TUMOR CELL SURFACES AND MALIGNANCY

Richard O. Hynes, Organizer

March 18 – March 23, 1979

Plenary Sessions

Poster Sessions

Cellular Parameters of Malignancy

405 THE IN VITRO TUMORIGENIC SYNDROME, Robert Pollack, Department of Biological Sciences, Columbia University, N.Y.,N.Y.10027

What is the mechanism of in vitro transformation? Why does a cell become a tumor? These are certainly not identical questions, but in their overlap lies the chance to understand this disease. The in vitro transformations that are separately correlated with tumorigenicity form a subset of physiological, genetic and biochemical events, each linked to the other to form a syndrome. The linkages imply a common mechanism, and that, in turn implies that their normal counterparts share in a common mechanism to maintain normal growth control.

For early passage rodent fibroblasts transformed by Papova viruses, the syndrome includes increased production of the protease plasminogen activator, acquisition of the ability to grow without anchorage, reordering of the actin-containing cytoskeleton, and of course, cellular tumorigenicity.

We will discuss current work on the roles of promotors and viral genes in initiation and maintenance of this syndrome, and on the mechanism underlying it.

406 KINETIC AND BIOCHEMICAL PROPERTIES OF NORMAL AND TRANSFORMED CELLS, Arthur B. Pardee, Peter W. Rossow, and Veronica G. H. Riddle, Sidney Farber Cancer Institute and Department of Pharmacology, Harvard Medical School, Boston, MA 02115.

The first part of this talk will present biochemical and kinetic evidence suggesting that cultured fibroblasts emerging from the quiescent state are quite different from exponentially growing cells entering the cycle following cell division. Several inhibitors that do not affect cycling cells block G_0 cells from reaching S phase (DNA synthesis). Biochemical differences include protein phosphorylation patterns and specific proteins that are synthesized rapidly following growth stimulation of G_0 cells but that do not show periodic synthesis in exponential cells transiting G_1. There are also morphological differences including ciliation of centrioles. We conceive of G_0 cells as being out of cycle, and requiring extra events to return them to cycle; e.g., we divide the return pathway of quiescent cells into two stages, G_0 to G_1, followed by G_1 to S.

The second part of the talk will deal with the response of cells to conditions suboptimal to growth. Normal cells become sensitive to the depletion of growth factors about 2 hr before S (the restriction point). This time remains relatively constant when cell growth is slowed by the presence of low levels of cycloheximide. Thus the elongation of the generation time occurs prior to the restriction point. Cells transformed by different agents are affected differently by low serum concentrations and various drugs that affect cell growth. The growth factor requirements of many of these transformed lines are also quite different from one another, in some cases the lines having lost nearly all of the factor requirements of normal cells and in others having lost only a few. We conclude from these experiments that the "transformation" and the specific retention of some degree of growth control does not have meaning unless at the very least, the transforming agent is specified. In particular, transformation by DNA tumor viruses such as polyoma and SV40 is of an extreme sort with abolished growth control and few growth factor requirements. In contrast, transformation with RNA viruses and chemicals generally has much less drastic consequences.

407 CONTROL OF CELL DIVISION BY CELL TO CELL CONTACT, L. Glaser, M. Lieberman, D. Raben, J. Salzer, B. Whittenberger, and R. Bunge, Departments of Biological Chemistry and Anatomy and Neurobiology, Division of Biology and Biomedical Sciences, Washington University School of Medicine, St. Louis, MO 63110.

We have investigated contact inhibition of 3T3 cells by examining the inhibition of growth of sparse 3T3 cells by a plasma membrane enriched fraction obtained from confluent 3T3 cells. The inhibition of growth by plasma membranes is reversible by trypsinization, and is not the result of a depletion of growth factors from the medium. Membranes at saturation arrest about 50% of the cells at each generation early in the G_1 portion of the cell cycle. The inhibition of cell growth by membranes is competitive with high serum concentration or by the addition of defined mitogens such as FGF and dexamethasone. The inhibitory components present in membrane fractions are destroyed by heating at 60°. ^{125}I-labeled membranes bind to 3T3 cells, but heat inactivated membranes fail to bind. Cells whose growth has been inhibited by addition of membranes show a decreased rate of α-amino isobutyric transport, but normal rates of glucose and phosphate transport. The inhibitory components present in membranes can be extracted with octyl glucoside and remain active after removal of the detergent by dialysis.

Schwann cells from dorsal root ganglia (Wood, Brain Research 115, (1976) 361-375) remain quiescent unless in contact with axons from dorsal root ganglia. A membrane vesicle fraction prepared from such neurites is mitogenic from Schwann cells. The membrane component is trypsin and heat sensitive, appears to be located on the external surface of the neurite membrane, and is not present in the soluble fraction of the neurites.

The general conclusion from these experiments is that both homologous (3T3) and heterologous (Schwann cell-neurite) cell contact can act to stimulate or inhibit cell growth, by mechanisms that could be at least superficially considered analogous to the interaction of a hormone with the cell surface. (Supported by Grants GM 18403, NS 009923, BM 77-15972 from NSF)

408 THE APPEARANCE IN CONFLUENT VASCULAR AND CORNEAL ENDOTHELIAL CELL MONOLAYERS OF A SPECIFIC CELL SURFACE PROTEIN (CSP-60) NOT PRESENT IN ACTIVELY GROWING ENDOTHELIAL CELLS OR IN CELL TYPES GROWING IN MULTIPLE LAYERS, Denis J. Gospodarowicz*, Israel Vlodavsky*, and Laurin K. Johnson§,* Cancer Research Institute*, Endocrine Research Division§, and the Department of Medicine*, University of California San Francisco, San Francisco, Ca. 94143.

The formation of a highly organized vascular and corneal endothelial cell monolayer is associated with the appearance of a 60K molecular weight cell surface protein (CSP-60) which, when reduced, gives rise to a 30K component. This major cell surface protein is not detectable in either rapidly growing endothelial cell cultures seeded at low density or in subconfluent cultures which do not yet exhibit the strict pavement-like morphology of a confluent monolayer composed of closely apposed cuboidal cells. It is also absent in vascular smooth muscle cells, which grow in multiple layers at confluence, as well as from endothelial cultures that were maintained in the absence of fibroblast growth factor and which grow on top of each other at confluence. After disruption of a confluent endothelial cell monolayer, CSP-60 is no longer exposed on the cell surface, but it reappears as soon as the cells readopt their characteristic highly flattened and two-dimensional organization. There is no correlation between the appearance of fibronectin and the formation of a confluent endothelial cell monolayer, since such a configuration is readily adopted in the absence of fibronectin after its partial or complete removal from the cells by urea, EDTA, or trypsin. Maximal amounts of fibronectin and no CSP-60 are detected in subconfluent, but not yet organized endothelial cultures or in endothelial cells that no longer form a monolayer of non-overlapping cells at confluence. Likewise, cultures of vascular smooth muscle cells which at confluence grow in multiple layers contain fibronectin but no CSP-60. Actively growing vascular endothelial cells derived from low density cultures that were pooled and reseeded at a high density resemble subconfluent cultures in having an unorganized morphology. This morphology is correlative with the presence of large amounts of cellular fibronectin and the complete absence of CSP-60. These results suggest that CSP-60 is only present in cells which adopt the configuration of a two-dimensional monolayer of flattened and closely apposed cells.

409 BIOLOGICAL EFFECTS OF TUMOR PROMOTERS MAY BE MEDIATED BY ALTERATIONS IN THE STRUCTURE AND FUNCTION OF CELL MEMBRANES, I.B. Weinstein, L.S. Lee, R.A. Mufson, H. Yamasaki, and P. Fisher, Columbia University, New York, New York, 10032.

The potent tumor promoter 12-O-tetradecanoyl phorbol-13-acetate (TPA), and related compounds, elicit four unusual biologic properties in cell culture: 1) mimicry of transformation in normal cells, 2) enhanced expression of markers of transformation in tumor cells, 3) inhibition of terminal differentiation, and 4) enhancement of carcinogen or virus initiated cell transformation. In various types of cell cultures these effects are associated with the following changes in cell surface properties and membranes: altered morphology and adhesion, increased transport of 2-deoxyglucose, increased release of arachidonic acid and prostaglandins, altered cell surface glycoproteins, and inhibition of the binding of ^{125}I-epidermal growth factor to its cell surface receptors. Time course studies, the effects of phorbol ester analogs, and the effects of various inhibitors suggest that the pleiotropic effects of the phorbol ester tumor promoters are a result of their capacity to induce a rather generalized perturbation in cell membrane structure and function.

410 SELECTION AND PROPERTIES OF TUMOR CELL LINES WITH ALTERED METASTATIC POTENTIALS. Garth L. Nicolson, Department of Developmental and Cell Biology, University of California, Irvine, California 92717.

Animal tumor mocels for studying blood-borne metastasis have been developed by *in vitro* cloning of malignant tumor cell lines or *in vivo* selection of malignant cell populations to obtain distinct organ preferring tumor cell lines with altered metastatic colonization potentials (G.L. Nicolson, BIOSCIENCE 28:441-447, 1978). Selection schemes and cell surface properties of lung- (I.J. Fidler, NATURE NEW BIOL. 242:148-149, 1973) brain- (K.W. Brunson, G. Beattie and G.L. Nicolson, NATURE 272:543-545, 1978) and ovary-colonizing (G.L. Nicolson, K.W. Brunson and I.J. Fidler, CANCER RES. 38:4105-4111, 1978) metastatic B16 melanoma, liver-colonizing RAW117 lymphosarcoma (K.W. Brunson and G.L. Nicolson, J. NATL. CANCER INST., in press, 12/78 issue) and lung-colonizing MSV3T3 vasoformative sarcoma (G.L. Nicolson, K.W. Brunson and I.J. Fidler, CANCER RES. 38:4105-4111, 1978) variant lines will be discussed. Cloned subpopulations from unselected parental and *in vivo* selected variant lines have been used to confirm that specific cell surface modifications are related to the high metastatic phenotype (G.L. Nicolson, K.W. Brunson and I.J. Fidler, CANCER RES. 38:4105-4111, 1978; G.L. Nicolson et al., in CELL AND TISSUE INTERACTIONS, pp. 225-241, Raven Press, New York, 1977; G.L. Nicolson, in BIOLOGICAL MARKERS IN NEOPLASIA: BASIC AND APPLIED ASPECTS, pp. 227-239, North-Holland Publishing Co., New York, 1978).

