activity is precipitated from solution by the addition of pallidin. The carbohydrate-containing component is separated from pallidin by solubilizing the precipitate in 6M guanidine HCl and fractionating on Sepharose 4B. The material yields four peaks: a void and an internal OD280 peak and two carbohydrate-containing peaks. The first carbohydrate peak contains 75% of the inhibitory activity in the starting crude extract, whereas the other peaks contain negligible activity. Carbohydrate analysis of the inhibitory component by paper chromatography reveals two major components, glucose and rhamnose. Metabolic labelling experiments indicate that only the first of the carbohydrate peaks is synthesized by the slime molds. Preliminary experiments indicate the heterosaccharide may be developmentally regulated. High concentrations of this heterosaccharide block the agglutination of cohesive amoebae in gyrated suspension. Low concentrations augment agglutination. This substance may act as an extracellular "aggregation factor" by attaching to cell surface pallidin on opposing cells.

753 MULTIPLE DISCOIDIN I GENES IN DICTYOSTELIUM, Stephen Poole, Walter Rowekamp, and Richard A. Firtel, Univ. Calif. San Diego, La Jolla, CA 92093

Discoidin I is a tetrameric protein which may play an important role in cell cohesion in D. discoideum. On 2-D gels, in vivo synthesized Discoidin I shows three different isoelectric forms in approximately equimolar ratios. Immunoprecipitation of in vitro translation products of mRNA from developing cells also yields these three isoelectric forms.

Using recombinant DNA techniques we have shown that Discoidin I is encoded by a small multigene family. We have isolated recombinant phage and plasmids containing portions of several different Discoidin I genes. DNA sequence analysis of the protein coding regions shows very little sequence divergence, resulting in only a few amino acid substitutions between the various genes. The substitutions are not completely random, but show some clustering. There is very little substitution in the third position of the codons. At least three of the genes are transcribed and the 5' ends of the mRNA's of these genes have been localized by S_1 nuclease mapping. The 5' untranslated regions of the three genes show strong homolgy and have features shared by other Dictyostelium genes which have been isolated. Finally, more 5' hybridizing regions exist in the genome than 3' hybridizing regions when studied with Southern DNA blot filters.

754 MASKING OF THE PRONASE SENSITIVITY OF SEA URCHIN EMBRYO CELL ADHESION FORMATION, Robert S. Turner, Jr. and Donald R. Bertolini, Wesleyan Univ., Middletown, CT. 06457 Our previous studies show that adhesionsformed during reaggregation of dissociated sea urchin blastula cells are pronase insensitive Reaggregation is totally inhibited by pronase plus NaN_3 or pronase plus CCB, but not by pronase plus colchicine. NaN_3, CCB or colchicine do not affect reaggregation when used alone as markedly as they do in the presence of pronase. We tested the possibility that NaN_3 or CCB inhibit adhesive component replacement from an intracellular source, which makes the pronase able to inhibit re aggregation since the adhesive components it affects are not continuously replenished. We labeled intact embryos and determined the amount of labeled protein released during reaggregation of control cells, pronase treated cells, NaN_3 treated cells and cells treated with NaN_3 plus pronase. If our hypothesis is correct, less label should be released in the presence of NaN_3 plus pronase, since the former agent would block the replacement of the adhesive components affected by pronase. We found that cells treated with pronase plus NaN_3 release more label than cells treated with pronase alone or NaN_3 alone. We also found that a class of glycopeptides is released in the presence of pronase plus NaN_3 that is not released when either of these agents is used alone. We conclude that the adhesive components of these cells are protected from digestion by pronase by an energy and microfilament dependent process.

755 MONOCLONAL ANTIBODIES TO HUMAN TERATOCARCINOMA DIFFERENTIATION ANTIGENS. Peter G. Carroll and Robert S. Schwartz, Tufts university School of Medicine, Boston, MA 02111

Teratocarcinoma cells in high density culture undergo differentiation to form foci of early embryonic tissue. These cells have therefore been useful in the study of normal development. Cell surface recognition molecules seem to direct this differentiation pathway. We have raised monoclonal antibodies to specifically detect these recognition structures of embryogenesis by immunizing mice with human teratocarcinoma cells grown in serum free media. The monoclonal antibodies displayed three major specificity patterns: 1) specific reactivity for undifferentiated cells (cells grown at low density), 2) specific reactivity for differentiated cells (cells grown at high density), or 3) reactivity with both undifferentiated and differentiated cells. Monoclonal antibodies that reacted with the (HLA+) autologous lymphoblastoid cell line have been excluded from these groups. This selective reactivity indicates a spectrum of molecules on the cell surface, both temporal and persistent, that preceed and accompany cell contact-dependent differentiation of this embryonic cell line. The biochemical nature of these putative cell interaction molecules will be discussed.

This work was supported in part by grants NCI CA-24530 and NIAMDD AM-27232.

756 DIFFERENTIAL CELL COHESIVENESS OF PRESPORE AND PRESTALK CELLS IN DICTYSTELIUM DISCOIDEUM, C-H. Siu, T.Y. Lam, G. Pickering and J. Geltosky, University of Toronto, Toronto, Ontario. M5G 1L6

Pseudoplasmodia of Dictyostelium discoideum at the culmination stage were separated into two cell populations by sedimentation in a discontinuous renografin gradient. The two lighter fractions (I and II) had enzymatic activites characteristic of the anterior prestalk cells, while the heaviest fraction (III) showed enzyme activities characteristic of the posterior prespore cells. Prespore cells obtained from preculmination and culmination stages showed enhanced cohesiveness when compared with 10-hr cells and 18-hr prestalk cells. They were more resistant to dissociation by EDTA. However, Fab fragments prepared from antibodies directed against a specific cell surface glycoprotein gp150 were more effective in dissociating prespore cells than prestalk cells. Cells beyond the 13-hr stage were about 4-fold more sensitive to dissociation by these Fab fragments than cells at the 7-hr stages, suggesting that prespore cells acquired an increase in cohesiveness between 10 and 13 hr. In addition, prespore cells contained an approximately 2-fold higher concentration of the major endogeous carbohydrate binding protein discoidin-I than prestalk cells. These differences may account for the differential cohesiveness of these two cell populations and provide a basis for cell recognition and cell sorting at the slug stage.

757 INHIBITION OF FUSION OF EMBRYONIC MUSCLE CELLS IN CULTURE BY TUNICAMYCIN IS PREVENTED BY LEUPEPTIN, J. Brian Parent and Kenneth Olden, Howard University Cancer Center, Washington, D.C. 20059

The carbohydrate requirement for alignment and fusion of embryonic quail muscle cells has been examined in tissue culture by use of tunicamycin (TM). The mononucleated, spindle-shaped proliferating myoblasts were treated with TM at various times before fusion and differentiation into multinucleated muscle fibers capable of spontaneous contraction. TM blocked protein glycosylation and expression of glycoproteins on the cell surface, and strongly inhibited fusion when added to cultures of differentiating muscle cells before the fusion "burst", but had no apparent effect on cell alignment. The inhibition of fusion was partially prevented when TM was administered in the presence of protease inhibitors such as leupeptin and pepstatin, but the inhibition of glycosylation was not prevented. Both glycosylation and fusion were completely restored to normal by the removal of the antibiotic from the medium. These studies provide strong support for the idea that myoblast fusion is partially mediated by glycoproteins with asparagine-linked oligosaccharides. However, the requirement for the carbohydrate portion of the glycoprotein appears to be indirect in that it acts to stabilize the protein moiety against proteolytic degradation. Our findings do not rule out the possibility that oligosaccharide units of surface glycolipids have some role in myoblast fusion.

758 DEVELOPMENTAL MODULATION OF PROTEIN SYNTHESIS IN DROSOPHILA PRIMARY EMBRYONIC CELL CULTURES, Carolyn H. Buzin and Robert L. Seecof, City of Hope Research Institute, Duarte, CA 91010.

The patterns of proteins synthesized during embryonic development in Drosophila melanogaster have been examined by two-dimensional gel electrophoresis. Primary cell cultures prepared from donor embryos synchronized to ± 1 hr were labeled with [^{35}S]methionine at 5, 11.5, 14.5, and 26 hour after oviposition. Of approximately 400 to 500 proteins detected, the synthesis of about 50 is developmentally modulated. The greatest number of changes in the synthesis of stage-specific proteins occurs at 11.5 and 14.5 hr after oviposition, periods just prior to and during the times of the greatest overt morphological and biochemical changes. At 11.5 hr, 35 stage-specific proteins are synthesized, including 19 that are not present at the previous stage examined. At 14.5 hr, 34 stage-specific proteins can be detected, including 11 newly synthesized proteins. However, 12 proteins from the previous stage are no longer synthesized. At the completion of embryonic differentiation, at 26 hr, no new proteins are synthesized and the synthesis of many that were present in earlier stages has decreased or stopped. Comparison of patterns of embryonic proteins to those synthesized by two Drosophila continuous cell lines reveals that the majority of proteins are common to all. However, only about 40% of the embryonic stage-specific proteins are present in either cell line. In addition, there are several proteins unique to each cell line that are not observed in any of the embryonic stages.

759 ANALYSIS OF A SEQUENCE DETERMINING SPECIFIC TISSUE-TROPISM WITHIN THE GENOME OF THE MINUTE VIRUS OF MICE (MVM). Peter J. Tattersall, Dept. of Human Genetics, Yale University, New Haven, CT 06510

Two strains of the parvovirus MVM which are restricted reciprocally in their ability to infect fibroblasts and lymphocytes have been characterized. These strains, designated allotropic variants, are serologically indistinguishable and almost identical in the nucleotide sequence of their genomes. Cell fusion studies indicate that the restriction is recessive and that the establishment of viral infection depends upon the expression of developmentally-regulated host genes which are not extinguished in stable cell hybrids. Lack of complementation between the two virus variants suggests that each differentiated host gene product recognizes a cis-acting determinant within the genome of the appropriate virus strain in order to promote replication of that genome alone. Further variants of these viruses, which overcome the restriction, have been selected and the location of the allotropic determinant has been mapped within the viral genome. The location of this sequence, and its role in the replication of the virus will be discussed. Further cell studies have shown that the expression of the developmentally-regulated host function is inherited in a meta-stable manner in vitro and that cells may switch between phenotypes associated with dissimilar differentiated states with relatively high frequency. Factors affecting the control of expression of these host genes, and their possible functions during differentiation, will be addressed.

760 TRIFOLIIN IN CLOVER ROOT EXUDATE AND ITS RELATIONSHIP TO CELL SURFACE POLARITY OF RHIZOBIUM TRIFOLII, Estelle M. Hrabak, Frank B. Dazzo, John E. Sherwood, and Georges Truchet, Michigan State University, East Lansing, Michigan 48824

Trifoliin, a Rhizobium-binding glycoprotein from white clover, was detected in sterile clover root exudate by an immunofluorescent assay employing encapsulated cells of Rhizobium trifolii 0403 heat-fixed to microscope slides. Its presence in root exudate was confirmed by immunoaffinity chromatography and polyacrylamide gel electrophoresis. Trifoliin in root exudate bound uniformly to fully encapsulated cells after 1 hr incubation, but after 4-8 hr there was a progressive reduction in bound trifoliin resulting in a distinctive localization at only one cell pole. Trifoliin could no longer be detected on the surface of heat-fixed cells after 12 hr of incubation with the root exudate. However, trifoliin was detected on one pole of cells grown in situ for 4 days in the simulated clover rhizosphere of Fahraeus slide cultures. The substance(s) in root exudate which induced this alteration were high in molecular weight, heat-stable, trypsin-sensitive, and were antigenically unrelated to seed trifoliin. These results suggest that proteinaceous substances in root exudate affect the development of polarity in trifoliin-binding receptors which may account for the distinctive polar attachment of these cells to clover root hairs during Phase I attachment.

Chemotaxis

761 FRACTIONATION OF CHEMOTACTIC FACTOR FROM BY-PRODUCTS OF INTERFERON PURIFICATION. Amanullah Khan, Cindy Kiker, Syeda Shariff and N.O. Hill, Wadley Institutes of Molecular Medicine, Dallas, TX 75235.

Human leukocytes are stimulated with an inducing virus (Sendai) and cultured for 24 hours to obtain human leukocyte interferon. It is likely that other lymphokines are also produced during this culture. We have attempted to isolate other lymphokines that may be produced and then discarded during interferon purification procedures. It was reported earlier that the monocyte chemotactic factor is produced during this procedure. Two of the discarded fractions consisting of ethanol precipitates at pH 5 and 5.6 contained chemotactic factor for monocytes. Ammonium sulfate (50%) precipitation was used as a first step for purification. Further work has shown that 45% ammonium sulfate gives better yield with less protein contamination. The ammonium sulfate precipitate was further fractionated on controlled-pore glass bead columns (Electro-Nucleonics, Inc., mean pore size 116A). Four fractions were obtained by this procedure. Fraction 3, which had a small protein peak, contained chemotactic activity.

762 CHEMOTACTIC PEPTIDE RECEPTOR CYCLING IN POLYMORPHONUCLEAR LEUKOCYTES (PMNs). Susan J. Sullivan and Sally H. Zigmond, University of Pennsylvania, Philadelphia, PA 19104. Incubation of PMNs with the chemotactic peptide N-formylnorleucylleucylphenylalanine, FNLLP, at 37°C results in a dose dependent loss of receptors which are available for binding. Within 20 min, a new plateau level of receptors is reached. The cells continue to take up peptide in a receptor-mediated manner after the plateau has been reached. If peptide is removed, cells recover the "lost" receptors. Recovery is first order function of the number of receptors "lost" from the cell surface. We have investigated the kinetics of these processes to determine if the plateau receptor level could represent a steady-state in which the receptors are continually being internalized and resupplied to the cell surface. The rate constant for receptor internalization, k_{ur}, was determined from the rate of receptor-mediated peptide uptake. The rate constant for receptor recovery, k_r, was determined after preincubation at various concentrations of FNLLP. We find that the rate of receptor internalization, k_{ur} (RC), is similar to the rate of receptor reinsertion, k_r (R_i), where (RC) equals the number of surface receptors occupied and (R_i) equals the number of receptors inside the cell. This is consistent with the plateau being a steady state of receptor cycling.

763 HUMAN BLOOD MONOCYTE AND PERITONEAL MACROPHAGE RECEPTORS FOR CHEMOTACTIC PEPTIDE J. Brice Weinberg, Joseph J. Muscato, and James E. Niedel, VA and Duke Medical Center, Durham, NC 27705
Synthetic N-formylated oligopeptides, including N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys, induce various functional changes in human phagocytes. The ^{125}I-labeled peptide is biologically active and binds to human blood monocytes and peritoneal macrophages. Monocyte uptake of the labeled peptide is specific, rapid, and saturable, but bound peptide cannot be displaced by saturating doses of unlabeled peptide. Half-maximal uptake in monocytes and peritoneal macrophages occurs at about 3 nM, a concentration that corresponds to the maximally chemotactic concentration of the peptide for these cells. At saturation, the monocytes bind 8 fmol/10^5 cells indicating approximately 50,000 uptake sites per cell. Pretreatment of the monocytes with 2-20 nM unlabeled peptide causes a 20-60% decrease in uptake of ^{125}I-labeled peptide (down regulation); in parallel assays there is a comparable decrease in chemotactic responsiveness. Fluorescence microscopy gives visual evidence of receptor-mediated internalization of rhodamine-labeled peptide by the fresh monocytes and peritoneal macrophages. After monocytes are cultured in vitro for 7-10 days, they morphologically resemble tissue macrophages. The cultured monocytes display a variable decrease in their binding of the labeled peptide, but they have 60-90% less chemotactic response to chemotactic peptide or zymosan-activated serum irrespective of their ability to bind the labeled peptide. Thus, freshly isolated human blood monocytes and peritoneal macrophages have comparable receptors for and chemotactic reponses to the synthetic chemotactic peptide.

764 PARTIAL PURIFICATION OF THE AFFINITY-LABELED FORMYL PEPTIDE CHEMOTACTIC RECEPTOR, James Niedel, Duke University Medical Center, Durham, North Carolina 27710.
Chemotactic formyl oligopeptides are processed by receptor-mediated aggregation, endocytosis, degradation and release after binding to a specific receptor on the plasma membrane of human neutrophils. Seven techniques for the covalent affinity-labeling of this receptor have been compared. N-formyl-Nle-Leu-Phe-Nle-^{125}I-Tyr-Lys was crosslinked with either dimethyl suberimidate, ethyleneglycol bis (succinimidyl succinate) (EGS) or dithio-bis (succinimidyl propionate) (Lomant's reagent). N-formyl-Nle-Leu-Phe-Nle-^{125}I-Tyr-Lys-N$^{\epsilon}$-4-azido-2-nitrophenyl and -N$^{\epsilon}$-4-azidobenzoyl were crosslinked by photoactivation and N-formyl-Nle-Leu-Phe-Nle-^{125}I-Tyr-Lys-N$^{\epsilon}$-bromoacetyl and -N$^{\epsilon}$-maleimidobenzoyl reacted spontaneously. All methods labeled a polypeptide with an apparent molecular weight of 55,000 to 70,000 by SDS-PAGE. This labeling was shown to meet the same criteria of saturability, specificity and affinity used to characterize the receptor by radioligand binding studies. Six of the methods labeled the receptor and multiple non-receptor proteins as well, whereas EGS labeled only the receptor. Efficiency of crosslinking was lowest for the two arylazides (<0.5%) and highest with EGS (10-20%).

Approximately 50% of the affinity-labeled receptors can be solubilized with low concentrations of various detergents, including digitonin, Triton-X100, Nonidet P-40, Ammonyx-10 and octyl glucopyranoside. Potassium iodide, acetic acid, divalent ion chelators and changes in ionic strength, treatments which release peripheral membrane proteins, do not extract the formyl peptide receptor. The receptor can be partially purified by ion exchange, gel filtration and hydroxyapatite chromography. Studies of lectin specificity are underway.

765 STUDIES ON THE cAMP RECEPTOR OF D. DISCOIDEUM IN INTACT CELLS, PLASMA MEMBRANES, AND THE SOLUBILIZED STATE, Beth L. Meyers and William A. Frazier, Washington University School of Medicine, St. Louis, MO 63110.

Aggregating cells of the cellular slime mold D. discoideum produce relayed cAMP signals which promote both chemotaxis and further differentiation. We have shown that the chemotactic receptor for cAMP in intact cells and plasma membranes binds cAMP in an oscillatory manner. This receptor has been solubilized from plasma membranes with the nonionic detergent Emulphogene BC-720. The extremely rapid association and dissociation rates of the receptor have necessitated development of ^{3}H-cAMP binding assays in which bound and free label can be separated in less than 2 seconds. In one assay detergent solubilized receptor is bound to DE-81 filters (ion exchange) and a second method employs hydrophobic binding of the receptor protein to decyl-agarose beads at low detergent concentration. With both assay methods, solubilized receptor shows the expected rapid on and off rates. The approximate Kd is 10^{-7} M and cGMP has a 10 to 50 fold lower affinity for the soluble receptor, indicating both lower affinity and selectivity of the receptor in the soluble state. Sulfonyl fluorides inactivate both the detergent solubilized and membrane associated receptors, suggesting identity of these cAMP binding proteins. Multivalent receptor ligands, such as cAMP-BSA conjugates, are chemoattractants and bind to intact cells with nonoscillatory kinetics suggesting multivalent receptor interaction. Studies on receptor localization and internalization using these multivalent ligands are now in progress. [Supported by NS 13269 and PCM-78 04304, WAF is an Established Investigator ofthe American Heart Assn.]

766 SPATIAL VS. TEMPORAL MECHANISMS IN DICTYOSTELIUM CHEMOTAXIS, R. P. Futrelle, University of Illinois, Urbana, IL 61801

By using a moving micropipette source, it is possible to exert some independent control over the spatial and temporal characteristics of the chemical signal concentration seen by a cell. Experiments are described in which this is done for cyclic AMP signals presented to Dictyostelium discoideum amebas. The results are discussed in terms of the two current models for ameboid chemotaxis: the response is due to the spatial gradient at the cell or, the response is due to the time variation at the cell (upshift vs. downshift). The results are related to our recent findings that chemotaxis in Dictyostelium is labile and rapid. Cells respond within seconds to starting, stopping, or moving the cAMP source. Concentration upshifts also produce a transient (30 s) stopping response called "cringing". Between cAMP pulses, late cells stop translocating for many minutes, but exhibit substantial activity at their margins, unlike cringing. We propose that the function of this long duration stopping, called "quivering", is to stabilize the cell arrangements which have developed to that point, rather than losing the arrangements due to random cell motion between successive cAMP signals.

767 THE EFFECT OF CYCLIC AMP ON PROTEIN PHOSPHORYLATION IN DICTYOSTELIUM DISCOIDEUM, Donna Coffman, Ben Leichtling, H.V. Rickenberg, National Jewish Hospital and Research Center/National Asthma Center and University of Colorado Medical Center, Denver, CO 80206

The phosphorylated proteins of Dictyostelium discoideum were examined by SDS polyacrylamide gel electrophoresis of $^{32}P_i$-labeled amoebae. We observed that the pattern of phosphoproteins changed during the differentiation of the amoebae on Millipore filters. To explore the effect of cyclic AMP on protein phosphorylation, duplicate cultures of starving amoebae were exposed to pulses of cAMP or 5'AMP every seven minutes such that the nucleotide concentration after every pulse was 10^{-7} M. Approximately thirty phosphoproteins were detected. Labeled extracts were separated into membranal and nuclear fractions. We found a number of changes in the occurrence of phosphoproteins during development. Exogenous cAMP caused the precocious appearance of certain membranal phosphoproteins (apparent subunit m.w. 77,000 and 79,000) and the precocious disappearance of certain nuclear phosphoproteins apparent subunit m.w. 34,000 and 39,000). The majority of the phosphoproteins appeared to be unaffected by the addition of cAMP. Clearly additional changes of the phosphoproteins in response to exogenous cAMP may occur. Therefore we have initiated a two-dimensional analysis of the phosphoproteins of Dictyostelium discoideum and of their behavior in response to cAMP.

Cilia and Flagella

768 W. J. SNELL, A. CLAUSELL, and J. WEBB, The Univ. of Texas Health Science Center at Dallas, Dallas, Texas 75235.

We have recently shown (J. Cell Biol., 84:203) that during aggregation between gametes of opposite mating type (mt) of Chlamydomonas there is a rapid loss of flagellar adhesion molecules whose replacement is prevented by inhibitors of protein synthesis. Since these results suggested that there is an adhesion-induced synthesis of flagellar proteins we have attempted to identify such molecules by incubating mt and mt$^-$ gametes in $^{35}SO_4$ both before and after mixing. Analysis by SDS-PAGE and autoradiography of flagellar fractions isolated from such preparations revealed that two proteins of approximately 220K and 165K daltons show a high rate of ^{35}S incorporation only during aggregation. These molecules, which are just barely detectable by Coomassie blue staining for proteins on the gels but are detectable with periodic acid Schiff staining for carbohydrates, appear in a membrane fraction separable from intact flagella when the flagella are further fractionated. We are making antibodies against these two proteins to determine if they are indeed flagellar surface proteins involved in adhesion. In further attempts to characterize changes in flagellar molecules that occur during flagellar adhesion and deadhesion, we have used octyl glucoside (OG) extracts of flagella, which have been shown to contain mating type specific adhesion molecules (J. Cell Biol. 204: 599). We have found that cells are able to adhere to and deadhere from OG-extract coated substrata. Moreover, the substratum adsorbed extracts are rendered non-adhesive by interaction with live gametes of the opposite but not the same mating type. We are currently trying to determine what biochemical changes in the extracts might be correlated with these functional changes. Supported by NIH Grant #25661 to W. J. S.