Additional information on metastatic melanoma lines selected for organ specificity (K.W. Brunson and G.L. Nicolson), malignant lymphosarcoma selected for non-adherence to immobilized lectins (C.L. Reading, P.N. Belloni and G.L. Nicolson), malignant mammary adenocarcinoma selected for lymph node and lung metastasis (A. Neri, E. Ruoslahti and G.L. Nicolson) and the interaction of malignant tumor cell lines with vascular endothelial cells (R.H. Kramer and G.L. Nicolson) will be presented in the abstract sessions.

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411 TUMOR HETEROGENEITY FOR INVASION AND METASTASIS, Isaiah J. Fidler, Cancer Biology Program, NCI-Frederick Cancer Research Center, Frederick, Maryland 21701.

In clinical cancer it is the process of metastasis, the formation of secondary tumor foci at distant sites, that eventually defeats therapeutic efforts. The development of a metastasis is dependent on an interplay between intrinsic characteristics of malignant tumor cells and host factors. The process of metastasis is highly selective and the metastatic lesion represents the end point of many destructive events that only a few cells can survive. Neoplasms, which are predominantly heterogeneous, contain a variety of subpopulations of cells with differing metastatic potential. Furthermore, metastatic cell variants have been shown to preexist in murine neoplasms of old and recent origin. Tumor cell variants with differing malignant phenotypes selected in a variety of tumor systems could be useful tools for answering questions regarding the biology of metastasis and in particular for testing new therapeutic approaches to cancer. The possible existence of highly metastatic variant cells within a primary tumor suggests that we no longer should consider a neoplasm to be a uniform entity. Efforts to develop effective therapeutic agents and procedures against malignant tumors should be directed toward the fatal metastatic subpopulations of cells. (Research supported by the National Cancer Institute (NCI) under Contract No. NO1-CO-75380 with Litton Bionetics, Inc.)

412 TUMOR CELL VARIANTS WITH ENHANCED INVASIVE AND METASTATIC PROPERTIES, George Poste, Department of Exp. Pathology, Roswell Park Memorial Institute, Buffalo, NY 14263.

There is increasing evidence that metastases are caused by subpopulations of tumor cells endowed with specific phenotypic characteristics which enable them to successfully invade surrounding host tissues, penetrate into the lymphatics and/or the vascular system, survive dissemination within the host circulation, successfully arrest in organs distant from the primary tumor, undergo extravasation into the surrounding tissues and proliferate to form clinical metastases and, at each of these stages, survive assault by the defense mechanisms of the host. Recognition that not all cells in a malignant primary tumor possess the properties needed to complete all of these steps in the metastatic process has major implications for the choice and design of experimental systems for the study of metastasis. Studies on unselected heterogenous tumor cell populations isolated from primary lesions may offer little insight into the properties needed for a cell to successfully metastasize. Analysis of the malignant phenotype therefore requires isolation and characterization of those subpopulations of malignant cells which are endowed with the full complement of properties required for metastatic behavior. In addition to their demonstrated capacity to cause spontaneous and/or experimental metastases in vivo, it is of considerable value if the metastatic tumor cell subpopulations chosen for experiments can also be cultivated in vitro so that their morphological, biochemical and immunologic properties can be characterized in detail.

Current techniques for the isolation and characterization of cloned tumor cell populations with enhanced invasive and/or metastatic properties will be discussed and the suitability of existing models for the study of metastasis will be critically reviewed. Experimental data on the properties of cloned tumor cell variants with enhanced invasive and/or metastatic properties isolated from avian and mammalian tumors isolated in the authors laboratory will be presented.

413 INVASIVE CELL MOVEMENTS IN VITRO AND IN VIVO. J.P. Trinkaus, Department of Biology, Yale University, New Haven, CT 06520.

Until recently the locomotion of tissue cells has been studied mainly in vitro on planar substrata and, although we do not yet know how cells move under these conditions, we now have some good ideas, based on an expanding array of fact. Yet, even when we come to understand how cells move in vitro this may not tell us how they move in vivo, where the cellular environment is often three-dimensional and where adhesion is entirely to other cells or to extracellular materials. It seems clear, therefore, that if we are to learn how cells engage in locomotion within organisms they must be studied there. There have been a number of investigations of cell locomotion in vivo recently and, although all must be regarded as preliminary, considerable information has been gained. Cells surely possess the same locomotory machinery in vivo as they do in vitro, such as an adhesive fluid cell surface, a cortical system of contractile microfilaments, and structural elements such as microtubules; however, they appear to use this machinery in quantitatively different ways when confronted with their normal tissue environment. Although they may move by means of spreading lamellipodia which adhere at discrete loci or plaques, like cells on a planar substratum in culture, their predominant modes of movement appear to involve either the contraction of long adhering filopodia, which pull the cell along, or extensive cytoplasmic flow into blebs or lobopodia. These different modes of movement may well depend on the three-dimensional extracellular matrix through which cells move in vivo, and this can be tested in part by culturing cells in collagen gels. Such a dual approach, observing the same cells in vivo where they show normal locomotory behavior, and in vitro, where the environment can be controlled and various cell components can be labeled and where the optical conditions are ideal, is precisely the approach that is necessary, if we are one day to understand the normal mechanism of tissue cell movement, and, with this, how cells move directionally during morphogenesis and invasively during the spread of cancer.

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Molecular Biology of Cell Surfaces

414 SURFACE PROTEINS AND THE TRANSFORMED PHENOTYPE, Richard O. Hynes and Toni Destree, Department of Biology, Center for Cancer Research, M.I.T., Cambridge, MA 02139

The absence or reduction in amount of fibronectin at the surface of transformed cells is a fairly reproducible correlate of oncogenic transformation. Readdition of fibronectin to transformed cells causes restoration of some normal properties, in particular, increased adhesion, flattened morphology, reduced overlapping and alignment of cells, and reappearance of organized microfilament bundles. It seems likely that many of these alterations reflect effects of fibronectin on cell-substratum adhesion. The corollary of this hypothesis is that reductions in levels of fibronectin lead to reduced adhesion and thus to pleiotropic alterations in behavior.

We have been investigating the molecular basis for the effects of fibronectin on cells. Fibronectin interacts with proteoglycans (Perkins et al, this meeting). The arrangement of fibronectin is also related to that of actin, as observed by double label immunofluorescence. The patterns of correspondence between exterior fibronectin and internal microfilament bundles suggest that the two are connected across the membrane and that fibronectin is a constituent of the adhesion plaques by which cells attach to the substratum. This arrangement is consistent with the effects of fibronectin both on cell adhesion and on the cytoskeleton.

415 STRUCTURE AND FUNCTION OF THE FIBRONECTINS, Kenneth M. Yamada, Liang-Hsien E. Hahn, and Kenneth Olden, Laboratory of Molecular Biology, National Cancer Institute, Bethesda, MD 20014

The fibronectins are high molecular weight adhesive glycoproteins present on the cell surface and circulating in blood. The cellular form (also known as CSP or LETS) is often decreased after malignant transformation, due to both decreased biosynthesis and increased turnover (1-3). Direct comparisons of purified cell surface and plasma fibronectins from chicken and man reveal identical biological activities in cell attachment to collagen and in cell spreading. However, cellular fibronectin is 50-fold more active in restoring a more normal morphology to transformed fibroblasts than plasma fibronectin, and it is over 150-fold more effective as a hemagglutinin. These differences in biological activity are accompanied by structural differences as revealed by electrophoresis in comparisons of cellular fibronectins from cell surfaces or culture medium and plasma fibronectin (4).

A second approach to determining the mechanism of action of the fibronectins is to isolate active fragments of the fibronectin molecule. Biologically active fragments have been isolated from cellular and plasma fibronectins, including a common 40,000 dalton chymotryptic fragment that contains the collagen-binding site. Isolation, purification, and characterization of these fragments have provided means to analyze adhesive interactions defective in transformed cells in terms of specific active sites on fibronectin, as well as permitting detailed, direct chemical comparisons of cellular and plasma fibronectins.

Finally, the role of carbohydrates in the function of fibronectin has been analyzed utilizing the drug tunicamycin to inhibit glycosylation. Non-glycosylated fibronectin is not significantly less active than normal fibronectin in several biological assays including hemaglutination and capacity to restore a more normal morphology and alignment to transformed fibroblasts. However, the absence of carbohydrate does result in a substantially greater susceptibility to proteolytic degradation both in vivo and in vitro. These and earlier results (5) indicate that the carbohydrate moiety of fibronectin is not required for its synthesis, secretion, or biological function, but instead helps to protect the protein against proteolytic attack.

1. Yamada, K.M., and Olden, K. Nature 275: 179-184 (1978).
2. Vaheri, A., and Mosher, D.F. Biochim. Biophys. Acta 516: 1-25 (1978).
3. Hynes, R.O. Biochim. Biophys. Acta 458: 73-107 (1976).
4. Yamada, K.M., and Kennedy, D.W. J. Cell Biol. (in press).
5. Olden, K., Pratt, R.M., and Yamada, K.M. Cell 13: 461-473 (1978).

416 POSSIBLE ROLES OF FIBRONECTIN IN MALIGNANCY, Lan Bo Chen, Ian Summerhayes, Rosalind Segal, Joyce Jen, Marcia L. Walsh, Philip Hsieh and Selma Silagi, Sidney Farber Cancer Institute, Harvard Medical School, Boston, MA 02115 and Cornell Medical Center, NY, NY 10021.

Our first phase of work on the possible role of fibronectin in malignancy was to establish whether the fibronectin negative transformed cells have a higher probability of inducing tumors in animals. By first approximation one can generally state that the loss of fibronectin as assayed by the culture method is indeed a high probability event (70%) for tumorigenic cells. If fibronectin really is involved in the determination of oncogenic potential, one has to ascertain why there are significant numbers of tumorigenic cells expressing more than the expected amount of fibronectin in culture. Therefore, our second phase of work is to concentrate on those fibronectin positive tumorigenic cells. We ask whether passaging cells in vivo can lead to the selection of cells with increasing tumorigenicity yet decreasing fibronectin expression in culture. Indeed, we observed such a phenomenon in hamster cells transformed by Herpes simplex virus (Type 2). Unfortunately, we have been unable to extend this observation to other fibronectin positive tumorigenic cells. We then hypothesized that, perhaps fibronectin has nothing to do with solid tumor induction but merely influences the escaping probability of tumor cells from primary tumor. We observed an almost perfect correlation between the loss of fibronectin and increase in metastasis until we found that certain highly metastatic mouse melanoma cells are, in fact, fibronectin-abundant in culture. As a last resort, we finally assayed fibronectin in situ in frozen sections of tumors by immunofluorescence. The result of these studies show that the expression of fibronectin in cultured tumor cells do not correlate with the expression of fibronectin in tumors induced by injection of the same cells. None of the in-culture-fibronectin-abundant melanoma cells express fibronectin in tumors in situ. Also, none of the metastatic tumor lines tested so far express fibronectin in tumors in situ. Moreover, even those nonmetastatic in-culture-fibronectin-abundant tumor cells, with few exceptions, express little or negligible amounts of fibronectin in tumors in situ. In contrast, an abundant amount of fibronectin was detected in the connective tissue of various organs, blood vessel walls, and muscles. It is possible that the factors controlling the expression and fibrillogenesis of fibronectin may be different in vivo and in culture. Since cell shape plays a vital role in fibronectin assembly in-culture-fibronectin-abundant tumor cells (mostly in fibroblastic shape) may assume a totally different cell configuration in vivo which discourages fibronectin assembly.

417 EXPRESSION OF MALIGNANT PROGRESSION IN CULTURED HUMAN EPITHELIAL CELLS, Helene S. Smith, Adeline J. Hackett, E. Louise Springer, Peralta Cancer Research Institute, Lawrence Berkeley Laboratory, Berkeley, CA; John L. Riggs, State Department of Health, Berkeley, CA and Michael W. Mosesson, Department of Medicine, SUNY, Brooklyn, N.Y.

We have approached the problem of determining which in vitro parameters correlate with various stages of human malignancy by characterizing epithelial cell lines developed from nonmalignant human tissues, primary and metastatic carcinomas. All of the lines derived from metastatic lesions were distinctive in that they had little or no fibronectin in an extracellular matrix, while the lines derived from primary carcinomas and from nonmalignant tissues all produced fibronectin matrices of varying morphologies. In contrast, using transmission electron microscopy to define various aspects of nuclear ultrastructure, the lines derived from primary and metastatic specimens were identical albeit readily distinguishable from the nonmalignant lines. Carcinoma biopsies had ultrastructural markers similar to those found in the tumor derived cell lines suggesting that these markers were not artifacts of culturing. Other parameters known to correlate with transformation in murine systems, such as growth without anchorage, on contact inhibited monolayers, or in immunosuppressed mice were expressed by some but not all of the malignant lines. In most, but not all cases, lines derived from metastatic lesions tended to have more abnormal properties than those derived from primary carcinomas. Since the tumor derived lines did not consistently display these parameters, it is likely that the properties do not represent changes essential to the malignant state. However, the nonmalignant lines were all negative suggesting that the markers are somehow related to malignancy even if not essential. The fact that each tumor line had a unique combination of abnormal properties, suggests that there may be many different ways that cells become malignant. If various in vitro transformation systems each describe a valid pathway for a normal cell to become malignant, one might expect that the properties correlating with neoplasia could differ depending on the system studied.

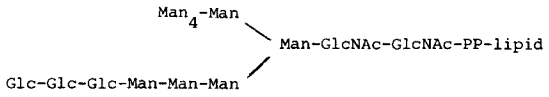
418 PLASMINOGEN ACTIVATOR AND ITS DIRECT EFFECT ON THE CELL SURFACE, James P. Quigley, Department of Microbiology and Immunology, SUNY, Downstate Medical Center, Brooklyn, New York 11203.

Chick embryo fibroblasts transformed by Rous sarcoma virus (RSVCEF) produce enhanced levels of the serine protease plasminogen activator (PA). Treatment of RSVCEF cultures with the tumor promoter, phorbol myristic acetate (PMA), further enhances (8-12 fold) the production of PA in already transformed cells. The net result is a 500-1000 fold increase in PA over untreated, normal CEF. Concomitant with the enhanced synthesis and secretion of PA, pronounced morphological changes occur in cultures under serum-free conditions. The PMA-treated cultures undergo cellular elongation, increased cell to cell adhesion, formation of star-like networks of cells and the eventual formation of clustered colonies composed of hundreds of cells. These morphological changes occur even when the cells are grown in plasminogen-free serum prior to the serum-free condition. A series of protease inhibitors were tested for their effect on the PMA-induced morphological changes, DFP (2×10^{-4} M), leupeptin (5×10^{-4} M), NPGB (1×10^{-3} M) and benzamidine (1×10^{-3} M) were among the compounds that prevented the formation of cell clusters and colonial aggregates. Trasylol (10-100 Units/ml), amino caproic acid (1×10^{-2} M) and soybean trypsin inhibitor (1-10 ugm/ml), all inhibitors of plasmin, were ineffective. A large number of other protease inhibitors were tested and also shown to be ineffective in preventing the morphological changes. The former compounds, which prevented morphological changes, were shown to be inhibitors of plasminogen activator by a direct fluorometric assay of the enzyme.

Use of the direct assay for PA to determine K_i's for the protease inhibitors and employment of ^{3}H-DFP to identify and characterize serine proteases indicate that PA itself is directly involved in altering the morphology of the PMA-treated transformed cells. The studies suggest that PA catalytically alters a cellular or extracellular substrate. This finding represents a new catalytic function for PA, independent of plasminogen, heretofore its only known substrate.

419 CELL SURFACE GLYCOPROTEIN SYNTHESIS AND PROCESSING, Phillips W. Robbins, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Mass. 02139

The asparagine-linked oligosaccharides of mature glycoproteins have a wide variety of structures, most of which can be assigned to two general families: the high-mannose class and the complex class. Both types contain $Man_3GlcNAc_2$ as a central core; the high mannose type contains additional mannose residues, and the complex type contains other sugars (galactose, fucose, and sialic acid). During the last year evidence has accumulated that both high-mannose and complex asparagine-linked oligosaccharides arise from a common precursor, identified as a lipid-linked oligosaccharide with the structure



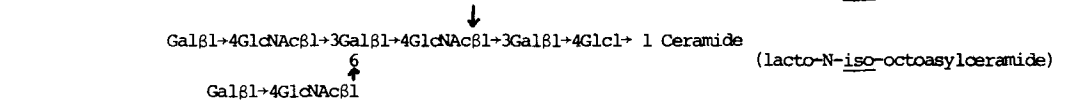
This species appears to be ubiquitous; it has been detected in 3T3 and Nil-8 cells, in chinese hamster ovary cells, in human diploid fibroblasts, and in normal, Sindbis virus-infected, and vesicular stomatitis virus-infected chick embryo cells.

Pulse-chase techniques, glycosidase digestion, and high-resolution gel filtration chromatography have been used to elucidate the early stages in the sequence of events ("processing") by which the lipid-linked $Man_9Glc_3GlcNAc_2$ species is converted to mature high-mannose and complex protein-linked oligosaccharides in chick embryo cells. The $Man_9Glc_3GlcNAc_2$ oligosaccharide is rapidly built up on the lipid carrier, then transferred en bloc to nascent polypeptide acceptors. The terminal glucose residue is removed so quickly (<2 min for isotopic equilibrium during pulse labelling with ^3H-mannose) that this, too, may be a nascent event. The second glucose residue is removed more slowly (5-10 min), and the third still more slowly (ca. 30 min). Only after all glucose residues are removed is the sequential excision of mannose residues begun. A family of smaller mannose-containing protein-linked oligosaccharides is generated, the smallest of which appears to be $Man_5GlcNAc_2$. It is probable that this species is the precursor of mature complex oligosaccharides.

420 GLYCOLIPID SYNTHESIS IN ONCOGENESIS AND ONTOGENESIS AND MODIFICATION OF ONCOGENIC PROCESS BY ANTI-GLYCOLIPID ANTIBODIES

Sen-itiroh Hakomori, Biochemical Oncology, Fed Hutchinson Cancer Research Center, and University of Washington, Seattle, Wash.98104

During the process of ontogenic development of human erythrocytes, the linear staright chain glycolipids (lacto-N-nor-hexaosylceramide and its analogs, structure see below) are converted to the branched chain glycolipids (lacto-iso-octaosylceramide and its analogs, strcture, see below).



The former represents the receptor for anti-i antibodies and the latter represents the receptor for anti-I antibodies. Thus, arborization of glycolipid carbohydrate chain in membrane may define a certain step of ontogenic phenotype of human erythrocytes (e.g. hemoglobin synthesis).

Two types of glycolipid changes associated with oncogenic transformation have been known: 1) a classical incomplete synthesis model with an often accumulation of the precursor glycolipids, and 2) activation of a new synthesis of a unique glycolipid(s) foreign to the host. The latter may cause a conversion of blood group in human tumor, such as: synthesis of PP_1-antigen in tumor of a host with blood group p (Levine et al, Proc.Soc.Exp.Biol.Med. 77, 403-405,51), A-like antigen synthesis in tomor of host blood group O/or B(Hakomori et al, J. Immunol98, 31,67), and Forsman synthesis in tumors of F^--individuals(Hakomori, ProcNat.Acd.Sc 74,3023,77). Many tumor cells are characterized by combination of these two glycolipid changes and these changes may reflect "retrogenetic"expression of ontogenesis.

The process of oncogenic transformation is inhibited or modified in the presence of anti-glycolipid antibodies Fab, as the process of ontogenesis is inhibitable by a ceratin antibodies to an embryonic antigen.

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421 CELL MUTANTS TO STUDY MALIGNANT TRANSFORMATION: ANALYSIS OF A SURFACE CARBOHYDRATE AND OF A GLUCOSE UPTAKE DEFICIENT FIBROBLAST LINES, Jacques Pouysségur, Centre de Biochimie, Université de Nice, Parc Valrose, 06034 NICE, France.

From 3T3 Balb/c cells, mutants with low adhesiveness to plastic substratum were selected. One of these selected clones, AD6, was analyzed and found to be deficient in glucosamine 6-phosphate N-acetylase. A result of this block is a decrease by 60% of cell surface carbohydrates (mannose, galactose, N-acetylglucosamine, N-acetylgalactosamine, sialic acid) and as a consequence there is a general reduction in the exposure of glycoproteins on the cell surface. This biochemical defect is fully reverted to normal, simply by growing the mutant cells in presence of 10 mM N-acetylglucosamine. This specific and reversible enzymatic block allows us to conclude that the abnormal properties of AD6 cells: low adhesion, round shape, increased agglutinability by lectins, loss of directional locomotion and absence of microfilament bundles are the result of the surface carbohydrate defect since reversion of glycoprotein synthesis to normal results in the general reversion of the altered phenotype.