769 THE PLASMA MEMBRANE OF SEA URCHIN SPERM: ISOLATION, ELECTROPHORETIC CHARACTERIZATION, AND ADENYL CYCLASE ACTIVITY. Nicholas L. Cross*, Marisa Mourelle, Meredith Gould-Somero* and Alberto Darszon. *University of California, San Diego, CA 92093, and Centro de Investigación y de Estudios Avanzados del IPN, México 14, D.F.

Two plasma membrane fractions were isolated from flagella of Strongylocentrotus purpuratus sperm by differential centrifugation. On SDS polyacrylamide gels, fraction 1 contained 9 major proteins and 14 minor species. Gels of fraction 2 were similar but had more protein of 55 k daltons as well as additional minor bands. Five proteins of ~200 k, 149 k, 120 k, and 59 k stained with PAS; these 5 can be externally labeled by lactoperoxidase-catalyzed radioiodination. Membranes of fraction 1 isolated from radioiodinated sperm are enriched 10-fold in the specific activity of ^{125}I, and comparison with gels of labeled, unfractionated sperm suggests that no major labeled proteins are lost during isolation. To this extent the membrane fraction is representative of the entire sperm plasma membrane. Both fractions contain adenyl cyclase activity which is stimulated by Mn^{2+} and insensitive to F^-. The specific activity of fraction 2 was 10-15 fold higher than in whole sperm and 3-7 fold higher than in fraction 1. The cyclase appears to be associated with the membrane since washing with buffers of high or low ionic strength or sonication did not release appreciable activity into the supernatant. The enzyme activity was inhibited by 10 μM to 12.5 mM Ca^{2+} when the Mn^{2+} concentration in the assay was 5 mM and ATP was 3.2 mM. Supported by USPHS Grant GM-07169 and NSF PCM 80-03759.

770 PAIR FORMATION IN TETRAHYMENA REQUIRES PRIOR CONTACTS WITH CELLS OF ANOTHER MATING TYPE, Peter J. Bruns, Cornell University, Ithaca, New York 14853

Tetrahymena thermophila forms mating pairs only after starved cells of different mating types physically interact (costimulate) for at least half an hour. Although there are seven mating types in this species, costimulation is not mating type specific; contacts with cells of one mating type prepare a cell for mating with cells of any but its own type. Both interacting cells must be ready for this activity (properly starved), and both must be mature (wild type cells must have grown for about 40 fissions after the last mating). A recessive early mature mutation has been isolated which eliminates the immaturity period; the scheme for its isolation and some of its characteristics are described. Supported by NSF grant PCM77-07056 and NIH grant GM27871-01

771 PROTEIN AND LIPID FUNCTION IN THE CILIARY MEMBRANE OF PARAMECIUM, David L. Nelson, Lea Eisenbach, Michael Forte, Todd Hennessey, Ching Kung, Rajeev Ramanathan and Yoshiro Saimi, University of Wisconsin, Madison, WI 53706

Antibodies raised against purified ciliary membranes immunoprecipitate several membrane proteins, including the immobilization antigen (i-antigen) (MW 250,000) and a family of acidic polypeptides of MW 42-45,000. Both the i-antigen and the 45,000 family are glycosylated, and are accessible to lactoperoxidase-catalyzed iodination with 125-I and to labeling with 3-H-$NaBH_4$ after treatment with galactose oxidase. The i-antigen is also very susceptible to proteolysis in intact cells. Either monovalent or bivalent antibodies against some membrane protein, probably the i-antigen, block Ca^{++} flux through the voltage-sensitive Ca^{++} channel of the ciliary membrane.

Lipids of the ciliary membrane also affect the function of the Ca^{++} channel. In a mutant with altered Ca^{++} currents and altered swimming behavior, there is a specific alteration in two ciliary membrane sphingolipids. Phospholipid vesicles prepared from the ciliary membranes of this mutant are more fluid, as measurd by fluorescence depolarization with diphenylhexatriene, than those from wild type cells. Addition of a sterol to the growth medium causes the electrical properties of the ciliary membrane, as well as its phospholipid composition and fluidity, to become indistinguishable from those of wild type cells.

772 LENGTH CONTROL IN THE CHLAMYDOMONAS FLAGELLUM, Jonathan W. Jarvik, Frederick Reinhart, and Sally Adler, Carnegie-Mellon University, Pittsburgh, PA 15213

We are examining length regulation in the Chlamydomonas reinhardtii flagellum in order to learn about the control of size at the cellular level. When the short-flagella mutant ts253 is mated to wild type to produce quadriflagellate dikaryons with two normal and two short flagella, the two short flagella grow to wild type length at a rate comparable to the initial rate seen after deflagellation of wild type. This result argues against passive models for flagellar length control, in which the rate of flagellar growth is an inverse function of flagellar length, and it argues for active models in which the cell monitors and regulates flagellar length.

253 cells have the peculiar property of regenerating their flagella after shear-deflagellation but not after pH shock deflagellation, whereas wild type regenerates after either procedure. This may mean that some cellular element involved in length control - perhaps the flagellar basal body - is particularly sensitive to pH shock in the 253 mutant. We are presently looking for biochemical or ultrastructural evidence of such sensitivity.

773 CHLAMYDOMONAS SEXUAL AGGLUTININS, W. Steven Adair, Washington University, St. Louis, Missouri 63130.

Fertilization in the unicellular flagellate Chlamydomonas is initiated by a rapid agglutination of gametes by their flagella. This cell-cell interaction is highly specific, occurring only between complementary mating types of the same species, and is presumeably mediated by recognition/adhesion components associated with the flagellar membrane. To probe the molecular basis for cell specificity and adhesion in this system we have devised procedures which employ the dialyzable non-ionic detergent octyl-D-glucopyranoside (OG) to extract the flagellar agglutinin(s) in a form which is amenable to bioassay using living cells. Using a quantitative form of this bioassay we have obtained preparations enriched for the active components by three different fractionation schemes: (1) gel filtration in the presence of Na-deoxycholate; (2) immunoaffinity chromatography; and (3) preparative agarose gel electrophoresis under non-denaturing conditions. In each case, agglutinin activity co-fractionated with two polypeptides of apparent MW 350 K and 20 K respectively. The 350 K component represents the major flagellar membrane polypeptide, is glycosylated, and is the major species labeled by lactoperoxidase/glucose oxidase catalyzed iodination. The 20 K component, by contrast, does not label under the same conditions and gives a negative PAS reaction for carbohydrate. Preliminary experiments indicate that neither 350 K nor 20 K alone has significant agglutinin activity; experiments to test whether the two components associate together to form an active complex are presently in progress.

774 CELL-CELL COMMUNICATION AND GENE EXPRESSION IN MATING TETRAHYMENA. Jason Wolfe, Biology Department, Wesleyan University, Middletown, CT. 06457.

The mating system of Tetrahymena includes a preconjugation courtship period during which there occurs cell-cell recognition, signal transduction and the development of the ability to form heterotypic pairs. Since the developmental events, which include changes in cell shape and in gene expression, can go to completion after reciprocal induction without continued interactions, the pre-pairing period consists of a specific and discontinuous cell-cell communication event. It is generally accepted that costimulation is mediated by fleeting direct contacts between the cell surfaces. In order to economically achieve specificity with seven mating types, surface-bound receptors and ligands may be organized in the form of triplet complexes. The interaction induces a change in the patterm of protein synthesis detectable between 15 or 30 min after mixing mating types, using ^{35}S-methionine labelling, SDS-PAGE and autoradiography. The predominant change is in transient synthesis of an 80 kd polypeptide (p80). Conditions which prevent costimulation also block the appearance of p80. However, induction of p80 is not inhibited by Actinomycin D at concentrations (25 µg/ml) which effectively block post-interaction development. Presumably its synthesis is under post-transcriptional control. There is evidence that p80 may be a nuclear protein which rapidly responds to different stimuli each of which effects a switch in gene programming.

This research supported by NIH grant GM 18014.

Cell Interactions in the Immune System

775 ANTIBODIES TO CHEMICALLY-SYNTHESIZED POLYPEPTIDES AS UNIQUE PROBES OF GENE EXPRESSION, Nicola Green, Thomas M. Shinnick, J. Gregor Sutcliffe, Richard A. Lerner, Molecular Genetics Group and Department of Cellular and Developmental Immunology, Scripps Clinic and Research Foundation, La Jolla, California 92037, Owen Witte, Molecular Biology Institute, University of California at Los Angeles, Los Angeles, California 90024

The retroviral envelope protein gp70 is both a recognitive molecule which allows virus particles to attach to cells, and a major differentiation antigen of certain normal mouse cells. One unresolved question concerning the viral expression of this protein is the mechanism whereby it is processed from its polyprotein precursor, inserted into the membrane of infected cells, and subsequently budded in the form of mature virus envelope. We analyzed this sequence of events using an antibody prepared against the polypeptide predicted by the right hand most 15 triplets of the Moloney MuLV genome coding region. This antibody recognizes 2 proteins in virally-infected cells -- the 80K env precurosr and a 17K molecule. Amino acid sequencing of the 17K molecule revealed that its N-terminus is that of p15e. Since the C-terminus is defined by antibody, this molecule must represent a composite of p15e and the recently-described R gene of the env precursor. Immune precipitation with ap15e sera showed that this 17K molecule is the predominant protein species containing p15e determinants in extracts of virally-infected cells. In virus particles, however, the major p15e species lacks the C-terminal R-reactive fragment and has an apparent molecular weight of 15K. These and other studies suggest a cleavage-exit model in which virus maturation is accompanied by removal of a C-terminal domain from the env precursor. In addition to providing insights into the process of retrovirus maturation, these studies offer a general method to access proteins predicted by nucleic acid sequence. Insofar as antibodies made in this way are sequence-specific, they become unique biochemical reagents.

776 BIOCHEMICAL ANALYSIS OF A MHC-LINKED LYMPHOCYTE CELL SURFACE ANTIGEN, Qa-2. Mark J. Soloski, Ellen S. Vitetta, and Jonathan W. Uhr. Department of Microbiology, University of Texas Southwestern Medical School, Dallas, Texas.

The region of the murine 17th chromosome telomeric to H-2D encodes a group of serologically defined cell surface antigens termed Qa-1-5. These antigens are of interest because their expression is restricted to several functional lymphoid cell subpopulations. In addition, the molecular weight and subunit structure (i.e., association with β-2 microglobulin) of Qa-2 molecules are similar to H-2 and TL antigens. In the present studies we have prepared isotopically labeled Qa-2 and H-2 molecules from mitogen stimulated C57BL/6 spleen cells. Comparative peptide mapping of tryptic peptides from Qa-2 and H-2 molecules (K^b, K^k, D^d) reveal that Qa-2 has a unique primary structure. However, considerable homology is indicated since 30-40% of the Qa-2 peptides co-chromatograph with peptides derived from H-$2K^b$, H-$2K^k$, and H-$2D^d$. Studies by other investigators have demonstrated that similar levels of structural homology are observed when H-2K, H-2D and H-2L tryptic peptides are analyzed. We conclude from these studies that the Qa-2 alloantigen is structurally related to a class of cell surface molecules (i.e. H-2) which play critical roles in immune recognition processes. These data further suggest that the genes encoding Qa-2 and H-2 molecules may have arisen from a common primordial gene.

777 CYTOLYTIC T CELL-TARGET CELL FUNCTIONAL BINDING IN SUSPENSION AND SPECIFIC REVERSAL, Steven P. Balk and Matthew F. Mescher, Harvard Medical School, Boston, MA 02115

The specific binding of target cells by cytolytic T lymphocytes (CTL) provides an excellent model system for studying cell-cell interactions mediated by surface proteins. An assay has been developed which allows measurement of the rate and extent of target cell binding in suspension, independent of the rate of lysis. Target cell binding was found to plateau in suspension within 3 hours. Unbound, functionally active CTL and target cells were present at this plateau, indicating that target cell binding is an equilibrium process. Scatchard plots could be used to derive values for Keq (apparent affinity) and bmax (maximum binding). Secondary in vitro stimulation of CTL by membranes resulted in the generation of effectors with a lower bmax and a higher apparent affinity than did stimulation with intact cells. Target cell binding in suspension was not blocked by purified plasma membranes.

Reversal of specific target cell binding was also examined. Functional adhesions between CTL and P815 target cells were stable in suspension for greater than 5 hours but were rapidly reversed by addition of free, unlabeled target cells. This reversal was specific in that no reversal was induced by tumor cells of other H-2 types. Similarly, binding of spleen cell targets was rapidly reversed by syngeneic spleen cells but not by cells allogeneic with the target. Comparisons of various syngeneic cells demonstrated that the rate of specific reversal is dependent upon the relative affinity of the CTL for the bound versus the free target cell.

778 GROWTH OF HUMAN T-LYMPHOCYTE COLONIES FROM WHOLE BLOOD: CULTURE REQUIREMENTS AND APPLICATIONS, S. J. Knox, F. D. Wilson*, M. Shifrine, and B. R. Greenberg†, LEHR, University of California, Davis, CA 95616; *Battelle-Columbus Laboratories, Columbus, OH 43201; and †U. C. Davis, Sacramento Medical Center, Sacramento, CA 95817.

Human T-lymphocyte colonies have been directly grown from whole blood following stimulation with PHA, Con-A or PPD. This technique eliminates the requirement for Ficoll-Paque gradient-separation, and provides a sensitive system for the study of T-lymphocyte progenitors that more closely approximates the in vivo milieu. Colony cells had typical lymphocytic/lymphoblastic morphology and were demonstrated to be T-lymphocytes on the basis of ANAE activity, and E rosette formation. Colony formation increased as a power function of the number of cells plated over a wide range of cell concentrations. The slope for the linear regression line for PHA-stimulated colony formation was 2.4, implying a requirement for at least two cells in such a response. Preliminary studies indicate that the whole blood lymphocyte cloning assay is an extremely sensitive technique for the measurement of in vitro radiation damage on the proliferative capacity of sensitive lymphocyte progenitor cells. Subpopulations of lymphocytes from patients with Fanconi's anemia were significantly more radiosensitive than lymphocytes from normal individuals. Abnormalities in whole blood colony formation are associated with certain disease states, and possibly may be useful as prognostic/diagnostic indicators. This method also has wide application for the study of cellular interactions, antigen recognition, and the induction and amplification of T cell function.

779 BOTH SPECIFIC AND NONSPECIFIC LYMPHOCYTE-MEDIATED CYTOLYSIS INVOLVES TARGET CELL HISTOCOMPATIBILITY ANTIGENS. Valerie W. Hu, Gideon Berke, Ella McVey and W.R. Clark. Univ. of Calif., Los Angeles,CA 90024, and the Weizmann Inst., Rehovot, Israel.

We have used lectin-dependent cell-mediated cytotoxicity (LDCC) to examine interactions between effector and target cells in cell-mediated cytotoxicity. We find that lectin does not mediate nonspecific lysis by simple bridging of effectors and targets via surface sugars, or by activation of effector cell function. Lysis occurs when target cells are pretreated with lectin, but not when effector cells are pretreated. Moreover, when cytotoxic T cells (CTL) are used as both effectors and targets, and only one is pretreated with lectin, lysis occurs only in the direction of the pretreated CTL target. We find that in LDCC, as in specific cell-mediated killing, target recognition proceeds through interaction of effector cell receptors (distinct from sugar moieties) and target antigens perhaps modified by, but distinct from, the lectin itself. We conclude that the target antigens recognized in nonspecific lysis are major histocompatibility antigens: 1) Cells that display reduced amounts of MHC antigens are poor targets in LDCC; 2) removal of H-2 by papain renders murine targets refractory to LDCC; 3) antisera to target cell MHC antigens block LDCC. The latter finding is also true in oxidation-dependent cell-mediated cytotoxicity. The obligatory involvement of MHC proteins in both specific and nonspecific lysis reconciles an apparent fundamental distinction between these two processes, and furthermore suggests a possible role for MHC proteins in post-recognition steps of cytolysis.

780 STUDIES OF IR GENE FUNCTION USING AN I-A MUTANT MOUSE STRAIN, Ted H. Hansen, C. Shirley Lin and Alan S. Rosenthal, Merck Institute for Therapeutic Research, Rahway, NJ 07065

The mechanism by which Ia molecules mediate Ir gene control was studied using the I-A mutant mouse strain, B6.C-H-2^{bm12}. Mutant mice were compared with "wild-type" B6 mice for their ability to respond in a T-cell proliferation assay against five soluble protein antigens. Two of these antigens, (TG)A-L and beef insulin, were tested under conditions of strict Ir gene control. Surprisingly, B6.C-H-2^{bm12} mice failed to respond to beef insulin while the wild type IA^b gene conferred responsiveness, and in contrast both mutant and wild type responded comparably to (TG)A-L. B6 and B6.C-H-2^{bm12} mice were also found to be indistinguishable in their responses to DNP-OVA, PPD and pork insulin. Therefore, these data demonstrate that the H-2^{bm12} mutation resulted in the selective deletion of an Ir gene function. Several approaches are being taken to investigate the mechanism causing the failure of B6.C-H-2^{bm12} mice to respond to beef insulin. The genetic basis of the Ir gene mutation was investigated using $(I^{bm12} \times I^k)F_1$ hybrid animals, two non-responders to beef insulin. Interestingly, these hybrid mice responded to beef insulin and studies are underway to determine the molecular basis of this complementation. The cellular nature of the H-2^{bm12} lesion is also currently being investigated by testing the ability of mutant and wild-type spleen cells to present antigen to $(I^{bm12} \times I^b)F_1$ hybrid T cells. The B6.C-H-2^{bm12} mice therefore offer new approaches to the study of the complexity of the genetic, molecular, and cellular mechanism of Ir gene function.

781 NCA IN MATURING MYELOID CELLS, Britta Wahren, Department of Virology, National bacteriological laboratory, S-105 21 Stockholm, Sweden

The normal colon- and granulocyte antigen (NCA) cross-reacts immunologically with carcinoembryonic antigen (CEA). NCA is a glucoprotein with terminal aminoacids in common with CEA. It is related to the differentiation of human myeloid cells.

To show whether NCA is synthesized during differentiation, stem cells from human bone marrow were cultivated in soft agar. After colony formation, NCA was identified in granulocytes, myelocyte-like cells and macrophages. It was located in the cellular cytoplasm and at the cell membrane.

Patients with chronic myeloid leukemia (CML) have high serum NCA. The in vitro formation of mature myeloid cells from healthy persons was inhibited by high levels of NCA but not by CEA. This inhibition may have a role in the regulation of leukemic myeloid cell differentiation in vivo in patients with chronic myeloid leukemia.

782 STUDIES OF TARGET CELL RECOGNITION BY CYTOTOXIC T LYMPHOCYTES, Joan M. Chapdelaine, T.V. Rajan, Stanley G. Nathenson and Frank Lilly, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Cytotoxic T lymphocytes (CTL) generated during a Moloney murine sarcoma virus (MSV) infection in *H-2^b* mice are specific for the *H-2^b* molecule recognized in association with a viral antigen shared by the Friend, Moloney and Rauscher (FMR) viruses. The *H-2D^b* specificity has now been studied using a heterozygous *H-2^b/H-2^d* Friend virus-induced tumor cell line which was mutagenized with ethyl methanesulfonate (EMS). These cells were treated with a hybridoma antibody directed against the *H-2D^b* molecule in the presence of rabbit complement. The surviving cells were no longer susceptible to the hybridoma antibody but were still lysed with conventional anti-*H-2D^b* alloantiserum plus complement. Sequential immunoprecipitation followed by gel electrophoresis indicated that both antisera recognized the same molecule. When the mutant cells were used as targets for MSV-specific CTL, the amount of cell lysis, compared to that seen with the nonmutant parent cells, was drastically decreased. However, they remained susceptible to allogeneic CTL raised against the *H-2D^b* molecule. The mutant cells also did not differ from the parent cells in their level of viral antigen expression. Preliminary biochemical data suggest that there may be several tryptic peptide differences between the mutant and the parental cell lines. These studies suggest that a possible mechanism of escape by virus-infected cells from destruction by CTL might be alteration by somatic mutation of the Class I *H-2* glycoprotein(s) that must be recognized by the CTL in order for them to be effective. This strongly implies that the mechanism of *H-2* restriction in this system works at the level of the target cell rather than that of the responder cell.

783 ANALYSIS OF THE ORIGIN OF THE ALLOREACTIVITY DETECTED IN SOME LONG TERM T CELL LINES REPEATEDLY STIMULATED WITH HAPTEN TREATED SYNGENEIC CELLS. A-M. Schmitt-Verhulst, A. Guimezanes and F. Albert. Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille Cedex 2, France.

When T cells from hapten-sensitized mice are restimulated in vitro with the same hapten on syngeneic spleen cells, T cell lines can be established which are dependent for their propagation on stimulation with that hapten on I region matched cells. Alloreactivity can generally not be detected in such T cell populations from the third restimulation on as measured by incorporation of (^{3}H) thymidine. One line however (C57BL/10 anti-C57BL/10-TNBS) after having shown such a classical pattern of specificity for restimulation up to stimulation 8 subsequently behaved differently : in addition to the previous pattern of stimulation, equal stimulation levels were detected for unmodified allogeneic cells presenting the s, q, f or p alleles in the I region which share the Ia5 specificity. Cells of the k or d haplotypes were not stimulating. Two possibilities could explain such behaviour : A/ alloreactive clones which were initially a minor component of the T cell line, maintained through trans-stimulation, could start to out grow the line, B/ among different T cell clones reactive to TNP + I-A^b, a selection could have occurred for clones reactive to both TNP + I-A^b and public allogeneic I-A specificities such that a given T cell can bear the two types of receptor, or that the same receptor specific for self I-A + TNP would also have affinity for foreign I-A antigens. To distinguish these possibilities, reactivities are being analysed under limiting dilution conditions. Direct antigen binding assays are being developed to study receptor specificity.

784 NOVEL PROTEINS ASSOCIATED WITH CHANGES IN MEMBRANE PROPERTIES DURING THE MATURATION OF THYMIC LYMPHOCYTES TO THE GLUCOCORTICOID-RESISTANT STATE, Donald A. Young, Mary L. Nicholson, and Bruce P. Voris, University of Rochester School of Medicine, Rochester, N.Y.14642

We have previously found changes in membrane properties (increased resistance to osmotic shock) that occur during the progression of rat thymus cells to immunologically-committed medullary lymphocytes. We report here the detection of new proteins that accompany this change. Resistant medullary thymocytes were obtained from rats treated with dexamethasone (10 mg/kg body weight/day) for 3 days. When the protein patterns from 2-D gel electrophoresis of glucocorticoid sensitive cells are compared to those from resistant cells the majority of the ≈ 1,000 proteins were similar. Yet 18 proteins differed. There were 13 proteins that increase in the resistant state and 5 that decrease. One major change is the appearance of a new protein of ≈ 36,000 daltons in resistant rat thymocytes. This protein is in the same position on the gels as is a protein found previously in corticosteroid resistant P1798 mouse lymphosarcoma cells but not in the corticosteroid-sensitive cells. These results suggest that there exists a set of proteins characteristic of the hormone-resistant state. Thus, similar mechanisms appear to be operative both for the emergence of resistance in tumor cells and for the normal maturation of thymocytes as they become resistant to glucocorticoids during immunological commitment.
(This work was supported by grants from NIH, AM16177, CA25655 and GM 07136.)

785 RECOGNITION OF ORGAN-SPECIFIC ENDOTHELIAL CELL DETERMINANTS BY B AND T LYMPHOCYTES, Eugene C. Butcher, Susan K. Stevens, and Irving L. Weissman, Department of Pathology, Stanford University, Stanford, CA 94305.