Two membrane polypeptides of MW 95K and 75K the synthesis of which is regulated by glucose, are present in increased amounts in AD6 and also in some transformed cells. We demonstrate that the altered pattern of these two Glucose Regulated Proteins (GRP95, GRP75) are induced in transformed cells secondary to glucose starvation.

Using [^{3}H]-2-Deoxyglucose suicide in Chinese Hamster Lung fibroblasts (CCl39) we selected DS7, a mutant impaired in glucose metabolism. The 10 min uptake of 2-Deoxyglucose represents only 10% of the wild type value. This very low activity is due to a 4-5 fold decrease in glucose transport activity (measured by the initial influx rates of 3-O-Methylglucose) and to an inhibition of the in vivo hexokinase activity. The glycolysis rate measured by the lactate produced per mg of protein synthesized is 28 fold lower in DS7. DS7 grows like the wild type in fructose, galactose but with a slightly increased generation time in glucose (17 H instead of 15 H). In spite of a very low glucose uptake and almost of a total deficiency in glycolysis, DS7, like the parental cells grows at high frequency in agarose. All the clones growing in agarose display the DS7 phenotype. Tumorigenicity tests are in progress.

A general conclusion of these studies is that the altered properties and cell behavior of transformed fibroblasts such as low adhesiveness, round morphology, increased agglutinability by lectins, altered motility, absence of microfilament bundles, increase in membrane protein GRP 95 and 75, increase in hexose transport and in aerobic glycolysis are dissociable from malignant transformation. These phenotypic alterations should therefore be considered as secondary events to the mechanism which leads to the loss of growth control.

422 TRANSGLUTAMINASE AND ε-(γ-GLUTAMYL)LYSINE ISOPEPTIDE BONDS IN NORMAL AND TRANSFORMED CELLS, Paul J. Birckbichler and M. K. Patterson, Jr., Biomedical Division, The Samuel Roberts Noble Foundation, Inc., Ardmore, OK 73401.

Transglutaminase catalyzes a Ca^{++}-dependent acyl transfer reaction between the γ-carboxamide group of peptide bound glutamine and primary amines. Inter- or intramolecular ε-(γ-glutamyl)lysine isopeptides are produced when the primary amine is the e-amino group of peptide bound lysine. Such isopeptides were reported in membrane proteins from eukaryatic cells (1). Transglutaminase activity was significantly reduced in cells transformed in vivo and in vitro with viruses and chemicals (2, 3). Human lung cells (WI-38) were used in subsequent studies. Reduced isopeptide levels were observed in virus transformed cells and in proliferating cells (4). Nonproliferating cells grown in the presence of dansylcadaverine, an isopeptide inhibitor, showed reduced isopeptide content and exhibited several criteria of proliferating cells. Immunofluorescent staining with antibody to guinea pig liver transglutaminase substantiated the differentials in transglutaminase activity (5). Following extraction with Triton X-100, normal cells but not virus transformed counterparts showed a residual fibrous network when incubated with antitransglutaminase antiserum suggesting an association of transglutaminase with cytoskeletal components. Additional studies showed parallels between transglutaminase and the transformation sensitive protein fibronectin. The results suggest isopeptide crosslinks contribute to a cellular architecture conducive to a nonproliferating state.

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Transmembrane Interactions

423 PLASMA MEMBRANE - CYTOSKELETON INTERACTIONS, Rick Ash*, Daniel Louvard** and S. J. Singer*, University of California at San Diego*, La Jolla, California 92093 and European Molecular Biology Laboratory**, Heidelberg, Germany.

In studies of the membranes of cultured fibroblasts our attention has focused on those regions of the plasma membrane which are adjacent to cytoplasmic fibers containing actin, myosin, filamin, tropomyosin and alpha-actinin (denoted X-sites for brevity). Using the fluorescence microscope with two color staining we have compared the distribution of actin, using a biotinyl-HMM system (1), with a variety of membrane proteins, using specific immunofluorescent reagents (2). Several integral membrane proteins are normally excluded from X-sites of the plasma membrane. However, when the same integral proteins are clustered with specific antibodies the resulting patches quickly move to X-sites and, therefore, line up over actin cables. Lectin receptors are not completely excluded from X-sites, but lectin-induced clusters are concentrated on X-sites (3). After trypsinization and replating of cells newly deposited extracellular fibronectin containing fibrils are spontaneously arrayed over X-sites (4). Clearly the X-sites are areas of several transmembrane interactions which suggests that the actin based cytoskeleton has an important organizing and controlling function for the plasma membrane. These results will be reviewed and work in progress on the identification of integral and peripheral components of the X-site complex will be discussed.

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424 ASSOCIATIONS BETWEEN SURFACE PROTEINS AND MICROFILAMENTS, Gordon L.E. Koch, MRC Laboratory of Molecular Biology, Cambridge, CB2 2QH, England.

The P815 mouse mastocytoma line possesses a cell surface densely covered by long microprojections refered to as filopodia. The filopodia can be detached from the cell body by gentle shearing and relatively pure preparations isolated. The purified filopodia contain actin as the major protein. During the shedding of the filopodia a substantial proportion of the cell surface major histocompatibility antigen H2 is also released. When filopodia are stripped of plasma membrane with detergent the antigen remains associated with the microfilament bundle. A specific affinity technique for microfilaments, the myosin affinity technique, confirmed that the H2 and microfilaments are associated in the filopodia.

The myosin affinity technique has also been used to analyse the putative associations between surface Ig and microfilaments during the "capping" of the former. The analyses show that cross-linking of surface Ig results in the formation of a stable association between the surface Ig and microfilaments. These in turn indicate that valency modulation of surface receptors could serve as a mechanism for transmitting transmembrane signals across the plasma membrane through perturbations of the cytoskeleton of the cell.

425 IDENTIFICATION AND PROPERTIES OF TRANSMEMBRANE PROTEIN FROM MOUSE CELLS, Louis M. Fink, Francis G. Grillo and Robert M. Evans, Department of Pathology, University of Colorado Medical School, Denver, CO 80262.

We have analyzed plasma membrane proteins from cultured mouse cells with respect to topographical distribution using latex-filled phagosome preparations as a model of membrane with an "inside-out" orientation. We have also studied the effects of cytochalasin B and colchicine on the protein composition of phagosomal membranes.

The approaches used to detect which proteins could span the bilipid layer all include selective enzymatic labeling of the cell surface proteins and selective labeling of the cytoplasmic plasma membrane proteins on preparations of phagosomes. After transglutaminase catalyzed dansyl cadaverine labeling of the cytoplasmic surface proteins of phagosomes, isolated from cells whose external proteins had been iodinated by lactoperoxidase labeling, antibodies directed against the dansyl moiety were used to separate the proteins labeled on the cytoplasmic surface. Analysis of the dansyl cadaverine labeled proteins for I^{125} by SDS-PAGE revealed 3 major bands containing both labels. Because this approach is somewhat restrictive in the number of proteins which are labeled, other methods have also been investigated.

We found that the carbohydrate moieties of the glycoprotein of phagosomes by lectin affinity chromatography on ConA and WGA Sepharose and by periodate borotritide labeling were essentially all on the external surface of the membrane. Using lectin affinity chromatography of solubilized membrane proteins and SDS-PAGE of the proteins from phagosomal membranes, either labeled selectively on the external surface or the internal surface, we have found that most of the membrane proteins which bind to the lectins are iodinatable from either side of the membrane. These molecules are strong candidates for having a transmembrane configuration.

Another method developed to analyze the position of specific proteins involves the resolution of proteins iodinated from the external or internal surfaces of the plasma membrane on two-dimensional gels. Analysis of these proteins reveals a class of proteins which are iodinatable only on the external cell surface, a class iodinatable only on the cytoplasmic face and a class iodinatable from either side. Some of these proteins appear to exist in several types of mouse cells. Double-labeling with S^{35} methionine and P^{32} suggests that one acidic protein of 125,000 daltons iodinatable from both sides of the phagolysomal membrane is also heavily phosphorylated.

The extensive resolution of the S^{35} methionine labeled proteins from plasma membrane or phagosome preparations has allowed us to analyze the effects of cytoskeletal disrupting chemicals on the protein composition of membranes. Cytochalasin B causes specific deletions of proteins from the phagosomes; however the effect is different in the various cell lines and macrophages tested. There are no detectable alterations in the protein composition of phagosomes prepared from colchicine treated cells analyzed on 2-D gels. (This work was supported in part by USPHS Grant CA-15823 and R.J. Reynolds Industries, Inc.)

426 ASSOCIATION OF α-ACTININ WITH THE PLASMA MEMBRANE. Keith Burridge, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724.

Since the discovery of the Z-line protein α-actinin in nonmuscle cells, it has often been suggested as a candidate for mediating the attachment of actin filaments to membranes. We are investigating this hypothesis. Indirect immunofluorescence with antibodies against α-actinin indicate that, along with other proteins of the contractile complex, it is found underlying the "patches" and "caps" of aggregated cell surface molecules. More direct evidence for the association of α-actinin with the plasma membrane has been sought by analysis of purified plasma membranes. Plasma membranes from lymphocytes and HeLa cells have been analyzed on SDS gels. When these are reacted with antibody to muscle α-actinin a prominent band at 100,000 daltons is labelled. Some of this membrane-associated α-actinin can be extracted from the membranes easily, but a fraction remains which can only be extracted under conditions which also remove the majority of the actin (for example, by extracting with a high pH, low ionic strength buffer containing chelating agents). This α-actinin which is closely associated with the plasma membrane could be linked in two different ways: either it is interacting with membrane components directly and forms a bridge for the attachment of the actin filaments, or alternatively it is binding directly to or cross-linking the actin filaments that are themselves linked to the membrane via molecules other than α-actinin. At present we are trying to distinguish between these alternatives.

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Cellular Adhesion and Migration

427 INTERACTION OF FIBRONECTIN WITH COLLAGENS, AND ITS ROLE IN CELL ADHESION, Erkki Ruoslahti, Edward G. Hayman, and Eva Engvall, Division of Immunology, City of Hope Medical Center, Duarte, CA 91010.

Fibronectin binds to different types of collagens. Of the native type I, II, III, and AB chain collagens, type III is the most active binder of fibronectin, and the AB chains, the least active. Denatured collagens are far more active than the native ones. Fibronectins from different species and different sources (plasma, amniotic fluid, cultured fibroblasts, and epithelial cells) show similar collagen-binding properties.[1,2,3,4]

Limited proteolysis allows cleavage of the fibronectin molecule into biologically active fragments. We have isolated collagen-binding fragments with molecular weights of about 70,000 and 30,000. The latter fragment is electrophoretically homogeneous. Attachment of trypsinized fibroblasts is greatly enhanced by coating of microtiter plate wells with fibronectin. The collagen-binding fragments did not enhance attachment of cells in this assay, whereas the non-collagen-binding fragments were active. Gel filtration of the non-collagen binding fragments allowed isolation of fragments with an activity equal to that of the whole fibronectin molecule in the cell adhesion assay. These results suggest that fibronectin has two dissimilar binding sites, one for collagen, and another for cell surfaces. The availability of a homogeneous collagen-binding fragment with a molecular weight of less than 10% of the whole molecule may facilitate studies on the molecular basis of the fibronectin-collagen interaction.

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428 COMPOSITION OF THE SUBSTRATE ADHESION SITE OF NORMAL AND VIRUS-TRANSFORMED MURINE CELLS, Lloyd A. Culp, Barrett J. Rollins, Ben A. Murray, Dept. of Microbiology, Case Western Reserve University, School of Medicine, Cleveland, Ohio 44106.

When normal or SV40-transformed Balb/c 3T3 cells are treated with the Ca"-specific chelator EGTA, they round up and pull away from their footpad adhesion sites to the serum-coated tissue culture substrate as shown by scanning electron microscope studies. Elastic membranous retraction fibers break upon culture agitation, leaving adhesion sites as substrate-attached material (SAM; cells leave "footprints" of substrate adhesion sites during movement by a very similar process). SAM contains 1-2 percent of the cell's total protein and phospholipid content and 5-10 percent of its glucosamine-radiolabeled polysaccharide, most of which is glycosaminoglycan (GAG). There is considerable enrichment in SAM for specific GAG's; for the LETS glycoprotein; and for the cytoskeletal proteins actin, myosin, and the subunit protein of the 10nm-diameter filaments. The GAG composition in SAM has been examined under different cellular growth and attachment conditions. Heparan sulfate content correlates with glycopeptide content (derived from glycoprotein). Newly-attaching cells deposit SAM with principally heparan sulfate and LETS and little of the other GAG's. Hyaluronate and chondroitin proteoglycans are coordinately deposited in SAM as cells begin spreading and movement over the substrate. Cells attaching to serum-coated or CIg-coated substrates deposited SAM with identical compositions. The proteoglycan nature of the GAG's in SAM has been examined, as well as the ability of proteoglycans to form two classes of reversibly-dissociable "supramolecular complexes" - one class with heparan sulfate and glycopeptide-containing material and the second with hyaluronate-chondroitin complexes. Enzymatic digestion of "intact" SAM with trypsin or testicular hyaluronidase indicate that (A) only a small portion of longterm-radiolabeled LETS and cytoskeletal protein is bound to the substrate via hyaluronate or chondroitin classes of GAG; (B) most of the LETS, cytoskeletal protein, and heparan sulfate coordinately resist solubilization; and (C) newly-synthesized LETS, which is metabolically labile in SAM, is linked to SAM by hyaluronate-and/or chondroitin-dependent binding. All of our studies indicate that heparan sulfate is a direct mediator of adhesion of cells to the substrate, possibly by binding to both cell surface LETS and substrate-bound CIg in the serum coating; hyaluronate--chondroitin complexes in SAM appear to be most important in motility of cells by binding and labilizing LETS at the periphery of footpad adhesions with subsequent cytoskeletal disorganization.

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429 METABOLIC REGULATION IN SUSPENDED AND REATTACHED ANCHORAGE-DEPENDENT FIBROBLASTS, S. Penman, Massachusetts Institute of Technology, Dept. of Biology, Cambridge, Massachusetts 02139

A model system has been developed for the study of the metabolic control of cells exerted by the interaction of cell surfaces with the external milieu. Anchorage-dependent fibroblasts are incubated in methocel suspension or reattached to a solid surface. Suspended cells are profoundly altered in macromolecular metabolism. Nucleolar synthesis and processing cease abruptly. Messenger production, but not hnRNA synthesis, is inhibited to 20% of control within 5 hours. Messenger RNA turnover essentially ceases, the molecules are withdrawn from active translation and stored in a form free of the cytoskeleton. mRNA is altered and no longer translatable in a cell-free translation system. Cell reattachment results in rapid restoration of messenger translatability and cellular protein synthesis. Only later nucleolar function and messenger production resume.

Recovery depends primarily on cell-surface contact and not on subsequent cell spreading. Concanavallin A prevents cell spreading but permits a full restoration of protein synthesis. Colchicine and cytochalasin also inhibit spreading and give similar results. Reattachment after prolonged suspension culture results in a supranormal production of actin. Gamma actin, normally a minor component in fibroblasts, is now synthesized at the rate of beta actin. Other major structural proteins such as tubulin and 58K intermediate filament protein are regulated by cellular architectural organization but through very different mechanisms. Models of their control systems will be presented.

The 3T6 fibroblasts examined here are an established line and have properties intermediate between fibroblasts from primary explants, which exhibit much more prompt inhibitions of macromolecular processes, and anchorage-independent cells which are indifferent to the mode of culture.

430 CELLULAR AND MOLECULAR SPECIFICITY IN THE INTERACTION OF ADHESION PROTEINS WITH COLLAGEN AND WITH CELLS. Hynda K. Kleinman, A. Tyl Hewitt, John P. Pennypacker, Ermona B. McGoodwin, George R. Martin and Peter H. Fishman*, NIDR and *NINCDS, NIH, Bethesda, MD 20014.

It is well established that fibronectin mediates the adhesion of fibroblasts to collagen, binding first to the collagen substrate and subsequently to the cells. The binding site for fibronectin in the α1(I) chain of type I collagen has been localized to a specific sequence of amino acids that lacks carbohydrate and contains the bond cleaved by animal collagenase.

In addition to fibronectin, there may be other cell specific adhesion proteins. For example, chondrocytes in a cartilage matrix do not synthesize fibronectin and since the tissue is avascular, they are probably not exposed to it. We have found that purified fibronectin does not stimulate chondrocyte adhesion, whereas whole serum and fibronectin-free serum do. The chondrocyte attachment activity in serum has been separated from that for fibroblasts by DEAE-cellulose column chromatography. The serum factor for chondrocyte adhesion loses 50% of its activity after incubation at 52° for thirty minutes, while fibronectin loses only 7% of its activity at this temperature. In addition, chondrocytes in culture synthesize a factor which promotes chondrocyte adhesion. We therefore conclude that chondrocytes adhere via a serum component other than fibronectin and propose the name chondronectin for this material.

Recently we have also attempted to identify the receptor for fibronectin on the cell surface. Incubation of mixed brain gangliosides with the fibronectin-collagen complex prior to the addition of cells blocks cell adhesion. An inhibition of cell attachment was observed with the oligosaccharide portion of the ganglioside, although it was not active to the same extent as that observed with the intact gangliosides. Ceramides, the lipid portion of the gangliosides, and free sugars including sialic acid, glucose, galactose, N-acetyl glucosamine and N-acetyl galactosamine did not inhibit cell adhesion. Glycosaminoglycans, such as heparin, heparan sulfate, keratan sulfate, dermatan sulfate, chondroitin sulfate and hyaluronic acid, were also ineffective. Of the gangliosides tested, GM_1 was inactive while GD_{1a} and GT_1 produced an equal inhibition of cell attachment and were more effective than the mixed gangliosides. Modification of terminal sialic acid residues by mild periodate treatment destroyed the activity of both GD_{1a} and mixed gangliosides. Our studies indicate that specific gangliosides (GD_{1a} and GT_1^a) are able to interact with fibronectin and block cell attachment to the fibronectin-collagen complex. Thus, the oligosaccharide chains of certain gangliosides (or other glycoconjugates) on the cell surface may serve as the binding site for fibronectin.

Hormones and Growth Factors

431 GROWTH FACTOR INTERACTIONS WITH NORMAL AND TRANSFORMED CELLS, George J. Todaro, Laboratory of Viral Carcinogenesis, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014

An hypothesis is presented that states that tumor viruses produce their transforming action on cells, at least in part, by the production of endogenous polypeptide growth stimulatory factors. Cells transformed by murine sarcoma viruses have been found to have reduced or absent cell surface receptors for epidermal growth factor (EGF) as compared to the untransformed parental cells and to cells transformed by other viruses. Sarcoma virus transformed cells are shown to release a family of polypeptide growth factors into the supernatant fluids in cell cultures. These, with molecular weights of approximately 25,000, 12,000 and 7,000 daltons, stimulate cell division and compete for EGF receptors. The new sarcoma growth factors (SGFs) are not produced by untransformed cells or by DNA virus transformed cells. SGFs induce normal fibroblasts to grow in soft agar and to express some of the phenotypic properties of transformed cells; these effects are dependent on the continued presence of the factor. A human fibrosarcoma was found that had normal levels of EGF receptors but no apparent multiplication stimulating activity (MSA) receptors. Since normal fibroblasts have MSA receptors, it was decided to test whether the receptor-negative cell was producing a related growth factor. In this case, a family of related growth polypeptides were found that interacted with MSA receptors, but not with EGF receptors, and stimulated cell division in cells of various species. The activities of both the SGFs and the MSA-related human fibrosarcoma growth factors are heat stable, protease sensitive and sensitive to disulfide reducing agents. The endogenous production of polypeptide growth factors by cells that are able to respond to their own products may represent a general mechanism for cell transformation.

432 THE PLATELET-DERIVED GROWTH FACTOR, Russell Ross, Elaine Raines, Beverly Kariya and Arthur Vogel, Department of Pathology, School of Medicine, University of Washington Seattle, Washington 98195.