The interaction of lymphocytes with specialized endothelial structures can be assayed quantitatively both in vivo and in a simple in vitro system -- it is an excellent model for the study of heterotypic cell-cell recognition. We have previously demonstrated that normal and neoplastic lymphocytes can selectively bind to specialized venular endothelium in frozen sections of murine lymph nodes and Peyer's patches. Some lymphomas have been described that interact almost exclusively with lymph node venules, and others that bind only to Peyer's patch venules. It was proposed that lymphocytes can express surface receptors for organ-specific endothelial cell determinants, and that these complementary lymphocyte-endothelial cell recognition structures mediate the organ specificity of lymphocyte emigration from the blood. We have now extended these studies by comparing the in vivo migration of B and T lymphocytes with their in vitro specificity for binding to lymph node and Peyer's patch high endothelial venules (HEV). It is shown that 1) B cells from any organ source preferentially recognize and bind to HEV in Peyer's patches, whereas T cells adhere preferentially to those in peripheral nodes; 2) these endothelial preferences are paralleled by (and presumably determine) a preferential in vivo migration of B and T cells to Peyer's patches and peripheral nodes, respectively. For instance, under conditions in which an equal number of B cells and T cells localize in (or bind to HEV in) peripheral nodes, there will be 5-6 B cells for every T cell in Peyer's patches. These results are presented in the context of our current model of lymphocyte-endothelial cell recognition.

786 HELPER AND SUPPRESSOR ACTIVITIES FROM UNSTIMULATED T CELLS REGULATE POLYCLONAL B CELL RESPONSIVENESS, Michael G. Goodman and William O. Weigle, Scripps Clinic and Research Foundation, La Jolla, CA 92037

Polyclonal activation of murine splenic B lymphocytes by lipopolysaccharide was found to be subject to regulation by helper and suppressor influences from T lymphocytes. In the normal adult spleen, only helper influences were exercised over polyclonal B cell activation; this influence is a property of Lyt 1^+23^-, slowly sedimenting T cells. Suppressive influence evidently is latent, for it exists at such a low level (or the cells are so few in number) that its effects are difficult to detect. Suppressor T cell function may be evoked by culturing spleen cells at high ratios of T to B cells, by activating splenic T cells with Concanavalin A, or by sonicating unstimulated splenic T cells in order to liberate a suppressive potential which is not expressed by these unstimulated cells when intact. The soluble fraction of resident splenic T cell sonicates exerts both helper and suppressor regulatory influences. The soluble helper activity is derived from Lyt 1^+23^- slowly sedimenting T cells, whereas suppressor activity is generated from a distinct subpopulation of Lyt 1^-23^+ rapidly sedimenting T cells. The thymus contains cells capable only of helping but not of suppressing polyclonal activation of splenic B cells. Helper and suppressor activities contained in splenic T cell sonicates were separated by gel chromatography; the suppressive activity was found to elute with a molecular weight between 68,000 and 84,000 daltons, and the helper activity eluted with a molecular weight between 15,000 and 23,000 daltons. The data indicate that helper and suppressor activities are distinct molecular entities derived from distinct splenic T lymphocyte subpopulations.

787 CYTOPLASMIC INCORPORATION OF ^{3}H-THYMIDINE IN MURINE SPLENOCYTES, Phyllis R. Strauss, Ellen Supple and James Schwalbe, Northeastern University, Boston, MA 02115

In preceding reports we have described the incorporation of thymidine into a cytoplasmic-plasma membrane product by murine nonadherent spleen cells from Concanavalin A stimulated mice. This product is greatly reduced in spleens of unstimulated mice. The same high molecular weight material can be obtained in greater yield by NP40 lysis of splenocytes or thymocytes. After a 2 hr pulse with ^{3}H-thymidine (4-6 μCi/ml, 80-100 nM), the NP40 soluble material (cytoplasmic component) accounts for 40% of all the high molecular incorporation in the cell. Sepharose 2B elution patterns and gel electrophoresis in 2% agarose reveal a heterodisperse size distribution with discreet molecular weights between 10^6 and 2×10^5 daltons. Phenol extraction in the presence of chloroform-isoamyl alcohol completely extracts the material into the aqueous phase. The gel and column chromatography patterns of the phenol-extracted material are the same as the cytoplasmic-plasma membrane product described earlier. Sensitivity of the phenol extracted cytoplasmic component to proteinase K, pronase, RNase, DNase I with and without Mg, exonuclease I and phosphodiesterase with or without bacterial alkaline phosphatase and to nuclease S-1 has been examined. The results differed from the behaviour of nuclear DNA. Since the appearance of the high molecular weight material is not sensitive to ethidium bromide, we conclude that short-term exposure to ^{3}H-thymidine incorporation in stimulated murine splenocytes yields a highly significant cytoplasmic component of non-mitochondrial origin. Supported by NIH CA 24283. PRS is the recipient of RCDA CA 00460, DHEW.

788 ASSOCIATION OF MEMBRANE BOUND IMMUNOGLOBULIN WITH INTRACELLULAR ACTIN. Allen J. Rosenspire and Yong Sung Choi, Sloan-Kettering Institute, Rye, New York 10580 USA.

In lymphocytes, the capping of surface immunoglobulin (as well as other receptors) is well known to be dependent on the cytoskeletal system. Immunofluorescent experiments have shown that actin will localize in the vicinity of capped membrane immunoglobulin (mIg), suggesting a link between actin and mIg. We have begun to investigate the relationship of the specific antigen receptor in lymphocytes (mIg) to the cytoskeleton by looking directly at the biochemical association of actin with mIg in chicken lymphoid cells. Lymphoid cells were biosynthetically labeled with ^{3}H-leucine and lysed in 1% Nonidet P-40 (NP-40). A myosin affinity technique was used to isolate ^{3}H-labeled actin and any cellular proteins associated with actin. We have found that in addition to actin, several different polypeptides (m.w. 63K, 95K, 105K, 200K) as well as mIg can be isolated with the myosin affinity technique, under conditions where mIg has not been specifically cross-linked or capped.

The association of mIg with actin appears to be specific because secreted ^{14}C-Ig added to the cell lysate does not co-isolate with ^{3}H-actin to any appreciable extent. Furthermore, addition of cold actin to the cell lysate competitively inhibits the isolation of mIg (as well as the other four polypeptides) by the myosin affinity technique. On the other hand, serological precipitation of NP-40 lysate with anti-F(ab)$_2$ yields the 63K peptide and actin in addition to Ig. These results suggest that a fraction of cellular actin is linked to mIg along with the 63K peptide while the other actin-associated polypeptides are bound to actin through interactions that are independent of mIg.

789 DETECTION OF Ia ANTIGENS ON THE SURFACE OF THREE SPONTANEOUS MURINE MELANOMA CELL LINES BY MONOCLONAL ANTIBODIES. Timothy V. Updyke and Garth L. Nicolson, Dept. Developmental and Cell Biology, University of California, Irvine, California 92717 and Dept. Tumor Biology, University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

Ia antigens play an important role in recognition, communication and regulation of cellular interactions within the lymphoid system. Ia antigens which map within the mouse H-2 locus, are polymorphic, integral membrane glycoproteins with a dimeric structure (α-chains 32-34K daltons, β-chain 25-28 K daltons in mice). Varying levels of the Ia-like antigens have been detected on human melanoma lines by several groups. We have used three monoclonal antibodies, Ia^K (specificity 2 and specificity 17) (Becton-Dickinson), and Cl. 49-12 antibodiy produced by fusing NS-1 myeloma cells with spleen cells from a rat immunized with murine Bl6-Fl cells (from Dr. K.M. Miner). These antibodies were used to immunoprecipitate NP-40 lysates from the following metabolically labeled (^{35}S-methionine, 1100 Ci/mmole) melanoma lines: KHDD, melanoma (C3H, H-2^K), Kl735P melanoma (C3H, H-2^K), and Bl6-Fl (C57BL/6J, H-2^b). Labeled spleen cells from C3H and C57BL/6 mice were used as Ia-positive controls and EL-4 lymphoma cells (H-2^b) as Ia-negative controls. After electrophoresing the immunoprecipitates on reducing 10% SDS-PAGE gels, the bands characteristic of the Ia dimeric structure were detected by an ultrasensitive protein staining procedure ("silver stain") and by fluorography. These results provide a preliminary basis for studying the role that these molecules play in melanoma-host interactions and metastasis.

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790 *THE UNEXPECTED ISOLATION OF AN Ly1$^+$Qa2$^+$ T CELL CLONE FROM AN IgM$^+$ B CELL LYMPHOMA,* *Carol L. Reinisch, Sidney Farber Cancer Institute and Harvard Medical School, Boston.* *Lymphoid tumors are characterized by cell surface antigens which reflect their ontogeny. For example, we have recently described independent clones isolated from MSV-MuLV induced leukemias which express H-2, brain-associated theta(BAT) and Qa2. In this system, the target cell of viral transformation appeared to be a stem cell. We now present a model of an immature B cell lymphoma from which an Ly1$^+$ Qa2$^+$ T cell clone has been isolated. C57BL/6(B6) mice, when injected at three weeks of age with MSV-MuLV, develop tumors two or more years later which histologically resemble Waldenströms Macroglobulinemia in man. Intraperitoneal inoculation of syngeneic B6 mice with mesenteric lymph node or spleen cell populations from MSV-injected mice results in the development of tumors characterized by 80% IgM$^+$ cells in the spleen, elevated levels of IgM and IgG and a predominance of Ly1$^+$ cells in the T cell compartment of splenic lymphoid cells. Attempts to clone the malignant B cell in vitro led to the unexpected isolation of an Ly1$^+$ Qa2$^+$ T cell. The phenotype of this clone, clone A, has remained stable even after the cell was recloned twice in vitro. Of greater interest was our finding that clone A promotes CFU-E differentiation in vitro and interacts with purified B cells by inducing IgM secretion. Both in vitro functions reflect the immunopathology of the tumor in vivo. Based on these results, we would argue that characterization of tumor cells by cell surface antigens defines the cells involved in neoplasia but does not elucidate the cellular interactions which govern tumor cell growth. Our findings underscore the necessity of identifying, but, more importantly, functionally characterizing populations of cells which interact during tumor cell proliferation.*

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791 STRUCTURE AND BIOSYNTHESIS OF A FAMILY OF LARGE MEMBRANE GLYCOPROTEINS EXPRESSED ON MURINE LYMPHOCYTES. Brian Dunlap, Andrew Watson, Beverly Koller and Fritz H. Bach, University of Minnesota, Minneapolis, Minn. 55455

Murine lymphocytes express a group of antigeneically related, large (170,000-240,000 daltons) membrane glycoproteins (LMPs), seriologically defined by αT200 and Ly5, whose relative distribution varies on different subpopulations. A 200,000 (200K) MW component appears restricted to T cells, while a 220K component is found on B cells. The MW distribution of these LMPs on allogeneically activated T lymphocytes differs from that of precursor resting T cells, while cloned T lymphocytes exhibit a distribution which varies with the type of clone. Peptide mapping of the LMPs from ^{125}I labeled cells demonstrates considerable structural homology between the protein moieties of these molecules. Our laboratory is now investigating (1) if the difference in molecular weight between these proteins is due to differential glycosylation or to undetected protein differences, and (2) the biosynthesis of the different MW forms. To study (1), the sialic acid terminating residues of the LMPs were labeled using the periodate/^{3}H borohydride method. The LMPs were isolated by immunoprecipitation, separated by SDS-PAGE and the resulting bands removed and digested <u>in situ</u> with pronase. The resulting glycopeptides were separated on Sephadex G50 columns. The 187K and 200K proteins have both small and large oligosacchararide moieties. The 200K component has a greater proportion of large oligosaccharides, consistent with the MW differences in the LMPs being due to glycosylation. Similar experiments are in progress using 220K and 240K MW LMPs. (2) Biosynthetic studies indicate rapid synthesis of a 170K MW precursor (not expressed on the cell surface) which is sequentially processed to the 187K and then to the 200K LMP.

792 MONOCLONAL ANTIBODIES WHICH CHARACTERIZE NEW DIFFERENTIATION ANTIGENS IN MOUSE BONE MARROW AND ALTER THE HOMING PATTERNS OF PROGENITOR CELLS. Joan W. Berman and Ross S. Basch, Dept. of Pathology, N.Y.U. Medical Center, New York, N.Y. 10016

Monoclonal antibodies reactive with a variety of hematopoietic progenitor cells have been produced. The hybridomas secreting these antibodies were made by fusing the Balb C myeloma SP-2 with spleen cells of Wistar rats that had been hyperimmunized with mouse (AKR/J) brain. Mouse bone marrow cells were used as the target in a fluorescence screening assay, using an Ortho 50H Cytofluorograf. Only clones which react with some, but not all, bone marrow cells were selected for further study.

Antibodies with specific reactivity for macrophage-granulocyte precursors, peripheral T-cells and immature T-cells have been identified. One of the T-cell reactive antibodies resembles Thy-1 in its activity but another reacts preferentially with mature, thymus-derived cells. Several antibodies react with immature thymocytes and T-cell precursors. These can be distinguished by their reactions with T cell tumors.

The ability of these monoclonal antibodies to interfere with stem cell repopulation of murine bone marrow, spleen and thymus has been investigated. One of these hybridoma products blocks the ability of T-cell precursors in the marrow to home to the thymus of a lethally irradiated mouse. (Supported by Grant CA 24472 of the N.I.H., U.S.P.H.S.)

793 MODULATION OF INTERACTIONS BETWEEN HEMOPOIETIC CELLS AND THE BONE MARROW STROMA. Dov Zipori, Dept. of Cell Biology, The Weizmann Institute of Science, Rehovot,Israel. Stromal cells from mouse bone marrow regulate the growth of both leukemic cell lines and normal mouse hematopoietic progenitors. The ultimate result of the interaction between stromal cells and lymphoblastoid cell lines depended on the differentiated functions expressed by the latter. Similarly, the proliferation of normal myeloid progenitors (CFU-C) was promoted by stromal cells, but their differentiation (i.e. formation of granulocyte/monocyte [G/M] colonies) was restrained. This inhibitory effect could be relieved either by detachment of the CFU-C from the stromal cell or following addition of specific methyl glycosides, suggesting a role for surface glycoprotein or glycolipid receptors. Colony formation in the presence of stromal cells could also be induced by high concentrations of the free sugar D-glucose. It appeared that glucose controls the production of stromal cell derived activity required for colony formation. The target cell for this activity differed in a number of features from CFU-C demonstrable under conventional culture conditions. Stromal cells cultured in medium in which sodium pyruvate replaced glucose, lost their capacity to support colony formation following re-addition of glucose while their ability to inhibit G/M colony formation was retained. Thus, stromal cells from mouse bone marrow concomitantly produce target cell-specific, differentiation-inhibiting and promoting activities. The molecular nature of these activities is presently investigated.

794 RECOGNITION OF HUMAN HISTOCOMPATIBILITY ANTIGENS BY CYTOTOXIC T LYMPHOCYTES, Michael S. Krangel*, William E. Biddison+ and Jack L. Strominger*, *The Biological Laboratories, Harvard University, Cambridge, MA. 02138 and +N.I.H., Bethesda, MD. 20205

HLA-A and -B antigens are cell surface glycoproteins consisting of a membrane embedded 44K heavy chain, in noncovalent association with a 12K light chain, β_2-microglobulin. These molecules display a high degree of serologic polymorphism, which is due to amino acid sequence variability among heavy chains. HLA antigens present on target cells may be recognized as self determinants by HLA restricted, virus immune cytotoxic T lymphocytes (CTLs), or as foreign determinants by alloimmune CTLs. The reactivities of HLA antigens in serologic and in cellular assays usually correlate, suggesting that the site(s) on the molecule recognized by these reagents may be identical. However an individual has been identified who has been typed as HLA-A2 serologically, yet whose peripheral blood lymphocytes (PBLs) cannot be recognized by HLA-A2 specific alloimmune CTLs, and whose influenza infected PBLs cannot be recognized by HLA-A2 restricted, influenza virus immune CTLs. The HLA-A2 heavy chain synthesized by the B cell line derived from this individual (M7) was compared to those synthesized by other HLA-A2 cell lines by isoelectric focusing and was found to differ by a single charge. Double label tryptic peptide analysis utilizing HPLC suggests that this structural difference is confined to a single peptide. Microsequence analysis is being used to identify this peptide and thus localize the structural difference between the HLA-A2 heavy chain synthesized by M7 with that synthesized by the cell line JY, for which extensive sequence information is available. These results suggest that the sites on HLA molecules recognized by alloantibodies and CTLs are not identical, and should help to clarify which regions of the molecule are important for CTL recognition.

795 MECHANISM OF ALLOANTIGEN-INDUCED CML SUPPRESSIVE ACTIVITY, Charles G. Orosz, Benny Leshem and Fritz H. Bach, Immunobiology Research Center, University of Minnesota, Minneapolis, Minnesota 55455

Suppressor T cells are among the functional subsets of lymphocytes thought to recognize and respond to foreign histocompatibility antigens in murine mixed lymphocyte culture. To investigate various aspects of suppressor cell generation and function, we developed an *in vitro* suppressor assay which allows ready detection of alloantigen-induced suppressor cells, but eliminates effects of concommittantly developed cytotoxic activity. In contrast to the *in vitro* generation of primary cytotoxic activity, alloantigen-induced CML suppressive activity develops only after activation with an allogeneic proliferative stimulus, such as H-2 I region encoded antigens; stimulation with cytotoxic target determinants, such as H-2K or H-2D/L region encoded antigens does not result in suppressive activity, if a proliferative stimulus is not present. When alloantigen-induced suppressive populations are fractionated on an 1g velocity sedimentation gradient, only fractions containing lymphoblasts suppress subsequent CML generation; furthermore, the suppressive population continues to proliferate during the 5 day assay for suppressor activity. Additional data suggest that apparent CML suppression results from (a) competition for soluble helper signals normally generated in primary MLCs and (b) dilution of newly generated cytotoxic cell pools by the expanding suppressive population. These observations raise the question of whether or not antigen non-specific, alloantigen-induced suppressor cells exist. Suppressive activity normally attributed to such a cell type may result from the ability of proliferating T cells, with other primary functions, to compete for and respond to soluble CML helper activity generated in mixed lymphocyte cultures.

796 PHOSPHOLIPID METHYLATION AND PHOSPHOLIPASE A_2 ACTIVATION BY HUMAN NATURAL KILLER CELLS, Thomas Hoffman, Phillipe Bougnoux, and Fusao Hirata, NIH, Bethesda, MD 20205

Phospholipid methylation and phospholipase A_2 activity associated with human natural killer (NK) function was studied using specific inhibitors or direct measurement. Pretreatment of peripheral blood effector cells with micromolar concentrations of a methyltransferase inhibitor, 3-deazadenosine (DZA), in the presence of homocysteine thiolactone, reduced cytotoxicity in a dose-dependent fashion. This effect was attributable to inhibition of methylation of lipids and not nucleic acids or proteins. Interaction with NK-susceptible (but not NK-resistant) tumor targets caused measurably increased phospholipid methylation by peripheral blood mononuclear cells. Inhibitors of phospholipase A_2 such as tetracaine, mepacrine, Rosenthal's inhibitor, as well as corticosteroids, impaired NK function by peripheral blood mononuclear cells. Rosenthal's inhibitor was also shown to exert its inhibitory effect on a purified NK cell population obtained by the isolation of large granular lymphocytes (LGL) on Percoll gradients. Peripheral blood mononuclear cells and LGL were also directly shown to display phospholipase A_2 activity, as measured by the decrease in radioactive arachidonate from prelabeled effector cell phospholipids (phosphatidylcholine). Phospholipid methylation apparently plays a part in recognition of susceptible cells by NK effectors. Consequent activation of phospholipase A_2 may also be involved in the mechanisms leading to lysis of the target cells.

797 MAJOR HISTOCOMPATIBILITY COMPLEX-ASSOCIATED NATURAL RESISTANCE AS A MODEL FOR SELF-RECOGNITION IN THE MAMMALIAN LYMPHOID SYSTEM, George A. Carlson, The Jackson Laboratory, Bar Harbor, ME 04609

Nonimmunized *H-2* heterozygous F_1 hybrid mice are able to recognize and reject parental strain cells of hemopoietic origin in apparent violation of the classical laws of transplantation. To study this natural resistance, transplantable leukemia cells were labeled with the thymidine analogue ^{131}I-IdUrd and injected i.v. into mice either *H-2* identical or nonidentical with the tumor. The survival and metastic distribution of the leukemia were determined by whole-body γ counting and by counting various organs after the hosts were killed. Increased tumor cell death was seen when the parental tumor and the F_1 host were not identical at the major histocompatibility complex. The initial seeding of the tumor in various organs was the same in both *H-2* identical and nonidentical host-tumor combinations, but elimination of *H-2* nonidentical cells from the spleen began as soon as 4 hr after injection. The spleen was a favored site for survival and growth of *H-2* identical cells. The leukemic cells survived well in the liver and poorly in the lungs regardless of *H-2* haplotype. A classical immune response was not involved in natural resistance against *H-2* nonidentical cells; neither T cells nor natural antibody could account for the rejection observed. Natural resistance can be further distinguished from an immune response in that specific memory can not be demonstrated. One interpretation of these and similar data is that *H-2* associated natural resistance is related to primitive cell-mediated alloreactivity seen in many invertebrates in, or leading to, the protochordate line. These primitive rejection systems may be based on the failure of the effector cells to find an appropriate self-marker on the allogenic cells.

Neuronal and Non-Neuronal Cell Interactions

798 POTENT MITOGENIC ACTIVITY FOR SCHWANN CELLS IN BRAIN AXOLEMMA, Dan Cassel, James L. Salzer, Patrick M.Wood, Richard P. Bunge and Luis Glaser, Washington University School of Medicine, St. Louis, MO 63110.
Previous studies in this laboratory have shown that membranes derived from dorsal root ganglia (DRG) neurons are mitogneic for cultured Schwann cells. Searching for a source of membranes more suitable for biochemical characterization of the mitogen, we found that bovine brain axolemma membranes, prepared by the method of DeVries et al. (Brain Res. 147:339-352) are highly mitogenic for Schwann cells. Using freshly replated cells, the mitotic index was increased by the addition of axolemma from 0.5-1% to 30-50% during 48 h incubation in the presence of ^{3}H-thymidine. Half maximal effect was obtained at 0.5 µg axolemma per microwell containing 2-4 x 10^{3} cells. Like the DRG neurite mitogen, mitogenic activity of the axolemma was abolished by treatment with trypsin. However, notable differences between the properties of the two preparations were also observed. Unlike the neurite preparation, the mitogenic activity of the axolemma was only partially inactivated by heat treatment (by 60-70%). Furthermore, axolemma membranes failed to stimulate the proliferation of Schwann cells in defined, serum free medium (N-2) whereas neurite membranes were highly mitogenic in this medium. These findings indicate a possible difference between DRG neurites and brain axolemma in either the mitogen itself or in a recognition unit involved in the attachment of the membranes to the cells.