The platelet-derived growth factor is a mitogen present in whole blood serum, that is derived from the process of platelet aggregation, or adherence, and release during the process of serum formation. This mitogen is principally responsible for the growth promoting activity present in whole blood serum that is missing in cell-free, plasma-derived serum. Isolation and purification of the platelet factor has resulted from a series of chromatographic steps utilizing CM-Sephadex, Biogel P-150, and DEAE Sephadex followed by Hydroxylapatite. The material eluted from Hydroxyl-apatite columns presents a doublet of active material o- acid urea gels. Elution of this material from the acid urea gels and examined on SDS anyalytical gels demonstrate three molecular weight regions, 18,000, 34,000 and 60,000. The material is stable in 7 M guanidine, 8 M urea, 2% SDS and Ph 4. Its activity is destoryed by trypsin and also is destroyed by reduction with dithiothreitol and alkylation with iodacetamid. The platelet factor induces mitogenesis cooperatively with molecules in plasma. In the absence of plasma, it will induce cells to enter S, however cell doubling will not occur. Increased cell doubling is correlated in a dose response fashion with increased amounts of plasma. If plasma is limiting (less than 0.5%) excess amounts of platelet factor will not induce multiple cell doubling. Experiments with limiting amounts of platelet factor and/or plasma demonstrate that the molecule that limits saturation density for 3T3 cells in culture is the platelet factor and not molecules present in plasma. Cells shown to be susceptible to the mitogenic effects of the platelet factor include fibroblasts, 3T3 cells, smooth muscle cells and glial cells. One important exception to this observation is the arterial endothelial cell which grows equally well in plasma containing medium in the presence or absence of platelet factor.

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433 ROLE OF THE CELL SURFACE IN THROMBIN-STIMULATED CELL DIVISION, Dennis D. Cunningham, Darrell H. Carney, and Kevin C. Glenn, Department of Microbiology, University of California, Irvine 92717.

Addition of highly purified thrombin (TH) to serum-free cultures of early passage human, mouse, or chick fibroblastic cell strains produces a 40 to 80% increase in cell number (1). To determine the cellular site of TH action, we linked ^{125}I-TH to carboxylate-modified polystyrene beads via a peptide bond. The TH beads stimulated cell division, while beads with nonmitogenic proteins linked to them did not. The stimulation by the TH beads was not a result of release of TH into the culture medium or directly into the cells. Thus, thrombin can initiate cell division by action at the cell surface (2). This prompted us to examine the cell surface for interactions and changes involved in the mitogenic stimulation by TH. Binding studies with ^{125}I-TH led to the identification of a single affinity class of cell surface receptor that was specific for TH. Modulation of the occupancy of this receptor by TH led to corresponding changes in the initiation of cell division, indicating that TH must bind to its receptor to cause cell division (3). Inactivation of TH with either DFP or PMSF did not change its binding properties, but did eliminate its ability to produce an increase in cell number. This requirement for proteolysis led us to look for cell surface cleavages that are involved in the initiation of cell division by TH. Our studies revealed that TH cleaved a cell surface component of 43,000 daltons (43k) on cells that were responsive to its mitogenic action. An apparently similar cell surface component was present on four separately isolated populations of cells that were unresponsive to TH but fully responsive to the mitogenic action of serum. However, on each of these unresponsive cell populations, 43k was not cleaved by TH, indicating that proteolysis of it is necessary for TH-stimulated cell division. Experiments in which the concentration of TH and the duration of TH treatment were varied revealed a close relationship between removal of 43k and stimulation of cell division. We radiolabeled the cell surface receptor for TH with a photoreactive derivative of TH and found that the receptor has a molecular weight of 43,000 daltons. Taken together, these studies indicate that thrombin must bind to its receptor and cleave it to stimulate cell division. (Supported by USPHS Grant CA-12306).

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434 MITOGENIC HORMONE-INDUCED COMODULATION OF THE EGF RECEPTOR. C. Fred Fox, Michael Wrann and Ron Vale, Dept. of Microbiology, Molecular Biology Institute, University of California, Los Angeles, CA 90024

Incubation of many polypeptide hormones with cells induces a loss in the binding capacity for the hormone, a phenomenon termed "down regulation". This has been established in a number of cases, e.g., insulin, human growth hormone and EGF. EGF receptor down regulation is accompanied by the incorporation of EGF into a compartment inaccessible to protease and antibody to EGF (1). Fluorescent derivatives of EGF (2-3) and insulin (3) appear intracellularly in vesicles (2-3); both appear together in the same vesicles (3). In cultured cells treated to affinity label the EGF receptor (4-5), the EGF-receptor complex cofractionates with the lysosomal fraction (6-8) and appears there as lower molecular weight products (5-8). With the EGF receptor, down regulation can be quantitatively accounted for by intracellular uptake, and a major biological activity of EGF, induction of DNA synthesis, has the same EGF requirement as does down regulation (6-8). We have determined the influence of different mitogenic polypeptide hormones on EGF binding by cultured murine 3T3 cells. When cells are incubated with FGF or PDGF and EGF binding measured six hours later, no effect is observed (8). When FGF or PDGF is added to 3T3 cells and EGF is added one hour later, down regulation occurring during a subsequent six hour incubation is strongly inhibited. This does not arise by PDGF or FGF acting at the EGF binding site; neither of these hormones directly competes with EGF for binding to its receptor. When PDGF or FGF alone is added to cells, both induce a transient down regulation of the EGF receptor. This reaches a maximum approximately 1-2 hr following FGF or PDGF addition. The time of maximal enhancement of transient down regulation corresponds well with the time of maximal inhibition of nontransient EGF receptor down regulation by EGF. Transient EGF receptor down regulation by FGF and PDGF is enhanced at low concentrations of EGF, where EGF by itself produces no significant down regulation. These data best fit a model whereby FGF, EGF and PDGF share the same down regulation mechanism. FGF or PDGF initially enhances down regulation of the EGF receptor by promoting formation of common endocytic vesicles shared by these receptors. The inhibitory phenomenon provides an indication that some common factor is both necessary and limiting for receptor internalization. References: 1) Carpenter & Cohen 1976 J Cell Biol 71:159; 2) Haigler et al 1978 PNAS 75:3317; 3) Maxfield et al 1978 Cell 14:1805; 4) Das et al 1977 PNAS 74:2790; 5) Das et al 1978 in Cell Surface Carbohydrates and Biological Recognition, pub. Liss, NY, p 647; 6) Das & Fox 1978 PNAS 75:2644; 7) Fox & Das 1979 in Transmembrane Signalling, pub. Liss, NY, in press; 8) Fox et al 1979 Cold Spring Harbor, in press. Supported by grants from MDAA, ACS & USPHS. MW is a Max Kade Foundation fellow.

435 GLYCOLIPIDS: A CLASS OF MEMBRANE RECEPTORS, D.R. Critchley and S. Ansell, Department of Biochemistry, University of Leicester, Leicester, LE1 7RH.

Much experimental evidence suggests that changes in cell surface organization are associated with the malignant phenotype, one such change being loss of the more complex glycolipids[1]. Whilst the significance of loss of more complex glycolipids from the malignant cell surface remains unclear, glycolipids have been implicated as determinants of cellular interaction and as receptors for the glycoprotein hormones and certain bacterial toxins.[2] However, although much of the evidence is substantial, it is largely indirect. We have recently provided direct evidence that glycolipid GM_1 is indeed the cholera toxin receptor at least in mouse fibroblasts using immunoprecipitation. GM_1 is the only glycolipid recovered when intact surface labelled (galactose oxidase or periodate borotritiide) Balb/c or Swiss 3T3 cells are exposed to toxin and the toxin-receptor complexes isolated from NP-40 extracts of the cells by addition of anti-toxin followed by protein A-containing strains of S.aureus. Glycolipids GM_3 and GD_{1a}, the major cellular glycolipids, and the ones most strongly labelled by the periodate procedure, were not recovered by this method. GM_1 was not found adsorbed to the bacterial pellet if cholera toxin or toxin antibody was omitted during the isolation procedure. There would thus appear to be little doubt that GM_1 is the cholera toxin receptor at least in mouse fibroblasts, although following the tentative identification[3] of a cell surface galactoprotein in these cells that cross-reacts with antibodies to GM_1, it remains possible that a glycoprotein receptor for cholera toxin is yet to be discovered. However we have been unable to identify such a glycoprotein following SDS-PAGE of immunoprecipitates prepared as described above. We have also characterised the major species of toxin receptor on a lymphoid cell line as GM_1. Interestingly interaction of cholera toxin with its receptors on lymphocytes induces patching and capping in a manner reported to be inhibited by cytochalasin B and azide,[4] and we are presently looking at possible ways in which GM_1 might interact with the cytoskeletal system. In conclusion, given that glycolipids are a class of membrane receptors, then their loss from the cell surface may be an important factor in the malignant process.

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Transport

436 EARLY EVENTS IN GROWTH STIMULATION, Enrique Rozengurt, Imperial Cancer Research Fund Laboratories, Lincoln's Inn Fields, London, WC2A 3PX.

Normal, untransformed fibroblasts reduce their rate of entry into S(DNA synthesizing) phase of the cell cycle and accumulate in G_1G_0 when the culture medium becomes depleted of growth factors. Addition of serum to such quiescent cultures stimulates DNA synthesis and cell division. Furthermore, quiescent cultures of fibroblasts can be stimulated to enter S phase by growth-stimulating hormones, factors produced by cells and tumor promoters. Certain combinations of these agents act synergistically in serum-free medium.

A complex array of biochemical changes precedes the onset of DNA synthesis (9-15 hours) in serum-stimulated cells. Since it is possible to stimulate DNA synthesis in a completely defined medium, more critical questions can be asked regarding the biochemical events that occur between recognition of a defined proliferative signal and the actual onset of DNA synthesis. As a first step, it is possible to define which early events stimulated by serum, in fact, are also induced by highly purified mitogenic molecules.

A central problem in understanding the mechanism of action of growth promoting factors is to elucidate how, after binding to specific surface receptors, such factors elicit metabolic responses in the cell. We are approaching this question by examining the biochemical basis of the early metabolic responses induced by serum or growth-promoting factors in quiescent cells. Our attention has been focused on three prominent responses: the increase in the uptake of nucleosides, the acceleration of the glycolytic flow and the increase in the transmembrane flux of monovalent ions. Recently, advances have been made in defining the biochemical basis of these early events in quiescent 3T3 cells. The increase in the uptake of nucleosides results from an enhanced capacity of stimulated cells to phosphorylate nucleosides rather than from a change in the rate of uridine translocation across the membrane. The acceleration of the glycolytic flow in cultures of Swiss 3T3 cells appears to result from an increase in the activity of the phosphofructokinase, as revealed in cellular homogentates of stimulated cells. Finally, a model which is consistent with studies on $^{86}Rb^+$, $^{22}Na^+$ and Li^+ transport is proposed for the regulation of the Na^+-K^+ pump: (i) that there is a Na channel in the plasma membrane of fibroblasts; (ii) that growth factors increase Na^+ entry via the channel; (iii) that the activity of the Na^+-K^+ pump in fibroblasts is limited by the supply of internal Na^+; and (iv) growth factors appear to stimulate the Na^+-K^+ pump in quiescent 3T3 cells by increasing its supply of Na^+. These findings prompted us to test whether substances that promote Na^+ entry can regulate cell proliferation. We discovered that vasopressins are potent mitogens for 3T3 cells. This hormone provides a convenient model molecule to further studies on the mechanism of action of growth-promoting peptides.