799 SURFACE DIFFERENCES AMONG CELLS OF THE 14 DAY EMBRYONIC CHICK NEURAL RETINA, Joel B. Sheffield, Department of Biology, Temple University, Philadelphia, PA 19122
In order to test the hypothesis that the progressive layering and differentiation of cells during the development of the neural retina is associated with cell surface alterations, we have developed a procedure for the isolation of four different viable cell populations from the 14 day embryonic chick neural retina. Two of these populations contain > 80% each of a morphologically distinct cell type, while the others contain mixtures of other cells. Cells in each of the fractions differ in associative behavior, as judged by cell aggregation and stationary culture assays. The size and shape of aggregates and the cellular configuration in stationary culture were dependent on the particular cell fraction used as a source of cells. When cells of two distinct populations were mixed and allowed to aggregate for periods of up to three days, clear patterns of cell sorting were obtained. Direct evidence of cell surface differences was obtained by freeze fracture analysis and by specific labeling of cell surface glycopolypeptides. Intramembrane particles (IMP) were counted on membrane surface of cells from the different populations. Each population had a characteristic distribution of IMP's which was consistent with the suspected origin of each population in the tissue. Isotopic labeling with glucosamine, enzymatically applied galactose, or borohydride reduction after galactose oxidation, followed by SDS-PAGE and radioautography indicates that although there are many surface glycoproteins in common between the different fractions, there are some materials that may be unique, particularly in the most pure populations.
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800 IMMUNOCHEMICAL STUDIES OF AVIAN SENSORY NEUROGENESIS, Gary Ciment and James Weston, University of Oregon, Eugene, OR 97403

The embryonic neural crest appears to segregate several distinct cell lineages during normal development. These lineages ultimately produce all peripheral nervous system neuronal and glial cells, as well as endocrine, paracrine, skeletal and pigment elements. In order to deduce the order of segregation of various lineages, and the intermediate cell types in each lineage, we have developed a battery of monoclonal antibodies which bind to surface antigens found on subpopulations of neurons within embryonic sensory ganglia. One such antibody (E/C8) is of a class that binds to the cell bodies and fibres of some neurons. Using direct and indirect immunoperoxidase staining methods with this antibody, we have examined cultures, tissue "squashes" and tissue sections containing various crest-derived cells. This antibody mediates staining of a subpopulation of cultured, apparently undifferentiated neural crest cells. In vivo, a subpopulation of cells in nascent (4 day) sensory ganglia and in 7 day sympathetic ganglia stains. The subcellular localization of staining indicates that the antigen is present on the initial segment of the neurite as well as a patch of the adjacent cell body. As development of the sensory ganglia proceeds (days 8 through 13), the amount of neuronal staining increases and becomes more extensive. In contrast, staining of sympathetic neurons becomes more restricted and eventually disappears from the soma altogether. Ultrastructural analysis of the staining patterns on the various crest-derived intermediate cell types should elucidate the role of such cell surface antigens in the normal development of peripheral neurons.

801 MOLECULAR MECHANISM OF AVIAN NEURAL CREST CELLS MIGRATION AND AGGREGATION. Jean-Paul THIERY, Donald NEWGREEN, Annie DELOUVEE and Jean-Loup DUBAND
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The neural crest is a transitory structure which appears early in development at the dorsal border of the closing neural tube of vertebrates. Neural crest cells migrate away from the neural tube and sometimes traverse long distances before reaching well defined sites in the embryo. At this stage cells derived from the neural crest aggregate and differentiate into various tissues including the peripheral nervous system and in the head, the mesectoderm. In order to better understand the mechanisms that govern the migration and aggregation of neural crest cells, we have been studying the role of the cell associated glycoprotein Fibronectin (F) both in vitro and in vivo. FN has been shown to be one of the major components of the extracellular matrix through which neural crest cells migrate. At this early stages of development, FN was synthesized by most embryonic tissues with the remarquable exception of all the trunk and some cranial neural crest cells. Since the first cranial crest cells to leave the neural tube were able to synthesize and construct FN matrices ; this matrix might serve as a substrate for the cells which follow. In the trunk, neural crest cells migrated through a FN meshwork already deposited by surrounding tissues. Therefore, we propose that the inability of trunk neural crest cells to synthesize FN may render them more sensitive to this substrate. At their site of arrest, the rapid decrease in cell to substrate adhesivity and an increase in cell to cell adhesiveness is correlated with a local disappearance of FN and a concomittant appearance of the cell adhesion molecule (CAM).

802 MONOCLONAL ANTIBODIES AGAINST TORPEDO SYNAPTOSOMES. P.D. Kushner and L.F. Reichardt.
In order to gain a more thorough molecular understanding of synapses, their architecture, function and development, we have generated a library of monoclonal antibodies against the cholinergic synaptosome of the ray electric organ. Torpedo synaptosomes were prepared by a technique designed to ensure as pure a presynaptic fraction as possible (Deutch and Kelly, in prep.). Mice were injected over a two month period. Spleen cells were then fused with NS1 cell line. Media conditioned by hybridoma cells were assayed for antibody production using solid phase radioimmuno assay. We have isolated, cloned and stored 125 cell lines which produce culture media positive for antibodies against the original synaptosome. In collaboration with R. Kelly,tissue specificity tests were conducted on the antisera and results indicate that most of the sera are nervous system specific, with less than 5% cross reacting strongly with fish liver. There appear to be several different classes classes of sera, as revealed both by plate assay (Kelly) and immunocytochemistry. Many of sera cross react with the mammalian nervous system. Our current efforts are focused on those candidates showing nervous system specificity, cholinergic specificity, and neuronal terminal specificity. We anticipate using these antibodies to dissect synaptic and neuronal components.

803 MONOCLONAL ANTIBODY M2: CEREBELLAR CELL SURFACE SPECIFICITY, Carl Lagenaur and Melitta Schachner, University of Heidelberg, D6900, FRG.
A monoclonal antibody designated M2 arose from the fusion of mouse myeloma with splenocytes from a rat that was immunized with early postnatal mouse cerebellar particulate fraction. The expression of M2 was examined by indirect immunofluorescence on living monolayer cultures of cerebellar cells derived from 1-10 day old mice. During the first three days of culture M2 antigen appeared to be confined to the cell surface of two classes of glial cells: 1. GFAP containing astrocytes and 2. a population of largely immature oligodendrocytes. This latter population is characterized by the expression of O4 antigen, present on more immature oligodendrocytes (Sommer and Schachner, Schachner et al., Dev. Biol., in press) but is generally negative for galactocerebroside and O1 antigen, present on more mature oligodendrocytes (Sommer and Schachner, ibid). After four days in culture, M2 appeared on tetanus toxin binding neurons in addition to the above described glial cells. It appears that in early cultures, M2 may prove useful in examining the molecular relationships between astrocytes and immature oligodendrocytes and in defining steps in oligodendrocyte differentiation. The delayed expression of M2 on neuronal cell surfaces will be a further aspect of developmental studies

804 SYNTHESIS AND EXPRESSION OF IMMUNE RESPONSE ASSOCIATED (Ia) ANTIGENS BY BRAIN CELLS. Jenny P.Y. Ting, Leslie P. Weiner and Jeffrey A. Frelinger. University of Southern California, Los Angeles, CA 90033

This report provides the first immunohistochemical and biochemical evidence that murine brain cells synthesize and express Ia (Immune response associated) antigens. We used an extremely sensitive and specific, triple-layer immunoperoxidase method to reveal Ia^+ cells in frozen sections of mouse brains. Positive staining was observed only with appropriate anti-Ia sera and not inappropriate sera. Hence, B10.S brain sections stained with anti-I^S but not with anti-I^k serum. Specific staining was observed with two additional mouse strains, B10.K and AQR. Specificity of the reaction was confirmed by absorption studies which ruled out anti-Tla region reactivity. Finally, detectable Ia^+ cells constitute a very low percentage (<1%) of the total brain cell population. Biochemical evidence for the the active synthesis of Ia antigens by brain cells was obtained with ^{3}H-leucine/tyrosine labelled lysates and immunoprecipitation using monoclonal antibody. Only two characteristic molecular species of 25,000 and 35,000 daltons were isolated by gel electrophoresis. Their migration patterns were indistinguishable from Ia antigens obtained from spleen cells. Anti-μ serum did not precipitate any product, thus eliminating contaminating B lymphocytes as a source of Ia antigens. The accumulative evidence indicates a central role for Ia bearing cells from both lymphoid and nonlymphoid organs in T-B lymphocyte, and lymphocyte-accessory cell interactions which are required for antibody production and antigen-specific, T-cell responses. The importance and implications of Ia-bearing brain cells will be discussed in light of these findings.

805 BRAIN LIGATIN: A MEMBRANE-BOUND LECTIN THAT BINDS ACETYLCHOLINESTERASE. M. GASTON, R.B. MARCHASE AND E.R. JAKOI, DUKE UNIVERSITY, DURHAM, N.C. 27710.

Ligatin, a lectin that recognizes phosphorylated sugars, has been demonstrated in mammalian tissues to bind specific hydrolases to cell surfaces. Ligatin exists as a filament that can be released from membranes still complexed with its bound hydrolases by treatment of membrane preparations with $CaCl_2$ and/or pH 8.0. The ligatin-hydrolase complexes subsequently can be dissociated with EGTA, resulting in a concurrent depolymerization of the ligatin filament. From isolated membranes of cerebrum and cerebellum, this procedure also produces a cosolubilization of membrane-bound acetylcholinesterase (AChE, E.C.3.1.1.7.). Binding of the cosolubilized AChE to ligatin can be demonstrated in vitro by affinity chromatography using the immobilized lectin. Ligatin-hydrolase complexes have been shown to be dissociated by low concentrations of specific phosphorylated sugars (mannose 6 phosphate and glucose 1 phosphate). These sugars are also effective in eluting bound AchE from ligatin affinity columns. Analysis of labeled glycitols produced by tritiated borohydride reduction confirms the presence of phosphorylated sugars on the ligatin-cosolubilized material from brain.

806 C1 ANTIGEN IN NORMAL AND MUTANT MOUSE CEREBELLUM, Ilse Sommer and Melitta Schachner, University of Heidelberg, D6900, FRG.

A monoclonal antibody designated C1 was obtained from the fusion of mouse myeloma with splenocytes from a mouse immunized with bovine corpus callosum. When examined by indirect immunofluorescence on mouse brain sections, C1 was found to stain cerebellar Bergmann glia, retinal Müller cells and ependyma in the adult mouse neuroectoderm. C1 antigen is detectable in radially oriented structures in the telencephalic anlage at embryonic day 10, the earliest stage tested so far. In the first postnatal week, C1 antigen is found in astrocytes of the presumptive white matter, of the internal granular layer and in Bergmann glial fibers in the molecular layer of the cerebellum of normal and mutant mice (reeler, staggerer, weaver). In normal mice, C1 antigen expression subsides in the white matter and internal granular layer during the second postnatal week, but persists in Bergmann glia and ependymal cells throughout adulthood. In cerebella of mutant mice, however Bergmann glia also lose the ability to express C1 antigen during the second postnatal week. Bergmann glia of the ventromedial part of the cerebellum (which are known to mature earlier) lose the antigen first. C1 expression in Müller cells and ependymal cells is not affected by these mutations.

807 CHOLINERGIC SYNAPTIC VESICLES CONTAIN AN ANTIGENIC PROTEOGLYCAN, Steven S. Carlson, Ken Kassenbrock, and Regis B. Kelly, University of California, San Francisco, CA 94143

Antibodies raised to highly purified synaptic vesicles from fish electric organ cross-react with a cytoplasmic element of the mammalian nerve terminal, presumably the synaptic vesicle. The cross-reacting antigens are not present on the outside of a resting frog neuromuscular junction, but become exposed to the outside when the terminal undergoes exocytosis of synaptic vesicle contents (von Wedel, Carlson & Kelly (1981) P.N.A.S. in press). The antigens which are exposed on the outside of the active nerve terminal are presumably those which are inside the synaptic vesicle. We have demonstrated that electric organ synaptic vesicles contain large amounts of a material with the following characteristics. It does not enter acrylamide gels. It contains protein, carbohydrate and sulfate. It elutes from gel filtration columns containing SDS as a large molecular weight material between 335 and 70 kilodaltons. It binds in NP40 detergent to wheat germ agglutinin-Sepharose 4B beads and is selectively eluted by N-acetylglucosamine. We believe the material to be a sulfated proteoglycan. The proteoglycan appears to be inside the synaptic vesicle since intact vesicles fail to bind to wheat germ agglutinin beads whereas vesicles lysed by sonication bind very effectively. The antibodies used to detect exocytosis bind to synaptic vesicles and to no other major component of the electric organ. These specific antibodies immunoprecipitate the proteoglycan. The antigens which appear during exocytosis could therefore be the proteoglycan determinants, but this has not been shown directly. The proteoglycan may play a role in packaging the acetylcholine or ATP into the synaptic vesicle. Whatever its function, the observation that synaptic vesicle proteoglycans have unique determinants suggests that their structure is functionally important.

808 IDENTIFICATION OF MITOGENIC SIGNALS FOR CULTURED GLIAL CELLS. Rebecca M. Pruss, Perry F. Bartlett, and Robert P. Lisak. University College London, London WC1E 6BT England.

Glial cells in primary cultures of the central and peripheral nervous system divide slowly. It is necessary to define mitogens for these cells in order to propogate them for biochemical studies. We have examined a number of polypeptide factors which might provide mitogenic signals in vivo. These include a bovine pituitary extract, central myelin basic protein, fibroblast growth factor from both brain and pituitary, insulin, and epidermal growth factor. We have combined autoradiography with indirect immunofluorescence using neural cell type specific antisera to identify those cells which respond to a mitogen by incorporating ^{3}H-thymidine. In addition we have assayed the amount of ^{125}IUdR incorporated into mitogen treated purified rat Schwann cell cultures or rat corpus callosum cultures which are enriched in astrocytes. These methods showed that astrocytes and Schwann cells were able to respond to the pituitary extract while only astrocytes were able to respond to fibroblast growth factor. None of the other factors tested stimulated division in either Schwann cells or astrocytes.

Oligodendrocytes are rarely seen to divide in tissue culture. During our studies we observed large cells floating in suspension in long-term primary cultures of the rat central nervous system. These cells were tentatively identified as oligodendrocytes by the presence of cell-surface galactocerebroside and the absence of other neural cell markers. When these cells were harvested and grown on a surface of irradiated 3T3 cells they were positively identified as oligodendrocytes by their elaborate branched processes, and by the presence of intracellular basic protein, as well as surface galactocerebroside. Remarkable, nearly 50% of such cells were labeled by ^{3}H-thymidine during a 48 hour pulse even in the absence of mitogen.

809 ANALYSIS OF MEMBRANE DIFFERENTIATION DURING SKELETAL MYOGENESIS USING MONOCLONAL ANTIBODIES, Stephen J. Kaufman and Heide U. Lee, University of Illinois, Urbana, IL 61801

During the differentiation of skeletal muscle, mononucleate myoblasts proliferate, then cease replication, interact with one another and spontaneously fuse to form multinucleate, electrically excitable fibers. During this process of differentiation, changes in the membranes of myogenic cells presumably evolve and reflect the missions of these cells at their respective stages of development. Hybridoma cells producing monoclonal antibodies reactive with the myogenic clone of L_8E_{63} rat myoblasts were established. These antibodies are being used to study the biochemical differentiation of the myoblast membrane. Hybridomas were obtained by fusion of SP2/0 Ag-14 cells with spleen cells from BALB/C mice immunized with L_8E_{63} myoblasts and myotubes. These antibodies have been used to define stage-specific changes in the myoblast membrane by radioimmunoassay and immunofluorescence. The presence and distributions of the antigenic determinants on L_8E_{63} myoblasts and myotubes, on developmentally defective nonfusing fu-1 myoblasts and on primary cultures of rat and chick embryo myoblasts were compared (Lee and Kaufman, Devel. Biol., 81: in press). Some determinants present on myoblasts were absent or greatly reduced on L_8E_{63} myotubes. Identical results were obtained with primary rat myoblasts. Chick myoblasts have some but not all determinants recognized by these antibodies. Several determinants are almost totally absent on fu-1 cells; the distribution of some of these determinants on fu-1 cells are also different than on L_8E_{63} myoblasts. Studies are in progress to characterize these antigens and to define the qualitative, quantitative, and topographical changes on the myoblast membrane that are germane to the differentiation of these cells.

810 A CATALOG OF MOUSE BRAIN SYNAPTIC ANTIGENS, Larry D. McClain and Ronald T. Acton, University of Alabama, Birmingham, AL 35294

A variety of evidence suggests that membrane macromolecules play an important role in mediating cell-cell interaction phenomena in general and establishment of synaptic connections in particular. As a prerequisite to direct testing of this hypothesis, a unique subset of these cell surface components has been identified and selected for study. This subset is comprised of proteins and glycoproteins expressed on mouse cerebellar synaptosomes which are susceptible to surface labeling and which elicit a serological immune response. Synaptosomes were isolated from mouse cerebella of varying age by isopycnic density gradient centrifugation on linear metrizamide-sucrose gradients. The synaptosomes were lightly fixed using 0.5% paraformaldehyde and administered to rabbits according to conventional immunization protocols. The resulting antisera were heat inactivated and exhaustively absorbed with mouse liver membranes. Aliquots of crude cerebellar synaptosomal fractions prepared from mice of corresponding ages were surface labeled with ^{125}I using Iodogen or Bolton-Hunter reagent and with ^{3}H-borohydride using galactose oxidase or periodate. Following the labeling procedure intact synaptosomes were isolated by gradient centrifugation as before and solubilized using 0.5% NP-40, 0.01% SDS in 0.01 M Tris pH8. The radiolabeled antigens were immune precipitated using the rabbit antisera and gluteraldehyde fixed Staphlococcus aureus, resolved by SDS polyacrylamide gel electrophoresis, and detected by autoradiography. Synaptoplasm, microsomal, and plasma membrane fractions were similarly analyzed. Molecules which were detected exclusively on synaptosomes and which appeared to be expressed transiently in the course of development were cataloged numerically with the prefix MAS (mouse antigen synaptic).

811 DOES LAMINAR POSITION DETERMINE NEURONAL INTERCONNECTIONS IN THE NEOCORTEX? Alan L. Pearlman, Vance Lemmon and Peter Simmons, Washington University School of Medicine, St. Louis, MO 63110

The reeler (rl) mutation in the mouse produces a severe abnormality in the position of neurons in the laminated cortical structures of the brain, including neocortex, hippocampus, and cerebellum. We have analyzed the physiological properties of the neurons that project from the visual cortex to the superior colliculus, and found that the neuronal interconnections that determine the function of these cells are normal. We have also shown that thalamo-cortical and cortico-cortical connections are established properly in the visual cortical regions despite the abnormality in cell position. Thus the factors that determine cell-to-cell connections during development are not strongly dependent on the relative position of neurons within the cortex.

812 APPEARANCE OF SYNAPTIC MOLECULES IN ANEURAL MYOTUBE CULTURES. Nibaldo Inestrosa, Laura Silberstein and Zach Hall, Univ. of Calif., San Francisco, CA 94143.

As a first step in examining the synthesis of specialized synaptic components by nerve and muscle cells in culture, we have determined the appearance and cellular localization of 16S acetylcholinesterase (AChE) as well as synaptic basal lamina antigens in a mouse skeletal muscle cell line, C2. The endplate form of AChE (16S) appears in C2 myoblast cultures after fusion to form myotubes. Within two days after myotube formation, this form represents 20 to 30% of the total AChE activity present. The 16S AChE is at the cell surface, as evidenced by protection from DFP inactivation by the membrane-impermeable inhibitor, BW284c51, and solubilization by collagenase digestion. The 16S enzyme is extracted by 1 M NaCl, but not by Triton X-100; thus, it is probably associated with the extracellular matrix. Histochemical localization of AChE reveals a patchy distribution of the surface enzyme. Collagenase digestion, which removes only the 16S form, abolishes this staining pattern, indicating that the patches consist of the 16S enzyme.

We looked for other components of the synaptic basal lamina that might be present in myotube cultures by using antisera that are specific for three distinct components of the endplate basal lamina (Sanes and Hall, 1979). Each of these antigens appear in aneural C2 myotube cultures as localized areas on the cell surface, often coincident with acetylcholine receptor clusters. Work in progress concerns the relationship between the synaptic antigen clusters and those of esterase and receptor. We conclude from these experiments that muscle cells, in the absence of nerves, can synthesize synapse-specific components of the basal lamina.

We are grateful to D. Jaffe for making available to us the C2 cell line.

813 MONOCLONAL ANTIBODIES TO NEURONAL CELL-SURFACE GLYCOPROTEINS, Christo Goridis and Michel HIRN, Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille cedex 9, France.

Specific antibodies may provide the resolving power necessary to dissect cell surface components involved in cellular recognition phenomena. The possibility of obtaining antibodies against individual surface proteins has been greatly facilitated by the introduction of the myeloma hybrid technique for production of monoclonal antibodies. For the selection of antibodies reacting with the brain cell surface, we developed an indirect radioactive binding assay on live monolayers of mouse cerebellar cells. Using this test in conjunction with binding assays on liver membranes and thymocytes to discard antibodies against constituents present on all tissues, we obtained two monoclonal antibodies which recognize glycoproteins present at the neuronal cell surface. The monoclonal antibody H28.123.16, when tested by immunofluorescence on primary cultures of young postnatal mouse cerebellum stains only neuronal cell types. It immunoprecipitates a triplet of surface-iodinatable polypeptides of 180,000, 140,000 and 120,000 daltons, which bind to and are specifically eluted from lentil lectin columns. The monoclonal antibody H30.107.2 immunoprecipitates a glycoprotein of 48,000 daltons from lysates of surface-iodinated cerebellar cells. Electron microscopy of peroxidase-labelled sections of mouse cerebellum show the antigen to be present on granular and Purkinje cells and on a subpopulation of astrocytes. The antigens recognized by both antibodies are present in neonatal cerebellum but persist throughout adulthood.

814 NEURONAL DIFFERENTIATION IN CULTURES OF WEAVER (WV) MUTANT MOUSE CEREBELLUM. M. Willinger, D. Margolis and R.L. Sidman. Harvard Medical School, Boston, MA 02115.

The assembly of a neuronal network requires interactions between the cell surface and the environment to direct nerve cell movement and axonal growth. The development of granule cell neurons in dissociated cell cultures of normal and weaver (wv) mutant mouse cerebellum serves as a model system to study these events. The weaver mutation in the homozygous state results in the early death of the cerebellar granule cell neuron in situ. It has been postulated that the neuronal death is due to a block in axonal outgrowth and neuron migration caused by defective interactions with Bergmann glia. Neuron survival, neurite initiation and neurite elongation was quantitated in cultures of normal (+/+), heterozygous weaver (wv/+) and homozygous weaver (wv/wv) cerebellar cells to determine the dynamics of mutant phenotype expression. The majority of wv/wv neuronal precursors (cells labeled with a pulse of ^{3}H-Tdr six hours prior to sacrifice) die over the first three days in vitro. Neurite initiation and elongation of surviving neurons is severely impaired. Greater than 50% of these cells do not have fibers at day 3, as opposed to only 10-15% in +/+ cultures. Cultures of wv/+ neurons have values intermediate between +/+ and wv/wv for the number of cells of a given fiber length ($0, \leq 20, > 20\ \mu$) over the first six days. These results suggest that the rate of neurite outgrowth is affected by gene dosage. To determine whether the abnormal characteristics of weaver neurons are a result of defective cell interactions during differentiation, normal or weaver neurons are being co-cultured with astroglia of the opposite genotype. Preliminary results suggest that neuron survival and neurite outgrowth is altered by the astroglial environment.

815 MONOCLONAL ANTIBODIES DETECT CELL SURFACE CHANGES DURING MYOGENESIS, Barbara K. Grove and Frank E. Stockdale, Stanford University, Stanford, CA 94305.

The differentiation of skeletal muscle is characterized by the recognition, alignment, and subsequent fusion of myoblast cell surfaces to form large, multinucleated myotubes. Monoclonal antibodies were used to investigate antigenic changes in the cell surface membrane specific for the various stages of myogenesis. Chick embryonic skeletal muscle cells were cultured in vitro to the desired stage of differentiation and then injected into BALB/c mice. Spleen cells from the immunized mice were hybridized with NS-1 non-secreting mouse myeloma cells. Hybrid cell clones were selected in HAT medium and screened, using an indirect radioimmunoassay (RIA), for the production of monoclonal antibodies to myogenic cell surfaces. Target cells for the RIA included three stages of myogenesis (myoblasts, alignment stage myoblasts, myotubes) and chick lung cells as a control for polymorphic antigens. Sixteen clones were obtained which produced antibodies specific for myogenic cells. Initially, the supernatants were screened in gluteraldehyde fixed cells. To ascertain whether the antibodies actually recognize surface membrane components, RIA's were also performed on unfixed cells. Of the 14 immunoglobulins tested, all bound to unfixed cells although overall binding was lower than to fixed cells. For each antibody, the determinants were most prevalent on the myoblast stage of differentiation with binding ranging up to 14-fold over background. Thus, we have successfully used hybridomas to obtain monoclonal antibodies to skeletal muscle cell surface membranes and have identified at least 4 determinants which are more prevalent on myoblast cell surfaces than on myogenic cell surfaces at other stages of differentiation. Supported by NSF grant #PCM7904963.

816 INTERCELLULAR RECOGNITION: QUANTITATION OF INITIAL BINDING EVENTS, David R. McClay, Gary M. Wessel, and Richard B. Marchase, Duke University, Durham, N.C. 27706.
A cell binding assay has been developed that supports the hypothesis that intercellular adhesion can be subdivided into two separable phenomena, an initial recognition event and a subsequent stabilization. In addition, the assay provides a quantitative measure of intercellular binding strengths. Radioactive single cells are brought into contact with cell monolayers at $4^{o}C$. The monolayers are inverted and a centrifugal force is then applied tending to dislodge the probe cells from the monolayers. By varying the speed of centrifugation, the force maintaining associations between embryonic chick neural retina cells was determined to be on the order of 10^{-5} dynes. Specificities of single embryonic cells for monolayers of homologous tissue type and for topographic position within a tissue were detected with this assay and corresponded to those observed in more traditional assays at 37^{o}. Also observed were two time and temperature dependent stabilization processes in which the force required for dislodgment increased. One of the stabilization processes was sensitive to dinitrophenol and inactive at $4^{o}C$, while the second was still active in metabolically quiescent cells. The metabolism-dependent process resulted in interactions at least 13 times as strong as the initial binding. The metabolism-independent process resulted in about a two-fold increase in binding strength and had a temperature dependence similar to that of membrane diffusional phenomena.

817 CONTROL OF DIFFERENTIATION OF BC3-H1 CELLS, Kendra L. Caldwell, Robert Munson, and L. Glaser, Department of Biological Chemistry, Washington University School of Medicine, St. Louis, MO 63110.
BC3-H1 cells, a non-fusing smooth muscle-like cell line (Schubert et al., J. Cell Biol., 61, 398 (1974)), show dramatic increases in the level of creatine kinase and acetylcholine receptor (determined by the binding of α-bungarotoxin) when growth is arrested at confluency or when sparse cells are transferred to medium containing low levels of serum. The induction of these proteins is not due to the depletion of serum components, since medium recovered from such cells will not induce the synthesis of creatine kinase when added to sparse BC3-H1 cells. Induction of creatine kinase by lowering the serum concentration from 20% to 1% in sparse cultures is a function of cell density and appears not to be due to the depletion of serum components. Induction of muscle specific proteins in these cultures occurs over a period of several days and binding of ^{125}I-α-bungarotoxin to these cells followed by autoradiography show that this slow induction is a property of most cells in the culture, i.e. that at early times all cells are partially induced. Addition of high serum or a high molecular weight fraction of fetal calf serum to a partially induced culture results in an arrest of induction, in the latter case without initiation of growth. We hypothesize that induction of muscle specific proteins in BC3-H1 cells reflects a balance between inducing signals generated by cell to cell contact and repressing signals derived from the presence of serum components. The induction in these cells of muscle protein appears to a reversible process.
[Supported by grants from NSF PCM 77-15972 and NIH GM 18405 and GM 07067.]

818 INTERACTIONS BETWEEN MEMBRANES AS MEDIATED BY SPECIFIC LIGAND-RECEPTOR BINDING. Leaf Huang and Stephen R. Grant, Bruce P. Babbitt, Dept. of Biochemistry, University of Tennessee, Knoxville, TN 37916
We are attempting to develop a model system with which the binding between membranes as mediated by specific ligand-receptor association can be studied. Our approach is to covalently couple a water soluble ligand with a hydrophobic anchor such that the resulting amphipathic ligand can be incorporated into liposome membranes. The snake toxin,α-bungarotoxin (αBGT), has been chosen for this purpose because of its high affinity to the nicotinic acetylcholine receptor. αBGT was coupled to palmitic acid by using the N-hydroxysuccinimide ester of palmic acid. The major coupling product, mono-palmitoyl-αBGT (PBGT), was purified by the hydrophobic column chromatography. PBGT showed specific binding to the detergent solublized acetylcholine receptor with a K_D of $2x10^{-7}$M, as compared to $1.16x10^{-8}$M for ^{125}I-αBGT. PBGT can be incorporated into unilamellar liposomes of about 1000 A in diameter by a detergent-column method. PBGT-liposomes showed specific binding to the microsac membrane vesicles isolated from the electroplaxes of Torpedo Californica but not to the red blood cell ghosts. Furthermore, there was a threshold number of PBGT per liposome above which the binding of PBGT-vesicles with microsacs increased dramatically. This apparent positive cooperativity in binding is explained in terms of the multivalent binding theory. We conclude that this system provides a simple model to study the membrane-membrane interactions mediated by specific ligand-receptor binding. Supported by NIH (CA24553) and MDA.

819 NERVE-PARENCHYMAL CELL INTERACTIONS IN DEVELOPMENT, Judith Medoff, Saint Louis University, St. Louis, MO 63103

The growth of embryonic parenchymal organs is stimulated by coculturing dissociated cells from chick embryo kidney and liver with single cell suspensions of dorsal root and sympathetic ganglia cells on a rotary shaker. Mixed reaggregates of nerve and parenchymal cells synthesize 1½-3 times more DNA, RNA and protein than each tissue cultured separately or mixtures of parenchymal and non-neural cells. When the cell surface is modified with either concanavalin A (50µg/ml) or wheat germ agglutinin (10µg/ml), the enhancement of macromolecular synthesis in mixed reaggregates of nerve and parenchymal cells is abolished when compared to each tissue cultured separately in the presence of lectins. These results suggest that specific surface components and cell contacts may be required for growth stimulation. Other populations of cells with different neuronal properties were combined with parenchymal cells in an effort to identify the growth promoting factor provided by nerve. Dissociated cells from both a mouse neuroblastoma, which secretes choline acetylase and tyrosine hydroxylase, and a rat glial tumor clone, which secretes S-100 protein, stimulate macromolecular synthesis when cocultured with dissociated chick embryo parenchymal cells. These results lend support to the notion that cell-to-cell interactions between neuronal and non-neuronal cells may play an important role in certain phases of organogenesis.

820 Cultured Embryonic Chick Neurons and Glia Have Similar Surface Components, Richard Akeson and Edward Risden, Children's Hospital Research Foundation, Cincinnati, OH 45229.

Selective expression of cell surface components is one facet of cell phenotype. Embryonic chick telencephalon cells plated on poly-l-lysine coated tissue culture dishes in medium containing 10% horse serum yield cultures containing greater than 95% neurons. Cells from the same dissociate plated on collagen layers in medium containing 10% fetal calf serum yield cultures of mixed round neuronal and flat (presumptive glial) morphology. After one passage greater than 95% of the cells have a flat morphology. The cell surface components of neuronal and flat cells have been examined by selective labeling with pyridoxal phosphate and NaB^3H_4. Cultures of chick skin fibroblasts from the same embryos were labeled for comparison. The method labels approximately 20 polypeptides in each cell type separable on SDS-polyacrylamide gels. A minority of these are common to all three cell types indicating major differences in surface components accessible to labeling between fibroblasts and cells of neural origin. However only two of the major components found on neurons are not found on flat cells. Two components of flat cells were not found on neurons indicating substantial similarity of surface components (by this criterion) of neural cells from the same brain region regardless of phenotype. Metabolic labeling studies confirm that most of the labeled components are cellular polypeptides and not adsorbed serum proteins. These studies establish baseline data for comparison of surface components of cells of individual neuronal and glial phenotypes.

821 BASAL LAMINA OF THE NEUROMUSCULAR JUNCTION: FUNCTIONAL AND IMMUNOHISTOCHEMICAL ANALYSES. Joshua R. Sanes, Washington Univ. Medical School, St. Louis, MO, 63110.

A basal lamina (BL) ensheaths each skeletal muscle fiber, and extends between nerve and muscle at the neuromuscular junction where it comprises a substantial fraction of the synaptic cleft material at this synapse. The synaptic portion of the BL plays important roles in the formation, function, and maintenance of the neuromuscular junction: It contains acetylcholinesterase, which terminates transmitter action; it mediates adhesion of nerve to muscle; and it guides differentiation of both pre- and postsynaptic membranes when neuromuscular junctions regenerate following damage to nerve and muscle (Nature 271: 172,1978;JCB 78:176,1978;JCB 82:412,1979). These results motivate a molecular analysis of synaptic and extrasynaptic BL. Because muscle BL has not been isolated and because synaptic sites occupy only 0.1% of the muscle fiber surface, immunohistochemical methods have been used. Synaptic and extrasynaptic portions of the BL are indistinguishable by conventional EM, and immunohistochemistry shows that some proteins--e.g., laminin--are present in both regions. On the other hand, some molecules are concentrated in or excluded from synaptic BL. Antibodies recognize at least 3 determinants present in synaptic but not extrasynaptic BL. One synapse-specific antigen is acetylcholinesterase; the others remain unidentified (JCB 83:357,1979). Two other antigens are concentrated in extrasynaptic BL; antibodies to them stain synaptic sites lightly if at all. Thus, synaptic and extrasynaptic domains share some features, but both are specialized. We now want to know when the antigens appear during development, which cells make them, and whether any of them participate in functions ascribed to BL.

822 MONOCLONAL ANTIBODIES DIRECTED AGAINST RAT PANCREAS MEMBRANES, Gary A. Van Nest and William J. Rutter, University of California, San Francisco, California 94143

A series of 10 monoclonal antibodies has been generated against a plasma membrane enriched fraction isolated from rat pancreas. Six of the 10 antibodies react with the pancreas cell surface in immunofluorescence tests using intact pancreas cells. Three of these antibodies are specific to the pancreas while the other three react with membrane components common to the pancreas and several other organs including liver, gut, and lung. The four antibodies that do not react with the cell surface recognize internal components that associate with membranes upon fractionation. Two of these appear to react with lipase, a third with a 71,000 MW protein that may be associated with zymogen granules, and the fourth with an unidentified protein. Immunofluorescence tests with intact embryonic pancreas cells indicate that the non-specific surface antigens are present on the pancreas at the earliest time tested (14 days of gestation). In contrast, the pancreas specific antigens arise between 16 and 18 days of gestation, the same developmental period when exponential increases in the synthesis of digestive (pro)enzymes and insulin are detected. The molecular weights of several of the antigens have been identified using SDS gel electrophoresis combined with various procedures including immunoprecipitation and transfer to nitrocellulose for antibody treatment. Antibodies are also being generated against isolated intact pancreas cells and zymogen granule membranes.

Ligands to Extracellular Matrix

823 AN ESTABLISHED KIDNEY EPITHELIAL CELL LINE WITH POTENTIAL FOR TISSUE REGENERATION. Milton H. Saier, Jr., University of California, San Diego, La Jolla, CA 92093.
The MDCK dog kidney epithelial cell line, established in culture more than 20 years ago without exposure to oncogens, forms epithelial sheets in vitro which pump NaCl from mucosal to serosal surface. Cells grown in tissue culture have the morphological and enzymatic properties of distal tubular cells (M.J. Rindler, et al., J. Cell Biol. 81, 635 (1979)). When MDCK cells were injected into baby nude mice, continuous nodule growth was observed until adulthood was attained. Subsequently nodule size remained constant without detectable regression for up to 6 months. Histological studies revealed the presence of 2 cell types: normal mouse fibroblasts which comprised 90% of the solid nodule mass, and MDCK cells, which formed epithelial sheets lining internal fluid filled sacs. The tissue was morpholgically indistinguishable from normal dog kidney. Electron microscopic analysis showed that 1) all microvilli (on the mucosal surface) faced the lumen of the sacs; 2) adjacent MDCK cells were joined by tight junctions; and 3) the serosal surfaces of the epithelial sheets were characterized by smooth membranes which were in contact with a basement membrane and collagen fibers. In vitro cell aggregation studies showed that these cells exhibited adhesive properties which allowed them to form pure and mixed cell aggregates. The results lead to the conclusion that the MDCK cells retain 1) the capacity for both homotypic and and heterotypic adhesion; 2) regional differentiation of the cell surface membranes, and 3) potential for the regeneration of kidney tubular-like structures in vitro.

824 FIBRONECTIN INVOLVEMENT IN EMBRYONIC BASAL LAMINA RECONSTITUTION, A.G. Brownell, H.C. Slavkin, C.C. Bessem & P. Bringas, Jr., Univ. So. Cal., Los Angeles, CA 90007
Basement membranes in a number of developing organs contain fibronectin (Linder et al. (1975), J. Exp. Med.:142, 41-49). As basal lamina are purportedly synthesized by epithelial cells, we designed experiments to determine what role mesenchyme-derived fibronectin (FN) has on basal lamina organization. Using the developing mouse tooth organ, we dissociated the two tissues by trypsinization to remove the basement membrane and then cultured isolated mesenchyme or epithelial tissues under various conditions. A serum-free culture system is permissive for differentiation. Basal lamina reconstitution was assayed by transmission electron microscopy. In serum-free medium alone, isolated enamel organ epithelia did not reconstitute basal lamina between 2-24 hrs. Addition of 10% fetal calf serum, authentic mouse plasma FN, or mesenchyme-conditioned medium resulted in basal lamina reconstitution. Isolated tissues were incubated for 6 hrs. in serum-free medium. After homogenization and dialysis, proteins found within tissues and medium were chromatographed on gelatin-Sepharose 4B to analyze for FN. Three fractions were collected: unbound proteins, 4.5 M urea eluant and 8 M urea eluant. Each fraction was examined by SDS-gel electrophoresis. FN specifically binds to gelatin-Sepharose 4B and is eluted with 4.5 M or 8 M urea. Using the criteria of binding to gelatin and the subunit molecular weight on SDS-gels, we found: 1) enamel organ epithelia did not make FN; 2) dental papilla mesenchyme made FN which is released into the culture medium; and 3) epithelia cultured in mesenchyme-conditioned medium accumulates FN. These experiments support the hypothesis that mesenchyme-derived FN participates in the supramolecular assembly of epithelial basal lamina reconstitution. Supported by USPHS Research Grant DE-02848 and Training Grant DE-03569.

825 MONOCLONAL ANTIBODIES DIRECTED AGAINST FRAGMENTS OF FIBRONECTIN, Kenneth D. Noonan[1], Norine E. Noonan[2] and Kenneth Yamada[3], [1]Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL 32610, [2]Department of Metabolism, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610 and [3]Laboratory of Molecular Biology, National Cancer Institute, Bethesda, MD 20205.
Proteolytic fragments of chick cell fibronectin (CFN) were injected into Balb/c mice and the spleens of the immunized mice were used to prepare hybridomas by fusion with cells from the P3x63Ag8 myeloma cell line. We have developed monoclonal antibodies against the collagen binding fragment (40K) of fibronectin, the heparin sulfate binding fragment (50K) of fibronectin and the intact fibronectin molecule. Monoclonal antibodies derived from splenocytes isolated from animals immunized with either the 40K or 50K fragments of CFN recognized the appropriate fragment of CFN. Monoclonal antibodies derived from immunizations with the intact CFN molecule recognized the 40K and 50K fragments as well as the cell binding region of the CFN molecule. Most of the monoclonal antibodies bound to chick embryo fibroblasts describe the same filamentous arrangement of CFN which was detected with a monospecific anti-CFN serum. Approximately 75% of the monoclonal antibodies studied to date show species specificity in that they bind to chick FN but not FN derived from mammalian sources. The most interesting monoclonal antibody available to us is one which, while binding very efficiently to cell FN, binds only very weakly to plasma fibronectin (CIg). This antibody is being used to describe that region of the CFN molecule which is probably not conserved in the CIg molecule. (This work was supported by NIH grant GM-23227 to KDN.)

826 EFFECT OF EXOGENOUS GANGLIOSIDES ON FIBRONECTIN RETENTION AND ORGANIZATION BY GANGLIOSIDE-DEFICIENT CELLS, Peter H. Fishman*, David R. Critchley*, Joel Moss†, Betty E. Hom† and Kenneth Yamada§, *NINCDS, †NHLBI and §NCI, NIH, Bethesda, MD 20205
Previous studies demonstrated that complex gangliosides inhibited the fibronectin-mediated attachment of Chinese hamster ovary cells to collagen-coated substrates (Kleinman, Martin & Fishman, *Proc. Natl. Acad. Sci. USA 76*: 3367-3371, 1979). We have examined the fibronectin-ganglioside interaction further by using a line of transformed mouse fibroblasts (NCTC 2071 cells) that have been adapted to grow in chemically defined medium and are deficient in gangliosides. To detect the presence and organization of fibronectin, we first exposed the cells to antifibronectin antibodies followed by a fluorescent-labeled second antibody. NCTC 2071 cells cultured in the absence of serum or added gangliosides produced fibronectin, which attached to the substrate but was not retained by the cells. When the cells were cultured for 18 h in medium containing increasing amounts of a mixture of bovine brain gangliosides, the cells now retained fibronectin. An effect was observed with as little as 2 μg of gangliosides per ml. More dramatic effects were observed with individual gangliosides; of those tested, the disialoganglioside G_{D1a} was more effective than the monosialogangliosides G_{M1}, G_{M2} and G_{M3}. The effect of ganglioside treatment on fibronectin retention appeared to be reversible. When the ganglioside-treated cells were cultured in the absence of gangliosides, the cells lost their fibronectin. Finally, following exposure of the cells to gangliosides, there appeared to be increased fibrillar organization in the regions of cell to substrate contact.

827 ENDOTHELIAL CELL SULFATED GLYCOPROTEINS AND EXTRACELLULAR MATRIX, Aaron Heifetz and Alice Johnson. The University of Texas Health Science Center, Dallas, Texas 75235.
Human vascular endothelial cells incorporate $^{35}SO_4$ into glycosaminoglycans and N-linked oligosaccharide side chains of glycoproteins. These sulfated glycoproteins were separated from sulfated proteoglycans by DEAE-cellulose chromatography and the partially purified glycoproteins yield several major ^{35}S-components upon SDS-PAGE. The pronase derived ^{35}S-oligosaccharides of these glycoproteins are unique from the ^{35}S-glycosaminoglycans when analyzed by gel filtration and ion exchange chromatography, cetyl pyridinium chloride precipitation, and a variety of enzymatic and chemical treatments. The majority of all cell-layer associated ^{35}S-glycoconjugates are membrane bound and are differentially extracted by salt, urea, and detergents. When confluent endothelial cells are mechanically removed from a plastic substratum, 25% of the ^{35}S-glycoconjugates remain attached to the plastic. This substratum attached material contained 16% of the cellular [^{35}S]heparan sulfate, 24% of the cellular [^{35}S]chondroitin and dermatan sulfates, and 60% of the cellular [^{35}S]glycoproteins. Thus the synthesis of sulfated glycoproteins may be associated with endothelial cell basal matrix. Human pulmonary endothelial cells grown on a hydrated collagen matrix showed only small changes in the relative proportion of glycoconjugates synthesized. However, endothetial cells grown on a matrix derived from vascular smooth muscle cells displayed a preferential 4-fold increase in [^{35}S]heparan sulfate synthesis. Thus, endothelial cell interactions with extracellular materials may alter the surface properties of these cells exposed to the vascular flow. (Supported by grants from the NHBLI #1-RO1-HL25937 and the American Heart Association/Texas Affiliate)

828 CONTROL OF COLLAGEN BIOSYNTHESIS BY LYMPHOCYTE FIBROBLAST INTERACTION IN NORMALS AND PROGRESSIVE SYSTEMIC SCLEROSIS, Randall S. Krakauer, Martha Cathcart and Maureen Mayes, Cleveland Clinic Foundation, Cleveland, Ohio 44106

Since antigen stimulated lymphocytes have been shown to stimulate collagen synthesis, we investigated the ability of alloantigen stimulated human peripheral blood lymphocytes to regulate collagen synthesis by human embryonic lung fibroblasts in vitro. For the collagen synthesis assay, human embryonic lung fibroblasts were cultured with C14-proline in the presence of supernatant from alloantigen stimulated lymphocytes. After culture, protein is precipitated and hydrolyzed. Proline and hydroxyproline are separated on paper chromatography and C14 at each point is determined on a scintillation counter. With this technique it is possible to measure total protein synthesis and total collagen (hydroxyproline containing protein) synthesis. In this manner, supernatants of alloantigen stimulated lymphocytes increase collagen synthesis three-fold over control. Alloantigen stimulated lymphocytes from patients with progressive systemic sclerosis, an autoimmune disease involving excessive collagen accumulation, cause collagen synthesis three times that of normals with a significant increase in total protein synthesis and an increase in the fraction of total protein being represented by collagen.

We therefore believe that collagen synthesis is in some ways under immunologic control through lymphocyte fibroblast interaction and that in progressive systemic sclerosis the immunologic abnormality responsible for autoimmunity is also responsible for increased lymphocyte stimulation of collagen synthesis by fibroblasts. Thus we believe the immunologic abnormality is responsible for both the autoimmune and connective tissue aspects of this disease.

829 GLUCOSE CAUSES THE DISSOCIATION OF FIBRONECTIN-GELATIN COMPLEXES, T. Kent Gartner and Patricia P. Agin, Dept. of Biology, Memphis State University, Memphis, TN 38152

We previously reported that plasma fibronectin specifically bound D-glucose and that this bound glucose caused the dissociation of plasma fibronectin-gelatin complexes by a non-competitive mechanism (Gartner and Agin, Biochem. Biophys. Res. Comm. In press). Here we report that the binding of cell surface fibronectin to gelatin is controlled by a similar, if not identical, mechanism. That is, cell surface fibronectin bound D-glucose and this bound glucose caused the dissociation and/or inhibition of formation of cell surface fibronectin-gelatin complexes. Glucose and gelatin did not compete for the same binding sites on cell surface fibronectin. D-galactose also affected cell surface fibronectin-gelatin interactions, but with much less efficacy than D-glucose. In contrast, 2-deoxy-D-glucose, D-fructose, L-glucose, sucrose, maltose and lactose were without effect. Since plasma fibronectin and cell surface fibronectin bound D-glucose with the same affinity ($Kd = 2.3 \times 10^{-7}M$) and because the fibronectin-gelatin interactions of both types of fibronectins were affected by the same sugars, it appears as if both types of fibronectins have similar, if not identical, saccharide binding sites. Thus, these fibronectins seem to share a common allosteric control mechanism for modulating their interactions with gelatin.