437 REGULATION OF TRANSPORT AND MEMBRANE FUNCTION, Julia E. Lever, The Salk Institute, 10010 N. Torrey Pines Road, La Jolla, CA 92037

The functional interplay between regulation of membrane transport systems and regulation of intracellular processes, cell proliferation and gene expression has been investigated by diverse approaches using several types of cell culture systems as well as plasma membrane vesicles derived from these cultures.

Fibroblast cultures have been used as a model to investigate the transport changes which accompany growth regulation. These changes can be analyzed in terms of the transport mechanism using membrane vesicles. Fibroblast plasma membrane vesicles exhibited several nutrient transport functions (1). Active transport of neutral amino acids (A-System) and monovalent phosphate ion (P-system) (2) were energized by Na^+ gradients artificially imposed across the membrane. Interior-negative membrane potentials provided an additional driving force for A-system amino acid accumulation but Na^+-dependent phosphate transport was electrically silent. Whereas many characteristics of the transport systems of intact cells were also expressed in vesicles, important differences were observed -- notably in the case of glucose transport (3). Correlative studies using vesicles implicated protein kinase-mediated membrane phosphorylation as an element which rapidly modulates transport in fibroblasts (4).

Epithelial cell culture systems were used as a model for differentiation of plasma membrane transport properties (5). Specialized epithelial transport functions were manifest in both the Rama 25 rat mammary and the MDCK dog kidney epithelial cell lines as 'domes' of cells resulting from fluid accumulation beneath the cell monolayer. A broad spectrum of known inducers of mammalian cell differentiation stimulated dome formation in these cell lines. Dome formation was also increased by conditions expected to elevate intracellular levels of cyclic AMP. These results suggest a hypothesis that domes arise in these cultures by a process analagous to differentiation and regulated by cyclic AMP.

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438 USE OF CYTOCHALASINS FOR STUDYING SUGAR TRANSPORT AND CELL MOTILITY IN MAMMALIAN CELLS, Diane Chang Lin, Andrew Lees, and Shin Lin, Department of Biophysics, Johns Hopkins University, Baltimore, MD, 21218.

The fungal metabolite cytochalasin B (CB) is a potent inhibitor of sugar transport and cell motility in mammalian cells. Two derivatives of this drug are being used in our laboratory for identifying cellular components involved in the drug-sensitive processes.

Dihydrocytochalasin B (H_2CB), formed by reduction of CB with $NaBH_4$, has no effect on sugar transport even at high concentrations, but is similar to CB in affecting cell motility and morphology. In the human red cell, [^{3}H]H_2CB binds with high affinity to a high molecular weight complex peripherally associated with the cell membrane; SDS-acrylamide gel electrophoresis showed that the complex consists of spectrin, actin, band 4.1, and some minor protein components. The relative affinities of several cytochalasins (CB, CD, CE, H_2CB) for the binding complex correlate with their relative potencies in affecting cell motility and morphology in several biological systems. Preliminary data indicate that the complex, isolated from a low ionic strength extract of red cell membranes, can act as an initiator of actin polymerization in vitro, suggesting that it functions as a regulatory site for formation and membrane-attachment of actin-containing microfilaments in vivo.

The second derivative is CB-C7-monoacetate (CBAc), prepared by acetylation of the C-7 and C-20 hydroxyl groups of CB with acetic anhydride, followed by selective removal of the C-20 acetyl group by mild alkaline hydrolysis. This derivative is an effective inhibitor of sugar transport, but does not affect cell motility and morphology in fibroblasts even at high concentrations. [^{3}H]CB has previously been shown to bind with high affinity to sugar transport proteins in the human red cell. Competitive binding experiments showed that the affinity of CBAc for these proteins is 3 to 4 times less than that of [^{3}H]CB; in contrast, the affinity of this derivative for high-affinity binding sites associated with red cell spectrin and actin is 20 to 30 times less than that of [^{3}H]H_2CB. These results indicate that CBAc will be a useful tool for identifying sugar transport proteins in cells such as fibroblasts, where most of the high-affinity CB binding sites are apparently not related to sugar transport. (This work was supported by grants GM-22289, AG-00993, and RCDA-GM-00241 from the National Institutes of Health, and grant VC-288 from the American Cancer Society.)

Viral Proteins and the Cell Surface

439 THE TRANSFORMING GENE PRODUCT OF AVIAN SARCOMA VIRUSES, R.L. Erikson, J.S. Brugge, M.S. Collett, E. Erikson, and A.F. Purchio, Department of Pathology, University of Colorado Medical Center, Denver, Colorado 80262

The product of the avian sarcoma virus (ASV) transforming gene (sarcoma, src) is a phosphoprotein with a molecular weight of 60,000, denoted pp60^{src} (1-4). This protein appears to act as a protein kinase when assayed in specific immune complexes (5,6). Since all ASV strains examined to date encode a similar src gene polypeptide and since the pp60^{src}-kinase activity is growth-temperature dependent in cells transformed with an ASV temperature-sensitive transformation mutant (6) it is possible that ASV-induced oncogenesis may be mediated by aberrant phosphorylation of cellular proteins by pp60^{src}. It is interesting that pp60^{src} is itself a phosphoprotein because it is well-established that the phosphorylation state of various enzymes influences their function. Studies on the sites of phosphorylation of pp60^{src} and enzymatic activities that influence phosphorylation will be presented and discussed. A protein immunologically and structurally similar to ASV pp60^{src} has been identified in normal uninfected avian cells (7) and will also be discussed.

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440 ON THE BIOCHEMISTRY OF TRANSFORMATION BY ROUS SARCOMA VIRUS. S. J. Singer*, Roger C. Carroll*, J. F. Ash*, Michael H. Heggeness*, and Peter K. Vogt**, University of California at San Diego*, La Jolla, California 92093 and University of Southern California School of Medicine**, Los Angeles, California 90033.

We have been studying phenotypic properties associated with the transformation of fibroblasts by Rous sarcoma virus (RSV) employing a temperature-sensitive mutant (LA23) of RSV infecting a normal rat kidney (NRK) cell line. LA23-NRK cells grown at 33° exhibit the transformed phenotype: the cells are rounded, cell surface receptors are mobile in the plane of the membrane, and the cytoskeleton is comparatively disaggregated (1). When grown at 39°, they exhibit the normal phenotype: the cells are flat, surface receptors are not mobile over large distances, and the cytoskeleton (stress fibers and microtubules) extends the length of the cell. Furthermore, at 33° the cells show an enhanced rate of aerobic glycolysis compared to 39°, and this rate shows an altered sensitivity towards dinitrophenol and oligomycin compared to normal (2). If the LA23-NRK cells are grown at 33° in the presence of cycloheximide or other inhibitors of protein synthesis, within several hours they show a reversion of the transformed phenotype to the normal with respect to the characteristics described. These results led us to propose that transformation by the src gene of RSV is a pleiotypic and reversible process, such as is involved in a pleiotypic enzymic modification reaction and its reversal. These results conform with the recent demonstration (3) that the src gene product has, or is closely associated with, a protein kinase activity. Experiments designed to detect which cell components might be affected in vivo by such a kinase activity, and the possible mechanisms involved in generating the new steady state of the transformed cell, will be discussed.

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441 MOLECULAR FORMS OF POLYOMA VIRUS TUMOR ANTIGENS, Dr. Thomas L. Benjamin, Department of Pathology, Harvard Medical School, 25 Shattuck St., Boston, Ma. 02115

Sera from rats carrying syngeneic polyoma virus-induced tumors precipitate five species of T antigen from lytically infected or transformed cells. Estimates of molecular weight by SDS-PAGE are 100K, 63K, 56K, 36K and 22K[1]. The 100K protein, called the major or "big" T antigen, is located largely or entirely in the nucleus. It is thermolabile in cells infected by ts-a mutants[2,3]. The 56K is associated with the plasma membrane[4,5], and the other forms appear to be present predominantly in the 'cytosol' fraction. Hr-t mutants are affected in some or all of the minor T antigen species. Deletion hr-t mutants fail to induce all our minor bands, while non-deletion hr-t mutants induce the 56K and 22K (reduced amount) but not the 63K and 36K species[6]. It is likely that multiple RNA splicing events are involved in the production of the multiple T antigen species, and that sequences in the hr-t region of the viral DNA are involved in the splicing. These two early viral genes - ts-a and hr-t - define two distinct functions essential for cell transformation as well as viral growth.

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442 EXPRESSION OF MOLONEY AND ABELSON MURINE LEUKEMIA VIRUSES AT THE CELL SURFACE, O.N. Witte*, N. Rosenberg+ and D. Baltimore*, *Center for Cancer Research, M.I.T., Cambridge, Mass. and +Department of Pathology, Tufts University School of Medicine, Boston, Mass.

Murine leukemia viruses (MuLV) replicate in cells without affecting the normal host cell macromolecular synthetic processes. The assembly of their enveloped virions provides a model for the control of specific protein interactions at the cell membrane.

Moloney (M) MuLV is a replication competent virus that transforms "T" lymphoid cells in vivo. This virus encodes 3 large polyproteins. Precursors for the core structural proteins and the virion polymerase are translated on cytoplasmic ribosomes, rapidly bind to the cell membrane and assemble a budding structure. Cleavage of these precursors occurs during the final stages of the budding process. A separate precursor for the viral envelope proteins is translated on membrane bound ribosomes and is nascently glycosylated by high mannose groups. Its carbohydrate is subsequently processed and the protein cleaved during maturation to the cell membrane where it associates with core structures during the budding process.