830 SEPARATION OF RAWII7 LYMPHOSARCOMA TUMOR CELL METASTATIC SUBPOPULATIONS BY COUNTERCURRENT DISTRIBUTION IN DEXTRAN/PEG PHASES. Karen M. Miner, Harry Walter, and Garth L. Nicolson, Dept. Molecular Biology and Biochemistry, University of California, Irvine, Calif. 92717, Veterans' Administration Medical Center, Long Beach, Calif. 90822 and Dept. Tumor Biology, University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030.

Metastatic variant cells can be separated from tumor cell populations by countercurrent distribution in two polymer aqueous phase systems of dextran/PEG. The lymphosarcoma parental line RAWII7-P and subline (RAWII7-HI0) selected sequentially ten times for liver colonization were grown in suspension and subjected to countercurrent distribution. Both RAWII7-P and RAWII7-HI0 lines exhibited broad distribution profiles, but the distribution of cells from the more metastatic subline RAWII7-HI0 was shifted to the right with a higher mean partition coefficient. Cells from various partition fractions of the RAWII7-P profile were placed into tissue culture, allowed to double overnight, and then assayed in vivo by injection of 5×10^3 cells i.v. After 20 days in vivo RAWII7-P cells obtained from fractions of higher partitioning coefficients were more metastatic to liver. Analysis of surface proteins on cells from the separated fractions by SDS-PAGE and staining with ^{125}I-Con A indicated that the cell surfaces of the more metastatic cell subpopulations obtained from RAWII7-P differ from the original parental line. These results suggest that cell subpopulations of differing malignancy can be separated by countercurrent distribution.

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831 THE EFFECT OF TUNICAMYCIN ON IN VITRO AND IN VIVO BEHAVIOUR OF METASTATIC B16 MELANOMA CELLS. Tatsuro Irimura, Robert Gonzalez and Garth L. Nicolson, Department of Tumor Biology, University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030.

The effect of tunicamycin on experimental metastasis of B16-F1 and -F10 melanoma was examined by injecting cells intravenously into C57/BL6 mice. Cells treated previously with tunicamycin (0.5 g/ml) produced fewer lung tumor nodules did than untreated cells. In the presence of this drug, the synthesis of complex saccharide was inhibited about 90%, whereas protein synthesis remained at 50% of control levels. Cell surface proteins of B16 cells were identified by lactoperoxidase-catalyzed iodination-SDS-PAGE and showed little change after drug treatment, while surface carbohydrate chains detected by binding of Ricinus communis agglutinin were dramatically reduced. The drug also induced morphological alterations and reduction of growth rates. These changes were reversible, but approximately 12 hours were required for complete recovery. The results show that the effects of tunicamycin on melanoma cell blood-borne implantation was specific but was accompanied by several secondary effects. The results also suggested that the fate of injected tumor cells was determined prior to recovery of the usual cell surface properties. Adhesion of drug pretreated cells onto endothelial monolayers was slower and less extensive compared to untreated cells which may explain the reduced capacity to arrest and form pulmonary tumors.

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832 RETINAL LIGATIN: A CELL-SURFACE BASEPLATE FOR PHOSPHOGLYCOPROTEINS, R.B. Marchase, L.A. Koro, C.M. Kelly, and E.R. Jakoi, Duke Univ. Med. Ctr., Durham, N.C. 27710.

Ligatin is a filamentous, baseplate protein that binds and localizes other glycoproteins to the external cell-surface. In embryonic chick neural retina, affinity chromatography and chemical analyses suggest that ligatin binds glycoproteins bearing high mannose-type oligosaccharides containing penultimate mannose residues linked by phosphodiester bonds to terminal saccharides. Proteins co-isolated with ligatin and radiolabeled with ^{32}P are retained by an affinity column containing covalently bound retinal ligatin and resist elution by all monosaccharides, most phosphorylated sugars, and phosphate. Elution is achieved by millimolar levels of mannose-6-phosphate or glucose-1-phosphate. Treatment with endo-β-N-acetylglucosaminidase H, an enzyme that specifically removes oligosaccharides of the high mannose-type, prevents the proteins from binding to the column. The oligosaccharides prepared from these ligatin-associated proteins by Endo H contain ^{32}P and display a net negative charge but are unaffected by alkaline phosphatase unless pretreated under conditions sufficient for hydrolysis of phosphodiester-linked moieties, thereby exposing underlying phosphate. Enzymatic analyses detect mannose-6-phosphate as a constituent of the hydrolyzed oligosaccharide. Monomeric retinal ligatin at 20ug/ml has been found to inhibit the reassociation of single retinal cells. Ligatin's inhibitory effect is suggested to be mediated through binding to retinal cell surfaces in that preincubation of dissociated retinal cells with ligatin inhibited the cells' adhesiveness and removed the inhibitory activity from the media. Models for the involvement in intercellular adhesion of ligatin and the phosphoglycoproteins it binds will be presented.

833 INTERACTIONS OF COLLAGEN AND FIBRONECTIN IN EMBRYONIC CHICK HEART FIBROBLAST CULTURES, Charles D. Little and Wen-Tien Chen, University of California, San Diego, CA 92093

In order to study the interactions of collagen and fibronectin during formation of the extracellular matrix (ECM), we have examined their codistribution by double immunofluorescence (DImF) microscopy on whole mounts and frozen sections of embryonic chick heart fibroblasts (CHF). We prepared guinea pig antibodies specific to chick skin collagen (CSC) and rabbit antibodies against chicken fibronectin (CFN). In one group of studies, cells were immunolabeled at time points from 15 min to 3 days after trypsinization and replating. The results showed that in early spreading cells (15 min to 3 hr) there was intense intracellular labeling of both CSC and CFN; while beneath the cells non-superimposible patterns of extracellular CSC and CFN were seen proximal to the margins of spreading lamellipodia. Once cultures reached confluence (1-3 days) intense extracellular CFN labeling was observed but no labeling for extracellular collagen. Mild trypsinization of 3-day CHF cell layers produced a decrease in CFN labeling, and an appearance of intense extracellular CSC labeling. These data, suggest that an extracellular interaction between CSC and CFN exists at this time, which blocks the CSC binding sites for antibody, and that mild trypsinization exposes those sites. At later time points when "piled-up" cultures were examined by DImF, areas close to the cell were observed to show superimposition of CSC and CFN images; however, in areas away from the cells only CSC labeling was observed (see Vaheri, et al., PNAS 75:4944, 1978). These observations suggest that as a function of time in culture much of the secreted procollagen does not diffuse into the medium but is processed and assembled into ECM fibrils and that the relative amount of CSC to CFN in the ECM increases. Sup.by GM15971. CDL, Fellow, CA Affil. Am. Heart As., W-TC, Fellow Muscular Dyst. As.

834 STRUCTURAL ANALYSIS OF HAMSTER FIBRONECTIN WITH MONOCLONAL ANTIBODIES, Blair T. Atherton, Deena Taylor and Richard O. Hynes, Massachusetts Institute of Technology, Cambridge, MA 02139

We have developed a panel of monoclonal antibodies to map the location of a variety of fragments of fibronectin which contain different functional sites of the molecules. Antibody specificities were characterized by their reactivity with proteolytic and chemical cleavage products of fibronectin.

Antibodies have been obtained with binding sites scattered along most of the length of the fibronectin molecule. For example, we have monoclonal antibodies directed against (1) a 200 kd fragment which has gelatin, heparin and cell binding activities; (2) a chymotryptic fragment of 130kd with the same activities as (1) except it lacks the gelatin-binding site; (3) a 40kd tryptic fragment which contains a free sulfhydryl group and does not bind to gelatin.

Two antibodies are of particular interest because they have determinants in the same small region of NIL.8 cellular fibronectin, but one of them does not cross-react with hamster plasma fibronectin while the other does. These antibodies will be useful in defining the differences between cellular and plasma fibronectin.

835 FIBRONECTIN PROMOTES PHAGOCYTOSIS OF LATEX PARTICLES BY A MURINE MACROPHAGE LINE, Livingston Van de Water III, Scott Schroeder, E. Bryan Crenshaw and Richard O. Hynes, Massachusetts Institute of Technology, Cambridge, MA 02139

Studies on the clearance of various particles from the circulation have indicated that fibronectin may promote the recognition, binding and endocytosis of particles by phagocytes of the reticuloendothelial system. Using gelatin-coated latex particles, we have investigated in detail their uptake by a murine macrophage cell line ($P388D_1$).

Fibronectin promotes the association of gelatin-coated beads with these cells both in suspension and in monolayer cultures. This association is dependent upon fibronectin concentration, temperature, time of incubation and the presence of heparin as a cofactor. Proof that the beads were actually endocytosed was obtained by electron microscopy which showed beads internalized in membrane-bound vesicles, and by immunofluorescence analysis, using antibodies to fibronectin to stain external beads. Endocytosis of these particles by this cell line was inhibited by $F)ab')_2$ anti-fibronectin. These cells were also examined for the synthesis of fibronectin or the presence of fibronectin on their surfaces. Immunofluorescence analysis indicates that these cells do not have detectable levels of fibronectin. Furthermore, electrophoresis of metabolically labelled cells showed that $P388D_1$ cells do not produce fibronectin. Certain other macrophages (RAW 309 Cr.1, mouse peritoneal exudate cells) do synthesize fibronectin. This cell line should facilitate the study of the internalization of fibronectin with the cell surface and its involvement in endocytosis.

836 DUAL ADHESION MECHANISMS IN EMBRYONIC CHICK TISSUE, William A. Thomas, John L. Magnani, Susan M.Lobel, Arnold Breitbart, James Thomson, Malcolm S.Steinberg Princeton NJ 08544

Immunological and kinetic analyses show that embryonic (7-day) chick neural retina cells possess two distinct adhesion mechanisms, one Ca^{++}-dependent (CD), the other Ca^{++}-independent (CI). Rapid removal of the CI mechanism occurs with low [trypsin] with or without Ca^{++}, whereas removal of the CD mechanism occurs only with high [trypsin] but is blocked by Ca^{++}. Subsequent restoration of either mechanism begins after a 30-40 minute lag and requires several hours for completion. Adhesive activity of the CI mechanism is Ca^{++}-independent and temperature-insensitive while that of the CD mechanism is neither. CD adhesion is detected in our assay, however, only after treatment by trypsin and Ca^{++} together and is inactivated by subsequent removal of Ca^{++}. The two adhesion mechanisms do not cross-react to form adhesive bonds; in coaggregation experiments using FITC-labelled and unlabelled cell preparations, mixed aggregates are formed only when both coaggregating cell preparations share at least one adhesion mechanism. Similar kinetic, immunological and cross-adhesion experiments have been performed on other embryonic chick tissues as well. All CNS tissues investigated (cerebrum, optic tectum, cerebellum, and spinal cord) possess dual CI and CD mechanisms similar if not identical to those of retina. The non-neural tissues investigated (heart, liver, and limb bud) also possess distinct CD mechanisms but generally lack well-developed CI mechanisms. Each class, where present, reacts with the corresponding retinal mechanism. These results demonstrate regulation of cellular adhesive capacities through the presence or absence of distinct adhesion mechanisms and suggest that such capacities might be modulated physiologically through local changes in the concentrations of Ca^{++} and protease.

837 THE SYNTHESIS OF A PROTEASE INHIBITOR α_1-ANTICHYMOTRYPSIN BY HUMAN BREAST EPITHELIAL CELLS. Zoltan A. Tokes, Sandra J. Gendler, Curtis B. Winters and Gerald B. Dermer, Department of Biochemistry and the Comprehensive Cancer Center, University of Southern California, School of Medicine, Los Angeles, CA 90033.

Glycoproteins (gps) which may be important in maintaining the three-dimensional glandular structures were studied using human breast epithelium. Synthesis of gps was monitored by incorporation of 14 C-glucosamine and 14 C-leucine by breast surgical specimens which were maintained in short term organ culture and by established breast cancer cell lines. Five major families of labeled gps were identified from culture supernatants using two-dimensional gel electrophoresis.

Immuno-diffusion and - electrophoresis with culture supernatants and rabbit anti-$\alpha_1$antichymotrypsin produced characteristic radioactive arcs. 3-5% of the total labeled gps was precipitated with this antiserum. Two-D gel electrophoresis of the immunoprecipitate revealed a family of gps with an approximate mol. wt. of 65K and an apparent pI of 4.8-5.6 on urea gels. Similar characteristics were obtained with α_1-antichymotrypsin (Achy) purified from normal serum. Immunoperoxidase staining established the breast epithelial cells as the site of origin. The amounts of Achy that were observed varied among the different specimens of breast adenocarcinomas.

The synthesis of this protease inhibitor represents a new molecular parameter for breast epithelial cells. The designation of this gp as a protease inhibitor may be serendipitous to its in vivo function. However, Achy may provide a protective shield against neutral proteases released by leukocytes and may inhibit lymphocyte-mediated cytotoxicity. Achy synthesis may increase as a result of desmosome sparcity and protect normally unexposed cell surfaces. Alternatively, fewer desmosomes could result from decreased protease-inhibitor synthesis which could lead to the breakdown of junctional integrity.

838 ROLE OF FIBRONECTIN AND OTHER EXTRACELLULAR MATRIX MOLECULES IN HUMAN MUSCLE CELL DIFFERENTIATION, Hannah Friedman Elson, John E. Bergmann and Edward C. Elson, Univ. of California, San Diego, La Jolla, CA 92093.

It was previously shown that primary cultures of human fetal skeletal muscle cells could undergo growth and differentiation in a defined medium containing fibronectin (FN) and other components (Elson et al. (1980) J. Cell Biol. 87: 268a). Using this model system, we wished to further examine the role of FN and other matrix components in myogenesis. In the defined medium, FN was found to stimulate the attachment of cells to Petri dishes coated with collagen. Other extracellular molecules, such as some glycosaminoglycans, gelatin and fetuin, were either inferior to FN or inhibited the attachment of cells to collagen. None of these effects could be observed in an enriched serum-containing medium.

A small fraction of the cells was able to attach and, in rare instances, differentiate in the absence of added FN. This may have been due to endogenously synthesized FN. Indeed, we detected FN synthesized by these cultures using immunofluorescence microscopy, and followed its accumulation and distribution during development. In early cultures, fibrils of FN were most prominant at cell-cell contacts. Fibroblasts developed cage-like structures of fibrils around them, with fibrils extending to the dish surface. Myoblasts, on the other hand, had FN along cell processes and at points of contact with other cells, but little on their free cell surfaces. In more mature cultures, extensive networks of fibrils were found on the substratum and around fibroblasts, with almost none on myotubes. Thus, muscle cells appear to have less FN on their surfaces than fibroblasts at all stages, and bind it mainly at their points of contact to the substratum and to other cells.

839 REVERSE TRANSFORMATION OF CHO CELLS BY DIBUTYRYL CYCLIC AMP IS ACCOMPANIED BY AN INCREASE IN CONTRACTILE STRENGTH AND A FOCALIZATION OF CELL-SUBSTRATUM ADHESIONS

W. Mark Leader, David Stopak and Albert K. Harris, Department of Zoology, and
Arthur H. Lockwood, Department of Anatomy, Laboratories for Cell Biology, University of North Carolina, Chapel Hill, NC 27514

Many transformed fibroblasts regain features of normal cell growth and morphology when treated with agents that elevate intracellular cyclic AMP. Using elastic silicone rubber substrata (Science 208, 177, 1980) and interference reflection microscopy we have studied the effects of dibutyryl cAMP induced reverse transformation on the contractility and cell-substratum adhesions of CHO cells. We find that the cells' morphological conversion to a more spread and elongate shape is accompanied by strongly increased wrinkling of the rubber substrata and by replacement of broad close adhesions with many smaller focal tight adhesive contacts (adhesion plaques) to glass substrata. These changes in strength of contractility and morphology of adhesive contacts develop gradually over the six hours following addition of dibutyryl cAMP. Both these effects are fully reversible. There is an especially rapid decrease in contractility in the first hour following removal of dibutyryl cAMP. It has been shown that cAMP induces the organization of actomyosin containing microfilament bundles in CHO cells (Exp. Cell Res. 129, 31, 1980). Microfilament bundles attach to the plasma membrane at adhesion plaques and are likely responsible for contractile force. Therefore, an attractive interpretation of our observations is that a common cAMP dependent mechanism, possibly phosphorylation of specific proteins of adhesion plaques or microfilament bundles, co-ordinately regulates cell morphology, contractility and adhesion.

840 PURIFICATION OF EMBRYONIC CHICK LIVER ADHESION MOLECULES (LAM) FROM CHICK LIVER MEMBRANES, Edward J. McGuire, Malcolm Pitts, Sharon Grady and Larry Nielsen, National Jewish Hospital, Denver, CO 80206

Anti-aggregation antisera specific for embryonic chick liver cells have been used to develop an assay for the detection of embryonic chick liver adhesion molecules (LAM). The neutralization of the anti-aggregation immune sera and subsequent semi-quantitative assessment of the remaining activity is the basis for this assay. Embryonic chick liver membrane proteins have been solubilized using a number of detergents and the LAM activity has been partially purified using sucrose gradient sedimentation, isoelectric focusing, gel filtration, affinity chromatography and preparative PAGE procedures. The isolated LAM appears to be an intrinsic, trypsin resistant, membrane glycoprotein of approximately 65,000 daltons. The 65 K preparation of LAM is an effective immunogen in generating an aggregation blocking xeno-antiserum specific for embryonic chick liver cells. Purification of LAM to homogeneity has been hampered by an apparent lability by self association or loss by adsorption of the more purified LAM antigen(s).

841 FACTORS RESPONSIBLE FOR THE ADHESION OF CARTILAGE CELLS TO COLLAGEN, Gary Balian, Depts. of Orthopedics & Biochemistry, University of Virginia, Charlottesville, Virginia 22908

Cells from seventeen day old chick sterna were isolated by sequential treatment with hyaluronidase, and a collagenase/trypsin mixture. Attachment of these cells to collagen-coated petri dishes is mediated by a factor or factors obtained from various sources. The attachment activity was observed in fetal bovine serum, extracts of young sternal cartilage, conditioned medium from chick cartilage cells grown in culture and synovial fluid. Fractionation of synovial fluid by ammonium sulfate precipitation showed that the activity responsible for adhesion of chondrocytes to collagen precipitated between 10-15% ammonium sulfate (w/v). Although fibronectin mediates attachment of fibroblasts to type I collagen, the addition of human plasma fibronectin to chondrocytes did not facilitate attachment of cartilage cells to petri dishes coated with type II collagen. Chondrocyte adhesion to various collagen types isolated from lathyritic chick cartilage was studied. A mixture of type II and type V collagen produced greater attachment than type II by itself; type I was least effective in supporting attachment of chondrocytes. Preparations containing certain chromatographically purified collagen chains derived from type V were not as effective as intact type V collagen in supporting cell attachment. The ability of cells to bind to various collagen types through the intermediary of factors present in various biological fluids is being investigated in order to understand the role of collagen types and of adhesion factors in the chondrocyte environment.

842 CELLULAR FIBRONECTIN DEFECTIVE IN CELL ADHESION AND COLLAGEN BINDING, Albert J.T. Millis and Srinivasan Chandrasekhar, Biology Department, SUNY-Albany, N.Y. 12222.

Analysis of fibronectins synthesized by human fibroblasts, at different times during serial subcultivation, reveals biological differences. Fibronectin isolated from late passage cells is defective in promoting the adhesion of either late or early passage cells while the converse is true for fibronectin isolated from early passage cells. We establish via SDS-PAGE that the fibronectins have identical molecular weights. Further, we can show that both are present on the cell surface using indirect immunofluorescence. Fibronectin has been shown, in other laboratories, to be composed of discrete domains that interact with the cell surface, with components of the extracellular matrix, and act as a substrate for a transglutaminase. Our evidence indicates that the difference in the human fibroblast fibronectins is not in cell binding but rather in collagen binding. We used an enzyme linked immunoadsorbant assay to examine fibronectin-collagen interactions. Both direct and indirect assays support the conclusion that fibronectin from late passage cells has a 6-8 fold reduction in its affinity for type I collagen and for gelatin, but unaltered affinity for the other collagens tested. Our results establish that a single strain of non-transformed cells can synthesize distinctive types of fibronectin. Further, because it is the only isolated fibronectin shown to be defective in a critical step in the process of cell adhesion it will be useful in probing mechanisms of cellular interactions. (Supported by NIH-Ag00697.)

843 IDENTIFICATION OF INTEGRAL SURFACE MEMBRANE GLYCOPROTEINS INVOLVED IN CELLULAR ADHESION
C. H. Damsky, K. A. Knudsen and C. A. Buck, Wistar Institute, Phila. Pa. 19104

Several lines of evidence from work done in this laboratory and from work reported by others suggest the existence of a class of evolutionarily conserved glycoproteins of approximate 140,000 M_r involved in the maintenance and/or control of cellular adhesion in a wide variety of cell types. We have reported previously the production of antisera against surface membranes of hamster fibroblasts (Anti-M) and against material shed into serum-free tissue culture medium by mouse mammary tumor epithelial cells (Anti-SFM I). These antisera have been shown to cause a reversible disruption of cell substratum adhesion when applied to living cultures of their respective cell types (Wylie et al, 1979, J.C.B.80:385; Damsky et al 1981, J.C.B., in press). The development of an antibody-blocking assay and a biochemical fractionation scheme has led in each system to the isolation of a highly restricted group of glycoproteins of about 140,000 M_r which are able to block the effects of their respective antisera. (Damsky et al, 1981; J.C.B., in press; Knudsen et al, ms submitted). We have now tested the ability of the two antisera to induce rounding and detachment of other cell types. Cross reactivity in antiserum effects can be demonstrated although a higher concentration of antiserum is required to detach heterologous cell types. A similar cross-reactivity exists in the ability of purified active glycoproteins from each of the two cell types to block antiserum induced detachment of heterologous cell types. Reports from other laboratories (Thiery et al, 1977, J.B.C.252:6841, Takeichi 1978, J.C.B.75:464) have also implicated glycoproteins of about 140,000 M_r in cellular adhesion. These observations all support the hypothesis that there exists a class of adhesion-related surface glycoproteins of 140,000 M_r. ACS53A,CA 19144,27909.

844 EXPRESSION OF ADHESION-RELATED MEMBRANE COMPONENTS IN ADHERENT VS NONADHERENT MELANOMA CELLS.
K. A. Knudsen, C. H. Damsky and C. A. Buck, Wistar Institute, Phila. Pa. 19104

The existence of integral membrane components interacting with both intracellular and extracellular components involved in cell-substratum adhesion has been postulated. This laboratory has used a combination of immunological and biochemical methods to implicate a restricted group of membrane glycoproteins of about 140,000 M_r as being involved in the maintenance and/or control of hamster fibroblast-substratum adhesion (Wylie et al, J.C.B.80:485; Knudsen et al, ms sub). In order to support further the relevance of these glycoproteins to adhesion, we are employing a versatile cell system consisting of an adherent hamster melanoma cell line, CS 473, and its nonadherent varient, RPMI 3460, which can be induced to adhere to the substratum by BudR. Using this system we have shown that partially purified fractions of nonionic detergent extracts of the CS 473 cells will inhibit the rounding and detachment of cells induced by an antiserum, anti-M, made against hamster fibroblast membranes. In contrast, 10-fold more protein from similar fractions purified from the RPMI 3460 cells is required to block the morphologic changes caused by anti-M, suggesting that the nonadherent cells express either altered antigens or a reduced amount of the adhesion-related antigens. This contention is supported further by the observation that 40 times more nonadherent cells are required to absorb rounding activity from anti-M serum when compared to the adherent cells. Finally, when induced to adhere, RPMI 3460 cells appear to express the adhesion-related antigens. Supported by ACS 53A, CA 19144, CA 27909, CA 10815, CA 06517, CB 63984.

845 IN VITRO FORMATION OF CAPILLARY-LIKE STRUCTURES BY SKIN ENDOTHELIAL CELLS.
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Endothelial cells isolated from the capillary-rich region of human newborn foreskin grow in vitro as monolayers of closely apposed cuboidal cells in the plane of the growth surface and show little or no ability to orient outside this plane. Dramatic changes in the behavior and orientation of microvessel endothelial cells takes place when the luminal surface of an endothelial colony is exposed to collagen and fibrin.

Endothelial cells were isolated from the microvessels of newborn foreskin dermis and grown on the surface of an acid soluble collagen gel in Eagle's Minimal Essential Medium supplemented with human serum (10%), cholera enterotoxin (1×10^{-9}M) and isobutyl methylxanthine (3.3×10^{-5}M). After 2-3 cell divisions, the luminal surface of the endothelial cell colonies were exposed to a second layer of a gel of acid soluble collagen containing fibrinogen and thrombin.

Collagen and fibrin initiate a sequence of cellular changes that result in the formation of a central cavitation, reorientation of nuclei toward the center of the cavitation and junctions at the tips of adjacent cells. Formation of these structures occurs with collagen alone but is markedly stimulated by the presence of fibrin. Complete lysis usually occurs 5 days after stimulation.

Reformation of capillary-like structures under conditions frequently associated with de novo vessel growth after wounding suggests that this model may be appropriate in further studies of the factors that regulate blood vessel growth in vivo.

846 ROLE OF HYALURONIC ACID IN DECREASED ADHESIVE PROPERTIES OF TRANSFORMED CELLS, Susan P. Hawkes and John Blenis, Michigan Molecular Institute, Midland, MI 48640 and Michigan State University, East Lansing, MI 48824.

Development of the transformed phenotype has been studied in chicken embryo fibroblasts infected with a temperature sensitive mutant of Rous Sarcoma Virus. Cells exhibit a decrease in adhesive properties early in the process of transformation as determined by the ability of EGTA (5mM) to detach them from tissue culture dishes. One proposed mechanism of detachment involves the destabilization of the adhesive glycoprotein, fibronectin, by hyaluronic acid. Deposition of hyaluronic acid at the cell surface is considerably enhanced within five hours of the onset of transformation, followed by the continued release of the polymer into the culture medium. However, in this system loss of surface fibronectin does not occur until at least 24 hours after temperature shift. Hyaluronidase treatment of transformed cells at times prior to loss of fibronectin results in a significant restoration of adhesive properties. These results provide further evidence for the role of hyaluronic acid in cell detachment and particularly in the loss of adhesion which occurs as the result of transformation. Furthermore it can be suggested that the significant decrease in surface fibronectin may be a consequence of decreased adhesion rather than the primary cause. Supported by NIH Grant CA-27283-01 and the Elsa Pardee Foundation.

847 ANALYSIS OF SUBSTRATE ATTACHED MATERIAL DURING THE DEVELOPMENT OF THE TRANSFORMED PHENOTYPE, John Blenis and Susan P. Hawkes, Michigan Molecular Institute, Midland, MI 48640, and Michigan State University, East Lansing, MI 48824.

The development of the transformed phenotype has been studied in chicken embryo fibroblasts infected with a temperature sensitive mutant of Rous Sarcoma Virus. We have devised an assay to study the kinetics of changes in cell substratum adhesion which can be compared with the expression of other measurable parameters of transformation such as alterations in glucose transport, DNA synthesis and surface organization detected by fluorescamine labeling. Although fibronectin has been implicated in cell substratum adhesion, its loss from the cell surface occurs too late to account for the differences in adhesive properties we have measured. We have identified alterations in several endogenous proteins and associated glycosaminoglycans, in substrate attached material, which correlate with decreased adhesion of transforming cells and are possible candidates for the changes we observe.

Supported by NIH Grant CA-27283-01 and the Elsa Pardee Foundation.

848 EXTRACELLULAR MATRIX INFLUENCES ON ELASTIN GENE EXPRESSION, Robert P. Mecham, Pulmonary Division, Washington University School of Medicine, St. Louis, MO 63110

The extracellular matrix (ECM) plays a key role in the regulation of cellular development and in the synthesis of extracellular connective tissue macromolecules. As yet, little is known about the effects of the extracellular microenvironment on elastin gene control or elastic fiber formation. We have utilized the fetal bovine ligamentum nuchae to study the role of ECM in modulating elastogenesis. The ligamentum nuchae contains a single fibroblast cell type specialized to secrete an extensive extracellular matrix of which elastin is the major component. Our results show that ligament cells grown on tissue culture plastic are unable to form insoluble elastin despite continued synthesis of all known elastic fiber constituents, and synthesize less soluble elastin per ug DNA than cells in ligament tissue. Ligament cells cultured directly on nonviable ligament tissue, however, are able to utilize the tissue matrix to form insoluble elastin and are stimulated to synthesize more soluble elastin than cells grown on plastic. ECM also appears to play an important role in initiating elastogenesis in the ligament at the beginning of the last trimester of fetal development. Elastin production is initiated in ligament cells from young tissues not actively synthesizing elastin when the cells are grown on nonviable ligament tissue isolated after the onset of elastogenesis. These results provide strong evidence that the composition of the ECM influences the synthesis of elastin and that direct cell-matrix interactions are important in mediating elastogenesis *in vivo*.

849 INTERACTION OF PLASMA FIBRONECTIN WITH ACTIN, INTRACELLULAR MAMMALIAN MEMBRANEOUS STRUCTURES AND BACTERIA, Janos Molnar, Judy Froehlich and Brad Rovin, Department of Biological Chemistry, SMBS, University of Illinois at the Medical Center, Chicago, Il. 60612

The prominent role of plasma fibronectin (Pfn) in the host defense system as an opsonin for gelatin (collagen) coated colloids has been established. In the present study we had investigated the possibility that Pfn may also opsonize naturally occuring particles devoid of collagen.-Interactions with Pfn of test materials was determined: a. by an indirect method in which inhibition of Pfn-mediated uptake of ^{125}I-gelatin-coated latex beads by liver slices and/or macrophage monolayers was used as a measure of interaction; b. by a more direct method in which ^{125}I-labeled test materials were used in conjunction with macrophage monolayers in which phagocytosis mediated by Pfn confirmed such intraction.-It was found that actin from skeletal muscle was a potent inhibitor in both the liver slice and macrophage systems. Similarly all the membraneous subcellular fractions isolated from lung and peritoneal exudate cells and liver by sucrose density gradient centrifugation were inhibitory at very low concentrations. Besides these,E. coli and S. aureus strains of bacteria caused strong, concentration dependent inhibition in the macrophage system. The interaction of these materials with Pfn was confirmed by showing that Pfn promoted internalization of ^{125}I-labeled actin, membraneous subcellular fractions from tissues and bacteria.-The results revealed that Pfn can mediate phagocytosis, besides the already established collagen coated particles, of actin and actin associated particles, intracellular membraneous structures of tissues and certain types of bacteria. The data therefore suggest a very wide specificity and role of Pfn in the host defense system.

850 CELL DENSITY DEPENDENT REGULATION OF PHENYLALANINE HYDROXYLASE ACTIVITY IN HEPATOMA CELLS IN CULTURE, Ross Shiman, Don McClure, Richard E. Baker, The Milton S. Hershey Medical Center, Hershey, PA 17033.

The activity, but not the amount, of phenylalanine hydroxylase in Reuber H4 rat hepatoma cells in culture is regulated by the population density of the cells in the monolayer; and the effect is not due to conditioning of the growth medium (McClure, D., Miller, M. and Shiman, R. Exp. Cell Res. 98, 223-236, (1976)). Present data suggest that direct cell-cell contact mediates the effect, that the effect is cell-type specific and that low resistance junction formation between the cells is probably not involved. Three lines of evidence support these possibilities: 1. Clones of H4 cells which have a decreased cell-density dependence of hydroxylase activity also show a greatly increased tendency to aggregate with one another at low density. 2. Several rat liver derived cell lines which do not contain phenylalanine hydroxylase activity were co-cultured with H4 cells. None of these heterologous cell lines affected the activity of the hydroxylase in the H4 cells, implying that the effects are specific to the H4 cells and are not the result of general microenvironmental changes due to cell-crowding. 3. Although H4 cells do show some low-resistance junction formation, as indicated by their ability to transfer [^{3}H]uridine nucleotides from labeled to unlabeled cells (autoradiographic detection was used), the number of cells showing transfer was insufficient (only 5-10%) to account for the cell-density induced changes in phenylalanine hydroxylase activity.

851 USE OF MONOCLONAL ANTIBODIES TO DEFINE INTERACTIONS OF FIBRONECTIN WITH SOLUBLE & CELLULAR CONSTITUENTS, L.Furcht &D. Smith, Univ. MN, MPLS, MN

Fibronectin (FN)is a high molecular weight glycoprotein present in plasma, the cell matrix,& in the soluble supernatant of various cells grown in vitro. Specific regions of FN will bind togelatin or collagen,heparin, cell surfaces and staphlococci. Monoclonal antibodies were made to human plasma FN and the antibodies reacted with human FN as shown by competitive ELISA. The specific antigenic region of FN reacting with the hybridoma antibodies was determined. ^{3}H-FN was digested with trypsin and specific fragments were immunoprecipitated or purified on monoclonal affinity columns. One representative clone recognizes a 31K free sulfhydryl containing fragment and another recognizes an 80K free sulfhydryl containing fragment which also binds toheparin(Smith, Mosher, & Furcht submitted). Human confluent fibroblasts were labeled with 35-S methionine.Labeled media or cells were solubilized in SDS, triton, deoxycholate, NaCl, and Tris, with immune precipitates collected and run on gels. Immune precipitates from the medium showed a 220 FN band and a 180, and 160K proteins that co-precipitate with FN. Immune precipitates from the cell lysates showed these same bands and additionally bands of 200, 130, and 43 KD among others. This last set of bands co-migrates with known cytoskeletal components. These experiments demonstrate that hybridoma antibodies to FN will precipitate FN and FN associated proteins from cell.

852 SERUM, FIBRONECTIN, AND LAMININ INCREASE INTESTINAL EPITHELIAL CELL ATTACHMENT TO COLLAGEN, IN VITRO, Peter H. Burrill, Isa Bernardini, and Norman Kretchmer, National Institutes of Health, Bethesda, MD 20205

Rabbit intestinal epithelial cells were dissociated with hyaluronidase (Sigma, Type II). Washed cells (10^5 cells in 1 ml of medium, NCTC-135 and DMEM, 1:1) were added to pretreated 35 mm bacteriological dishes containing 2 ml of media without or with varying concentrations of calf serum (up to 10%) and incubated for one hr at 37°C. Attached cells were removed with 0.1% trypsin in phosphate-buffered saline and counted with a Coulter Counter (Coulter Electronics). Dishes, either plain or coated with 10 ug of Type I collagen film (Collagen Corp., Vitrogen 100), were pretreated for one hr at room temp with medium alone or medium containing 5% calf serum, purified fibronectin, or purified laminin. Cell attachment to collagen was stimulated at serum concentrations between 0.5% and 10% with maximal attachment at or above 5%. Cells did not attach to bacteriological plastic even in the presence of serum. Pretreatment of collagen coated dishes with varying concentrations of either fibronectin or laminin enhanced cell attachment in a concentration dependent manner. Attachment to substrate pretreated with either protein was increased by the addition of either 5% normal serum or fibronectin free serum in the incubation medium. These data suggest that intestinal epithelial cells can use both fibronectin and laminin to attach to a collagen substrate in vitro. Furthermore, serum possesses a factor, which is not fibronectin, that further stimulates epithelial cell attachment to these substrates.

853 DIFFERENCES IN CELL SURFACE GLYCOPROTEINS IN CONFLUENT AND SUB-CONFLUENT INTESTINAL EPITHELIAL CELL CULTURES, Annette Herscovics*, Wlodzimierz Sasak*, and Andrea Quaroni** Laboratory for Carbohydrate Research, Departments of Biological Chemistry and Medicine, and **Gastrointestinal Unit, Department of Medicine, Harvard Medical School at the Massachusetts General Hospital

The structure of the carbohydrate groups of membrane glycoproteins was studied in rat IEC-6 intestinal epithelial cells in culture [Quaroni et al., J. Cell Biol. 80 (1979) 248]. Subconfluent and confluent cells were labeled for 24 hrs with [2-^{3}H]mannose, and then treated with mild trypsin or Pronase to release cell surface constituents. Labeled glycopeptides obtained by exhaustive Pronase digestion of the cell surface and of the residual cell pellet were then fractionated on Bio-Gel P-6 before and after digestion with endo β-N-acetylglucosaminidase H to distinguish between high mannose and complex asparagine-linked oligosaccharides. The glycopeptides in the cell pellets contained mostly high mannose oligosaccharides, and showed no significant difference between confluent and sub-confluent cells. In contrast, the cell surface glycopeptides contained a much higher proportion of complex oligosaccharides. Subconfluent cells had a much higher ratio of high mannose to complex oligosaccharides than confluent cells. Treatment of sub-confluent cells with hydrocortisone or with retinoic acid, both of which inhibited cell growth in a different manner, caused little change in the glycopeptide pattern obtained from the cell surface or cell pellet. These results suggest that the changes in structure of the oligosaccharides at the cell surface are related to the density of the cell population, and not to the growth rate. (Supported by NIH grants CA-14294 and AM-03564).

854 PERICELLULAR MATRIX COMPONENTS OF NORMAL HUMAN EPITHELIAL CELLS AND OF HUMAN CARCINOMA CELLS Kari Alitalo, Esa Kuismanen, Hannu Halila and Antti Vaheri, Deparment of Virology, University of Helsinki, 00290 Helsinki, FINLAND

Human epidermal cells were grown in coculture with feeder Balb/3T3 cells. After selective removal of the 3T3 cells, the epithelial cell islands were studied for production of connective tissue proteins by metabolic labeling and analysis of collagenous proteins, fibronectin and laminin. The 3T3 cells were similarly analyzed and found to produce type IV collagen and laminin in addition to interstitial collagens and fibronectin. The epidermal keratinocytes produced only small amounts of matrix proteins in culture. The [illegible] effect of feeder cells or their conditioned medium may be to supply basal lamina proteins reported to promote the initial adhesion of epithelial cells in culture conditions.

In cultures of pure epithelial cells from uterine ectocervix, a pattern of matrix proteins very similar to that of epidermal cells was found. This was compared with matrix proteins of endocervical cells, that do not stratify in vivo or in vitro.

Human carcinoma cells in culture show distinct patterns of secreted matrix proteins that may be used in their classification and in studies of the biosynthesis of basal lamina components.

855 STRATEGIES FOR CLONING THE HUMAN THY-1 GENE. Susan F. Cotmore, Peter J. Tattersall, David C. Ward & Michael L. Waterfield. Imperial Cancer Research Fund, London, W.C.2, England & Yale University, New Haven, CT 06510, USA.
The rodent cell surface antigen Thy-1 is expressed on particular cell types at specific stages in their differentiation. High level surface expression of Thy-1 frequently coincides with the establishment of strong cell-cell contacts. Developmentally-regulated glycoproteins structurally homologous to Thy-1 have now been purified from a number of other mammalian species, including man. Sequence studies show that rat brain Thy-1 bears a strong structural and evolutionary relationship to immunoglobulin constant region domains. Many questions concerning the function, tissue-specificity and evolution of Thy-1 could best be examined by analysing the nucleotide sequences which code for these molecules. We are attempting to isolate these sequences by using two strategies. Firstly, short length polynucleotide probes are prepared from murine Thy-1 expressor cell mRNA, either from poly A^+ RNA or from sucrose gradient fractions enriched for translatable message. These probes are annealed to high RoT with affinity-labeled polynucleotide fragments prepared from mRNA of non-expressor human cells, and the common sequence hybrids removed by chromatography. The remaining single strand sequences are used to probe a cDNA-plasmid library derived from a human Thy-1 expressor cell. In addition, this library is being probed with cloned DNA known to code for other cell surface proteins which share a common evolutionary relationship with immunoglobulin. DNA from positive clones will be used to select message from mRNA of human expressor cell origin, and its in vitro translation product identified by immunoprecipitation.

Intercellular Junctions

856 SIGNAL FOR CONTACT AND MEMBRANE ADHESION MEDIATED BY GANGLIOSIDES, Gregory J. Brewer, Dept. Med. Microbiology, SIU Sch. Med., Springfield, Illinois 62708
In order to study membrane-membrane adhesion, I have made two spherical membranes in an apparatus which allows simultaneous monitoring of structure with fluorescent probes and functional conductance with electronics. Membranes were made of phosphatidylcholine and beef brain gangliosides dissolved in n-decane. Upon apposition, the area of contact increased from 0.05 to 0.26 mm^2 in 15 minutes. Adhesion was also indicated by difficulty in separation of the membranes, when this was tried. An indication of mobility of the glycolipids was obtained by binding fluorescein-concanavalin A. The measured fluorescence anisotropy in the contact region was double that observed on the rest of the membrane. Thus, the contact region showed restricted mobility. Furthermore, the conductance increased 10-fold across the contact region. Without gangliosides, these characteristics were not observed. When each membrane contained a different fluorescent probe, after adhesion we observed no mixing of the two probes at the junction. Therefore, the two membranes do not partially fuse, but form two adhering bilayers. Thus, gangliosides appear to mediate membrane-membrane adhesion and provide a transmembrane signal for this functional contact sensation.

857 THE GAP JUNCTION PROTEIN, PARTIAL SEQUENCE AND TURNOVER. S. Barbara Yancey, Bruce J. Nicholson, and Jean-Paul Revel, California Institute of Technology, Pasadena, CA 91125
The gap junction fraction we isolate from rat liver is highly purified as evaluated by electron microscopy of negatively stained preparations. SDS-Page, however, shows one major band with several minor bands at both higher and lower M.W. Two-dimensional peptide mapping of tryptic or chymotryptic digests of each band in conjunction with sequencing and other studies on the isolated components lead us to conclude that gap junctions as isolated by our procedure are comprised of a single protein of M_r 28 kD. The other components that are seen are derived from this protein with the exception of a 38 kD polypeptide which is probably a contaminant. The sequence of the N-terminal 58 amino acids of the junctional protein shows a stretch of hydrophobic residues consistent with a transmembrane portion of the molecule. This same N-terminal sequence is found in a 10 kD polypeptide characteristic of gap junctions isolated after treatment with proteases. The rate of turnover of the junctional protein has been estimated from the specific radioactivity in this 10 kD band after a single i.p. injection of ^{35}S-methionine. From the decay in the specific activity with time, the gap junctional protein has an apparent half-life of about 19 hours. This figure probably overestimates the true half-life and indicates a surprisingly rapid turnover of what has usually been believed to be a very stable structure. It suggests that in liver, the gap junctions may be very responsive to alterations in physiological demands.

858 DEVELOPMENTAL CHANGE IN CO_2 SENSITIVITY OF CHICK LENS INTERCELLULAR COUPLING. S. M. Schuetze and D. A. Goodenough Dept. of Anatomy, Harvard Medical School

Freeze fracture studies show that lens gap junctions differ from those in other tissues in that the connexons (subunits) are more disordered. We are studying gap junctions in early embryonic chick lens cells (Hamburger and Hamilton stages 12-22). Freeze fracture replicas of lenses from stage 12-14 embryos fixed in glutaraldehyde reveal small, infrequent gap junctions that contain tightly packed, ordered connexons, unlike their adult counterparts. Between stages 14-18, freeze fractured lens gap junctions contain small islands of tightly packed connexons within larger regions of loosely packed connexons. Between stages 18-22, the lens fiber junctions appear similar to the adult junctions. Lens cells are electrically coupled at all stages, shown by paired intracellular recordings. Typical resting potentials are -60 to -70mV. The cells are also dye coupled. One minute after iontophoretically injecting a single lens cell with the fluorescent tracer Lucifer Yellow (MW 443), dye can be found in dozens of adjacent cells. There is no striking change in dye coupling with age. We find that incubating stage 15-22 lenses for 20' in medium equilibrated with 50-80%CO_2 has no detectable effect on dye transfer. In contrast, incubating stage 12-14 lenses for 10' in the same media appears to decrease dye coupling, since only a few cells near the injection site contain dye. The appearance of CO_2-resistant coupling may be correlated with the appearance of loosely packed connexons in freeze fracture replicas. (Supported by NIH grants EY03011 and EY05337. We thank W. Stewart for the generous gift of Lucifer Yellow).

859 Structural studies of isolated communicating junctions from liver and bovine lens. G. Zampighi and S.A. Simon. Department of Anatomy UCLA and Department of Physiology Duke University.

A pure junction fraction in milligrams quantity was isolated from bovine eye lens without using partial detergent solubilization or exogenous proteolytic enzymes. SDS-PAGE shows a major band running at an apparent molecular weight of 27,000 d and a minor one at 21,000 d. Chemical analysis show phospholipid/cholesterol/protein having a molar ratio of ∿20:10:1 respectively.

Structural studies were performed on the isolated fraction by x-ray diffraction and electron microscopy (thin sectioning, freeze-fracture, and negative staining). The fraction is primarily composed of large (>10μm) wavy junctions. Each junction has an overall thickness of ∿150Å and exhibits in the frontal plane, a tetragonal array of protein oligomers spaced ∿65Å apart. Optical reconstruction suggests that each unit is made up of 4 identical subunits. No evidence of the usual hexagonal arrangement of connexons in these isolated junctions was observed. Moreover, the tetragonal lattices were observed in intact lenses by freeze-fracture. The random organization of particles previously described by others appears from our findings, to be produced from the paracrystalline lattice by the freeze-fracture techniques.

Detergent solubilized junctions were studied using circular dichroism. In calcium free buffer, the solubilized junction protein contains 40% α-helix and ∿15% β-sheet. When calcium was added, a definitive change in the molecular ellipticity was measured implying a conformational change.

860 OCCLUDING JUNCTIONS IN MONOLAYERS OF CULTURED EPITHELIAL CELLS. I.Meza, A. Martínez-Palomo, E. Stefani and M. Cereijido. Depts. of Cell Biology and Physiology. CIEA del IPN. Apartado 14-740 México 14, D.F.

MDCK cells plated at high density form occluding junctions (OJ) that separate two membrane domains endowed with specific structural and biochemical differences. OJ confere to the monolayer an effective electrical resistance of 80-600 Ω cm^2. This resistance starts to develop in 3-6 hrs after plating and reaches steady state values in 12-18 hrs. The formation of OJ and development of the electrical resistance can be inhibited by cycloheximide and puromycin. Once established, the OJ have a 9:1 Na/Cl selectivity, and a cation-cation selectivity following the order K>Na>Rb>Cs>Li. The number of strands found in freeze fracture replicas is heterogeneous, varying from 1 to 10 along the same junction. This correlates with measurements made with a voltage scanning procedure of high spatial resolution which shows sites of high conductance alternating with regions of low conductance along the perimeter of a given cell. As followed by changes in the transepithelial resistance, the removal and restoration of Ca^{++} causes opening and resealing of the OJ. Changes in OJ permeability are concomitant with changes in the integrity of the junctional strands and the cytoskeleton pattern. Immunofluorescent techniques indicate that actin filaments form a continuous ring at the periphery of the cells in close contact with the region occupied by the OJ. The organization of the actin ring is necessary for the maintenance of the junctions sealing capacity. (Supported by research grants of CONACyT of Mexico and AM26481 from the NIH).

861 REGULATION OF CELL TO CELL COMMUNICATION VIA GAP JUNCTIONS, Camillo Peracchia, Giovanni Bernardini, and Lillian L. Peracchia, Univ. of Rochester, Rochester, N.Y.
Physiological studies have shown that cell communication via gap junctions is abolished (uncoupling) by treatments which increase the intracellular concentration of divalent cations. Recent data indicate that cell uncoupling also follows a decrease in pH_i. Thus questions on whether divalent cations and/or H+ are the uncoupling agents and on their direct effect on the junctional gating mechanism have been posed. Some answers can be obtained from morphological studies. In various tissues cell uncoupling is paralleled by a change in gap junction particle packing from disordered to crystalline (Peracchia, Int. Rev. Cytol. 66, 81, 1980). Recently we have shown that Ca++, Mg++ and H+ affect independently, directly and reversibly the gap junction structure at concentrations ($5 \cdot 10^{-7}$M, $1 \cdot 10^{-3}$M, $3 \cdot 10^{-7}$M or greater respectively) similar to those believed to cause uncoupling. These data have been obtained on isolated lens fiber junctions. These junctions are capable of crystallizing also in the intact organ following treatments which increase cell calcium content (Bernardini and Peracchia, Inv. Ophthalmol., in press) and are able to uncouple following cell injury (Bernardini et al., J. Cell Biol. 87, 207a, 1980). Uncoupling of lens fiber junctions has been demonstrated by showing the rapid return of ^{42}K washout rate to control values following injury in presence of normal $[Ca^{++}]_o$. Gap junction crystallization is energy independent, as it occurs after ATP hydrolysis by hexokinase-glucose, and is not affected by glutaraldehyde. We suggest that junction crystallization and uncoupling result from neutralization of negative charges on the junctional protein by divalent cations or H+ followed by changes in protein conformation. Particle crystallization may result from blockage of repulsive charges. Supported by NIH GM20113

Secretory Vesicles and Endocytosis

862 ADSORPTIVE ENDOCYTOSIS OF *Wistaria floribunda* AGGLUTININ, M. Merion, R. Triemer and R. D. Poretz, Rutgers University, New Brunswick, NJ 08903

Exposure of murine fibroblast monolayers to *W. floribunda* agglutinin (WFA) results in the accumulation of large lysosomes. This effect, which is related to the carbohydrate binding site and valency of the lectin, is associated with endocytosis of WFA by the cells. Use of a WFA-ferritin conjugate in electron microscopic studies demonstrates that the lectin binds to both clathrin associated and free portions of the plasma membrane and is internalized within micropinosomes and clathrin coated vesicles. Continued incubation of the cells with the lectin conjugate results in fusion of lectin containing vesicles and localization of ferritin containing material within large lysosomes. To study the nature of the membrane glycoproteins involved in the internalization of WFA, experiments were performed to identify and determine the intracellular location of plasma membrane derived glycoproteins capable of interacting with the lectin. Affinity chromatography employing a WFA-sepharose adsorbant results in the isolation of six radioactive proteins from NP-40 solubilized plasma membranes of cells surface labelled with ^{125}I. PAGE of these proteins demonstrates that they range in molecular weight from 50 to 250 Kd. Isopycnic density centrifugation of homogenates of untreated cells results in a bimodel distribution of lysosomal enzyme activities. Exposure of the cells to WFA for increasing periods of time results in a progressive increase in the more buoyant pool of lysosomal enzyme activities with a concomitant decrease in the other pool. Results will be presented describing the location of plasmalemma derived glycoproteins capable of binding to WFA within various intracellular membraneous pools.

863 LIGATIN BINDS PHOSPHOHEXOSE ON ACIDIC HYDROLASES. E.R. JAKOI AND S.M. GASTON.

Ligatin, a lectin that recognizes phosphorylated sugars, has been isolated from plasma membranes of mouse macrophages, rat ileum and rat brain. Several acid hydrolases including N acetyl β D hexosaminidase are cosolubilized with this lectin. The cosolubilized hexosaminidase binds to ligatin in vitro as demonstrated by affinity chromatography using the immobilized ligatin. Hexosaminidase-ligatin complexes can be dissociated by low concentrations of mannose 6 phosphate (M6P) and/or glucose 1 phosphate (G1P). The effectiveness of these two phosphomonosaccharides varies characteristically: ileal hexosaminidase-ligatin complexes show a 4 fold preferential dissociation with M6P; macrophage complexes show a 160 fold preferential dissociation with G1P. Brain complexes dissociate with nearly equal preference for M6P and G1P. Heterologous complexes display the specificity characteristic of the source of the enzyme. Treatment of the cosolubilized hydrolases with endoglucosaminidase H releases phosphorus-32 label on hexosaminidase and prevents its binding to ligatin; yet treatment of the hexosaminidase with alkaline phosphatase affected its binding by no more than 30%. This apparent inability to dephosphorylate the hexosaminidase is consistent with the chromatographic behavior on QAE of ^{3}H mannose labelled acidic oligosaccharides isolated from the cosolubilized hydrolases. The oligosaccharides that contain ^{3}H M6P are less acidic than monophosphoesters and are insensitive to alkaline phosphatase until subjected to mild acid hydrolysis. These results suggest the presence of a phosphodiester on hexosaminidase analogous to the NAC glucosamine 1P 6 mannose on β glucuronidase isolated from murine lymphoma cells (Tabas and Kornfeld J. Biol. Chem. 255: 6633, 1980).

864 PROPERTIES OF HIGH-UPTAKE LYSOSOMAL HYDROLASES FROM DICTYOSTELIUM DISCOIDEUM, Hudson H. Freeze, William Novotny, and Arnold L. Miller, Univ. of Calif., San Diego,CA 92093 α-D-Mannoside (α-man) and β-D-glucosidase (β-glu) from Dictyostelium discoideum contain phosphohexosyl residues and are specifically endocytosed into human fibroblasts via a receptor mediated process. These enzymes were purified to homogeneity following secretion into phosphate buffer. Both enzymes contained 10-12% carbohydrate consisting of mannose (∿7%), N-acetyl glucosamine (∿2%) glucose (1.2%) and galactose (0.6%). Greater than 90% of each enzyme was adsorbed to Concanavalin A-sepharose (Con-A) and characteristic elution patterns were generated by stepwise elution with 25-800 mM α-methyl mannoside. Prolonged treatment of each enzyme with endoglycosidase H (endo H) reduced binding to <40% and significantly altered their Con A elution profiles. About 16% of α-man and 11% of the β-glu activities were bound to wheat germ agglutinin (WGA) and eluted by N-acetylglucosamine. Endo H treatment of β-glu had no effect on its binding to WGA, but increased the binding of α-man to 42%. Neither enzyme bound to columns of Ricinus communis. Our previous studies indicated that certain slime mold glycopeptides contained esterified sulfate residues. Aryl sulfatase treatment of β-glu, but not α-man, lowered the electrophoretic mobility of β-glu on native polyacrylamide gels suggesting that it may contain sulfate in sulfatase-sensitive linkage. These results indicate that α-man and β-glu are glycoproteins which may contain heterogeneous, high-mannose-like, endo H-sensitive oligosaccharide chains. (Supported by NIH grant 1 RO1 GM29262).

865 PURIFICATION OF COATED VESICLES USING PERMEATION CHROMATOGRAPHY, Suzanne R. Pfeffer and Regis B. Kelly, University of California, San Francisco, California 94143 Coated vesicles are thought to be vehicles for the intracellular transport of membranes. To identify the minor components which might be transported by the coated vesicles, it is essential that they be highly purified. Since coated vesicles have diameters in the range of 50-150 nm, they are included in glass bead columns of appropriate pore diameter. Bovine brain coated vesicles purified by the standard procedure of Pearse have been further purified using permeation chromatography on glass bead columns of average pore size 200 nm. At least four of the polypeptides present in the conventionally purified coated vesicles are absent from the final form. Six specific polypeptides and a family of polypeptides in the molecular weight range of about 100,000 appear to co-purify with clathrin, although four of the specific polypeptides are found in the contaminating membranes. To measure the extent to which coated vesicles are formed randomly during the homogenization procedure, pure radiolabelled cholinergic synaptic vesicles were added to pieces of bovine brain prior to homogenization. Considerable amounts of the exogenous membrane were found in the coated vesicle fractions in the first two steps of coated vesicle isolation. However, after permeation chromatography less than 0.007% of the exogenous synaptic vesicle membrane was found in the pure coated vesicles.

866 A CELL-FREE MODEL FOR COMPOUND EXOCYTOSIS: AGGREGATION AND FUSION OF CHROMAFFIN GRANULES IN THE PRESENCE OF CALCIUM, SYNEXIN AND CIS-UNSATURATED FATTY ACIDS
Carl E. Creutz, Janet H. Scott, Christopher J. Pazoles and Harvey B. Pollard
Cell Biology and Biochemistry Section, Clinical Hematology Branch, N.I.A.M.D.D., Bethesda, Maryland 20205

Synexin is a calcium binding protein originally extracted from the adrenal medulla that causes the calcium dependent aggregation of isolated chromaffin granules (J. Biol. Chem.253:2858-2866 and 254:553-558). When chromaffin granules that have been aggregated by synexin are exposed at 37°C to cis-unsaturated fatty acids such as arachidonic acid (20:4) or oleic acid (18:1), they fuse together to form large, membrane-bound vesicles that swell to enclose the maximum volume. Only limited fusion can be induced by isomers of oleic acid in which the cis double bond is displaced towards or away from the head group, and no fusion is induced by trans-unsaturated or saturated fatty acids, detergents or lysolecithin. During fusion the soluble core proteins of the granules are retained although some of the epinephrine escapes. The swelling of the vesicles can be inhibited by increasing the osmotic strength of the medium, and we are investigating the role of osmotic forces in the fusion event.

The regulators of this in vitro fusion - calcium, synexin and cis-unsaturated fatty acids - may be present in the cytoplasm of the chromaffin cell when it is stimulated to release epinephrine and granule proteins by exocytosis. Therefore, this fusion event may also occur between chromaffin granules undergoing compound exocytosis.

867 IN VITRO RECONSTITUTION OF CHROMAFFIN GRANULE-CYTOSKELETON INTERACTIONS: CALCIUM DEPENDENT F-ACTIN CROSS-LINKING BY PURIFIED CHROMAFFIN GRANULE MEMBRANES. Velia M. Fowler and Harvey B. Pollard. National Institute of Arthritis, Metabolism and Digestive Diseases, Bethesda, Md. 20205.

The secretory vesicles of the adrenal medulla (chromaffin granules) provide a useful model system for biochemical investigation of the proposal that actin plays a role in exocytosis. We have made use of a novel application of falling ball viscometry to measure actin binding by membranes (Fowler et al., and Luna et al., J. Cell Biol., in press). Purified chromaffin granule membranes cause large increases in **viscosity and gelation** of purified rabbit skeletal muscle F-actin under conditions which may approximate the resting intracellular environment (pH 6.8, 20 mM KCl, 1 mM $MgCl_2$, $[Ca^{++}]_{free}$ ca. 10^{-8}M, 30°C). These increases in viscosity reflect actin binding to the membrane since F-actin is, in fact, observed to cosediment with chromaffin granule membranes under conditions which do not pellet F-actin alone. The actin cross-linking activity of the membranes is thermolabile (100°C, 2 min) and trypsin sensitive, suggesting that F-actin may interact with a protein component in the chromaffin granule membrane.

The increases in viscosity of F-actin induced by the membranes are somewhat enhanced by inclusion of 1-2 mM $MgCl_2$, and are greatly diminished by increasing KCl concentration, or pH over the pH range from pH 6.0 to 8.0. Increasing the free calcium ion concentration from ca. 10^{-8}M to ca. 10^{-6}M at pH 6.8 results in a 50-90% drop in the increases in viscosity induced by the membranes over the viscosity of F-actin. Regulation of the interaction of F-actin with chromaffin granule membranes by parameters (pH, $[Ca^{++}]_{free}$) which are thought to regulate secretion in vivo suggests that actin may indeed play a role in exocytosis.

868 CALCIUM SPECIFIC BINDING OF SYNEXIN TO CHROMAFFIN CELL PLASMA MEMBRANE, Janet H. Scott, Carl E. Creutz and Harvey B. Pollard, National Institute of Arhtritis, Metabolism and Digestive Diseases, Bethesda, MD 20205

Synexin, a soluble calcium binding protein isolated from bovine adrenal medullary tissue, binds to chromaffin granules and causes them to aggregate in a calcium dependent manner (Creutz et al, J. Biol. Chem. 253, 2858, 1978 and 254, 555, 1979). It has been proposed that synexin may initiate membrane fusion during exocytosis by causing chromaffin granules to become closely juxtaposed to the plasma membrane. This hypothesis predicts that synexin can bind to the inner aspect of the plasma membrane in a calcium dependent fashion. We have demonstrated this point by using chromaffin cell plasma membrane isolated on polylysine coated polyacrylamide beads (Jacobson and Branton, Science 195, 302, 1977). Synexin was found to bind to plasma membrane coated beads and chromaffin granule membrane coated beads but not to uncoated beads nor to erythrocyte membrane coated beads, indicating membrane specificity. The analysis of enzyme markers showed that the binding of synexin to plasma membrane coated beads cannot be explained by contamination of the beads with granule membrane. The binding was calcium dependent ($K_d \sim 200\,\mu$M); magnesium, strontium and barium were ineffective substitutes. Synexin binding to beads was saturable and about half of the bound synexin could be recovered by incubation in the presence of EGTA. Synexin binding to membrane coated beads was also demonstrated by SDS polyacrylamide gel electrophoresis with comparable results. We conclude that **synexin** can bind specifically to the inner aspect of the plasma membrane of chromaffin cells and may therefore play a role in attaching chromaffin granules to the plasma membrane prior to exocytosis.

869 CHEMIOSMOTIC LYSIS OF CHROMAFFIN GRANULES IN VITRO AS A MODEL FOR OSMOTIC EVENTS IN EXOCYTOSIS IN VIVO, Christopher J. Pazoles, Carl E. Creutz and Harvey B. Pollard, Section on Cell Biology and Biochemistry, CHB, NIAMDD, NIH, Bethesda, MD 20205

Studies on several model systems of membrane fusion have suggested that the driving forces for fusion during exocytosis may include a gradient of osmotic strength across the site of plasma membrane-vesicle membrane interaction. Thus, regulation of the osmotic properties of intracellular compartments such as the secretory vesicle may be of importance during exocytosis. We have approached this potential regulatory mechanism by examining the molecular features of osmotic control in adrenal medullary chromaffin granules (CG).

Isolated CG could be induced to lyse and release their contents in an isoosmotic medium containing both MgATP and permeant anions such as chloride. This was due to an increase in CG osmotic content and subsequent water entry. Regulation of CG osmotic content by MgATP and anions reflected the involvement of several CG membrane components including a proton pumping ATPase and a selective anion transport site. Their concurrent actions resulted in electroneutral, coupled transport of protons and anions into the CG thereby elevating the CG osmotic content. This mechanism was elucidated by measuring the effects of osmotic strength, anions, MgATP and inhibitors of anion transport on CG lysis, transmembrane electrical and pH gradients, anion transport and ATPase activity in CG. The possible role of such chemiosmotic CG properties in exocytosis was supported by studies of secretion from isolated adrenal medullary chromaffin cells. Exocytotic secretion of catecholamines from these cells was dependent upon extracellular permeant anions and was supressed by elevated external osmotic strength and by the addition of proton ionophores (eg. FCCP) or of some anion transport inhibitors.

870 EXPRESSION OF THE MANNOSE/FUCOSE PINOCYTOSIS RECEPTOR BY PRIMARY MACROPHAGES AND MACROPHAGE HYBRIDS, Philip Stahl and Siamon Gordon Washington University Medical School, St. Louis, MO.and The Sir Wm. Dunn School of Pathology, Oxford, U.K.

Glycoproteins terminating in D-Mannose or L-Fucose and various lysosomal glycosidases are recognized and internalized by pinocytosis receptors on alveolar macrophages (MØ) [Stahl et al. (1980) Cell 19, 207]. Other primary MØ which express this trait include resident peritoneal MØ, thioglycollate-induced peritoneal MØ and bone-marrow derived MØ. Uptake of ^{125}I-β-glucuronidase into mouse thioglycollate-MØ is time and temperature dependent and is fully inhibited by yeast mannan, L-fucose-BSA or D-mannose-BSA. J-774 and P388D (macrophage-like lines) were negative for binding and uptake of ^{125}I-Mannose-BSA. Hybrids prepared by fusing rat alveolar macrophages with a HAT-sensitive clone of J-774 were positive for binding and uptake of ^{125}I-Man-BSA. Several clones derived from cultures of mixed hybrids express uptake activity. Hybrids prepared by fusing primary macrophages with a non-macrophage cell line were negative for receptor activity.

871 PURIFICATION OF CATACHOLAMINE CONTAINING SECRETORY GRANULES, John A. Wagner, Sidney Farber Cancer Institute, Boston, MA 02115

Catacholamine containing secretory vesicles were partially purified from the clonal cell line PC12. The purification procedure consisted of gentle lysis into isoosmotic buffer, differential centrifugation, and sedimentation on an isoosmotic ficol gradient. At this stage, the vesicles have been purified twelve to fifteen fold and 50 to 70 percent of the catacholamines were recovered with the vesicle peak. The specific activity of the vesicles at this stage of purification is 250 pm of dopamine per μg protein. The vesicle fraction also contains ATP, and the molar ratio of dopamine to ATP is about 10. The vesicle fraction is clearly enriched in a number of proteins when compared to the total cell proteins on SDS polyacrylamide gels and thus there is the possibility that there are vesicle specific proteins.

872 INHIBITION OF ENDOCYTOSIS IN A MACROPHAGE-LIKE CELL LINE BY GLUCOCORTICOIDS, Steven C. Miller and George Melnykovych, University of Kansas Medical Center, Kansas City, KS 66103 and Veterans Administration Medical Center, Kansas City, MO 64128

Glucocorticoids are known to alter the lipid metabolism of a variety of cultured cells. Such alterations may lead to specific impairment of specialized membrane functions such as endocytosis. As demonstrated by others, macrophages can internalize an amount of plasma membrane equivalent to ∿ 200% of their surface area per hour. Such surface active cells provide a model system for studying the effect of glucocorticoids on both fluid and receptor mediated endocytosis. We have used a macrophage-like cell line ($P388D_1$) to demonstrate that glucocorticoids inhibit the fluid-phase endocytosis of fluorescein-labeled dextran (FITC-dextran). Initial experiments demonstrated that the interaction of FITC-dextran with cells had all the features of fluid-phase uptake, i.e., the amount taken up was proportional to the concentration in the medium, the uptake proceeded continuously with time, and was blocked at 4°C. Dexamethasone (10^{-7}M) had no effect on endocytosis until 11 hours after addition of the steroid, where it inhibited the uptake of FITC-dextran by 35%. The amount of inhibition increased with longer exposure times to the hormone up to 50% after 22 hours. Although this effect on endocytosis was observed prior to any effect on growth of the cells, endocytosis as well as cell proliferation were inhibited by dexamethasone in a dose-dependent fashion. A preliminary survey of selected steroids has established that the inhibition of endocytosis was restricted to steroids of the glucocorticoid class. Cortisone was inactive suggesting that this cell line lacks the enzymatic ability to convert it to cortisol.

873 TRANSFER OF LYSOSOMAL MEMBRANE PROTEINS INTO PHAGOSOME MEMBRANES, W.J. Brown, W.A. Shannon, Jr., & W.J. Snell, Univ. of Texas Health Sci. Ctr. & Veterans Admin. Med. Ctr., Dallas, Texas.

Electron microscopic investigations have shown that during phagocytosis in polymorphonuclear leukocytes (PMN), cytoplasmic granules (lysosomes) fuse with nascent phagosomes. Because we have recently identified the externally disposed lysosomal membrane proteins from rabbit PMN by lactoperoxidase-catalyzed iodination procedures, it became possible to determine if these same membrane proteins are incorporated into phagosome membranes. Rabbit peritoneal PMN were pulsed with a paraffin oil emulsion (POE) meal for 2 min. after which the cells were washed from unbound, uningested POE and reincubated in buffer without POE. At various times after washing, aliquots of cells were removed and POE-phagosomes were isolated. These intact phagosomes were iodinated to label externally disposed proteins and analyzed by SDS-PAGE. The results showed that phagosomes prepared at t=2 min contained iodinated proteins which comigrated with lysosomal membrane proteins; and, the relative concentration of these proteins was found to be increased in phagosomes isolated later in the chase period. Interestingly, not all of the iodinatable lysosomal membrane proteins were found to have counterparts on intact phagosomes. This is the first biochemical evidence that lysosomal membrane proteins indeed become incorporated into phagosome membranes. In addition, the results suggest that there are fusion-induced modifications of some lysosomal membrane proteins rendering them inaccessible to surface iodination.

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874 INCORPORATION OF SPECTRIN AND GLYCOPHORIN A INTO CULTURED HUMAN FIBROBLASTS USING CELL FUSION, I. Virtanen, V.-P. Lehto, S. Stenman and C.G. Gahmberg, Dept Pathol., Bacteriol., and Immunol., Univ. Helsinki, Finland

The behaviour of the red cell membrane proteins, glycophorin A and spectrin was studied in indirect immunofluorescence microscopy during Sendai-virus induced fusion of human erythrocytes with cultured human fibroblasts.

After the initial attachment phase of erythrocytes to fibroblasts a rapid diffusion of glycophorin A took place to the fibroblast cell surface and after 24 h the fibroblasts contained glycophorin A all over the membrane.

On the other hand, in similarly fused cells, spectrin, a cell-membrane-associated cytoskeleton protein, found only in RBCs, behaved quite differently. Distinct plaque-like areas showing spectrin specific-fluorescence were seen in fused cells. The plaques were consistently associated with a nuclear distortion resulting in an apparent nuclear expulsion in some cells. After 24 h the cells were overall positive for spectrin.

The results show that spectrin and glycophorin behave in a distinctly different manner during fusion of erythrocytes with cultured human fibroblasts. On the other hand, they also suggest that spectrin organization could play a role in the nuclear phenomena involved in mammalian erythrocyte maturation.

875 A 140 KD GLYCOPROTEIN - A NOVEL, DETERGENT-RESISTANT CYTOSKELETON-ASSOCIATED MEMBRANE GLYCOPROTEIN, V.-P. Lehto, T. Vartio and I.Virtanen Depts Pathology and Virology, Univ. Helsinki, Haartmaninkatu 3, SF-00290 Helsinki 29, Finland

Surface labeled (galactose oxidase/NaB^3H_4 or metaperiodate/NaB^3H_4) cells were treated with 0.5 % Triton X 100 to study the cytoskeleton-plasma membrane interactions of cultured human fibroblasts. This approach is based on the property of nonionic detergents to preserve the protein-protein bonds while most of the membrane proteins and -lipids are extracted and to leave the cytoskeleton - intermediate filaments, microfilaments and microtubules - intact. Two major surface glycoproteins, a 220 kd gp and a 140 kd gp, were enriched in the cytoskeletal preparations and remained together with intermediate filaments in tne non-extractable residue also after disruption of microfilaments and microtubules. The 220 kd gp corresponds to pericellular fibronectin. The 140 kd gp is a novel surface glycoprotein which contains fucose and is collagenase- and trypsin resistant. It is hardly detectable in polyacrylamide gel electrophoresis if lactoperoxidase catalyzed iodination is applied as a membrane labeling technique. The 140 kd gp copurifies with the plasma membrane sheets in sucrose density gradient analysis of cultured fibroblasts. The results suggest that the 140 kd gp could serve as a transmembrane link between cytoskeleton and extracellular matrix.