Derived from M-MuLV, Abelson (A) MuLV is a replication defective virus which transforms "B" lymphoid stem cells. A-MuLV has retained homologous regions to M-MuLV at the 5' and 3' ends of its genome but has a large deletion-substitution region internally. Abelson MuLV expresses no functional structural polyprotein precursors like M-MuLV and hence it requires a helper virus. A-MuLV encodes a large polyprotein (120,000 MW) which represents a fusion protein between the N-terminal one-half of the Moloney core precursor and new information derived from the mouse genome. The P120 protein is strongly correlated with maintenance of the transformed state. P120 is not cleaved or incorporated into virions in mouse cells transformed by A-MuLV. Serum from mice rejecting a syngeneic A-MuLV lymphoid tumor contains antibody specific for the unique region of P120 which can stain viable A-MuLV transformed non-producer cells on the cell membrane.

Thus all the described proteins of M- and A-MuLV are found at the cell membrane. What mechanisms control the subsequent cleavages and assembly, or exclusion from budding structures are of continuing interest.

Cell Biology of Malignancy

443 CELL SURFACE COMPONENTS OF MALIGNANT MELANOMA VARIANTS SELECTED FOR ORGAN SPECIFICITY. Kenneth W. Brunson and Garth L. Nicolson. Department of Developmental and Cell Biology, University of California, Irvine, California 92717.

We have developed animal tumor systems for studying the organ specificity of blood-borne metastasis to brain (Brunson et al., NATURE 272:543, 1978) or ovary (Nicolson et al., CANCER RES. 38:4105, 1978). B16 melanoma line B16-F1 (from lung metastasis in C57BL/6 mouse) injected i.c. yielded tumors in brain (B16-B1) or ovary (B16-O1) which were cultured. Melanoma cells were re-injected i.c. or i.v. and this process repeated ten times to obtain lines B16-B10N and B16-O10 which were more metastatic to brain and ovary, respectively. To rule out simple organ adaptation selections were performed by direct intracerebral inoculation. In contrast to blood-borne selection for organ colonization, lines selected by organ inoculation (brain) formed the same number of metastases compared to parental lines. Lactoperoxidase-^{125}I-labeling of exposed surface proteins of intravascularly selected brain colonizing lines revealed increased exposure of components of ∿95,000 and ∿100,000 MW which correlated with enhanced brain metastasis, while lines selected for ovary metastasis showed increased labeling of components of ∿140,000 and ∿150,000 MW. Tumor lines selected ten times by direct brain inoculation were similar to parental cells in surface labeling. In vitro morphological changes associated with sequential selection to brain and ovary were revealed by phase contrast and SEM. Brain-selected lines (B16-B5, B16-B10N) were less spindle-shaped and flatter than B16-F1, and both had numerous long cellular projections. B16-O10 cells grew in colonies with round, rather smooth cell peripheries and were less pigmented than B16-F1.

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444 RELATIONSHIP OF FIBRONECTIN TO THE METASTATIC BEHAVIOR OF RAT MAMMARY ADENOCARCINOMA CELL LINES AND CLONES. Anthony Neri, Erkki Ruoslahti and Garth L. Nicolson, Department of Developmental and Cell Biology, University of California, Irvine, Calif. 92717 and Division of Immunology, City of Hope Medical Center, Duarte, Calif. 91010.

We have studied mammary tumor metastasis using 13762NF adenocarcinoma. Fisher 344 rats inoculated s.c. with tumor tissue showed no metastases at 23 days; however, at 40 days 13/21 and 4/21 animals had regional lymph node (RLN) and lung metastases, respectively. Cultures of primary and metastatic lesions were cloned. Biologic testing of clones revealed from 0/20 to 20/20 animals with lung and/or RLN metastases at 23 days. Clones from RLN or lung metastases preferred the lymphatic route rather than intravascular as judged by the number and size of positive RLN (40-90% animals at 23 days) prior to detection of lung metastases. In contrast, 3/4 primary tumor clones produced lung metastases (40-100% animals) without RLN involvement, and 1/4 primary tumor clones produced no metastases at 23 days. Subcutaneous tumor size was unique to each clone and not related to metastasis. In vitro morphology of clones varied from fibroblastic to epitheloid and was influenced by culture density. These observations indicate that tumor cell heterogeneity exists in the original parental tumor cell population with respect to growth and metastasis. Further studies were performed to determine the surface display and/or released amounts of fibronectin (LETS, CSP) using indirect IMF or competition RIA. Contrary to initial observations, these studies suggest that metastatic potentials of various clones are independent of surface exposed or released fibronectin.

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445 METASTATIC TUMOR CELLS INDUCE THE IN VITRO DEGRADATION OF ENDOTHELIAL CELL EXTRACELLULAR MATRIX. Randall H. Kramer and Garth L. Nicolson. Department of Developmental and Cell Biology, University of California, Irvine, California 92717.

The interaction of neoplastic cells with vascular endothelial cells and associated extracellular matrix has been studied in vitro. When B16-F1 metastatic melanoma cells are seeded on confluent calf aortic endothelial monolayers, a rapid retraction of the endothelial cells occurs at the site of tumor cell attachment. The effect of this activity on the integrity of the endothelial extracellular matrix (ECM) was examined by measuring the loss of radioactivity from $^{35}SO_4$-labeled endothelial monolayers. The ECM was isolated attached to the substratum by extraction of the endothelial monolayer with NP-40 detergent and is composed primarily of fibronectin and $^{35}SO_4$-labeled high molecular weight glycosaminoglycans (GAG's). A majority of the $^{35}SO_4$-labeled macromolecules are ECM-associated and are rapidly turned over by the endothelial cells. The introduction of B16-F1 melanoma cells onto $^{35}SO_4$-labeled endothelial monolayers results in an increase in the solubilization and degradation of ECM-associated GAG's which is related to the number of tumor cells added and correlates with time of endothelial retraction. In contrast, when tumor cells are seeded directly onto NP-40 exposed $^{35}SO_4$-labeled ECM, no significant solubilization or degradation of the ^{35}S-label is detected. These results suggest that: (1) tumor cells can induce the solubilization of endothelial basement membrane-like matrix; (2) this solubilization is mediated by the endothelial cells and (3) endothelial cells may actively participate in blood-borne tumor cell extravasation.

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446 IN VITRO GROWTH CHARACTERISTICS, MOTILITY AND ADHESIVE PROPERTIES OF METASTATIC VARIANT PW20 CELL LINES, Peter L. Salk and Robert P. Lanza, The Salk Institute for Biological Studies, San Diego, CA 92112.

In vitro growth characteristics, motility and adhesive properties have been examined in a family of tumor cell lines, derived from the polyoma-induced PW20 Wistar-Furth renal sarcoma, which vary in their spontaneous metastatic properties. Four of the lines are highly metastatic (88-100% of animals develop macroscopic metastases following excision of primary subcutaneous tumors), two are intermediately metastatic (67% incidence of metastasis), and three are poorly metastatic (0-20% incidence of metastasis). Cells from the poorly metastatic lines grow more rapdily in vitro and grow to higher density than cells from the metastatic lines. The motility of the cells in vitro, as judged both by colony morphology and by the formation of phagokinetic tracks on gold particle-coated coverslips, correlates directly with their metastatic activity. Cells from the metastatic lines are also more adhesive to each other and to plastic substrates, as determined by the degree of clumping of cell suspensions following trypsinization of cell monolayers, by the rate of attachment of cells to plastic tissue culture dishes following plating, and by the rate of trypsin-induced detachment of nonconfluent monolayer cells. Previous studies have shown that cells from the metastatic lines are more highly sialylated in vitro than cells from the poorly metastatic lines. Further experiments will be undertaken to examine the relationship between the surface sialylation of the cells and the in vitro properties observed in the present studies, and to determine the relevance of these parameters to the metastatic process.

447 SPONTANEOUS METASTATIC BEHAVIOR AND GLYCOSAMINOGLYCAN COMPOSITION OF B16 MELANOMAS. John S. Lazo, Charles A. Maniglia, Men Hui Lee, and Alan C. Sartorelli. Yale Univ. Sch. of Med., Dept. of Pharmacol. & Devel. Therap. Prog., Comp. Cancer Ctr., New Haven, CT 06510

Two non-selected B16 melanoma sublines (HM_3 and LM) were found to differ in spontaneous metastatic behavior. Subcutaneous implants of HM_3 and LM into syngeneic mice exhibited similar growth rates as measured by weight and host lethality as expressed by mean or median survival times. At death, 96% of mice bearing the HM_3 tumor had lung metastases while only 50% of animals with the LM tumor were similarly affected. No correlation existed between survival time and the number of lung metastases in mice bearing either tumor. Tumor glycosaminoglycan (GAG) composition was estimated by the amount of [^{3}H]glucosamine and [^{35}S] sulfate incorporated into cetylpyridinium chloride (CPC) precipitable material. HM_3 and LM tumors incorporated 850 ± 180 and 850 ± 270 dpm [^{3}H]glucosamine/mg dry tumor weight and 741 ± 170 and 730 ± 220 dpm [^{35}S] sulfate/mg dry tumor weight, respectively, into CPC precipitable material. Fractionation of this material with increasing concentrations of NaCl showed a significant difference in [^{3}H]glucosamine but not [^{35}S] sulfate distribution in the 2 tumors. Over 66% of total [^{3}H]glucosamine in GAG of the LM tumor was in the 0.4 M NaCl fraction compared to only 42% for the HM_3 tumor ($p \leq 0.01$). There was no significant difference in the amount of [^{3}H]glucosamine in the 0.8 M or 1.2 M NaCl fractions of HM_3 or LM tumors, but the LM tumor incorporated significantly less ($p \leq 0.05$) [^{3}H]glucosamine into the 2.0 M NaCl fraction. The spontaneous metastatic behavior of these 2 tumors may be related in part to an altered GAG composition.

448 PROPERTIES OF AN MSH-GROWTH RESISTANT VARIANT OF B16 MOUSE MELANOMA. Richard M. Niles and Mary P. Logue, Div. of Surgery & Dept. of Biochemistry, Boston Univ. School of Medicine, Boston, Mass. 02118

A variant of B16-F1 mouse melanoma was selected for its ability to survive and replicate in the presence of melanocyte stimulating hormone (MSH). Although the variant (MR-4) was completely resistant to growth inhibition by MSH, cyclic AMP was still able to block replication. Tyrosinase activity in MR-4 cells was considerably lower than in F1 cells. MSH induced a 2-3 fold increase in tyrosinase in both cell types, but the absolute activity from MR-4 remained significantly less than the parental cells. MR-4 cells were also found to have a markedly depressed cyclic AMP dependent protein kinase relative to F1 cells. The protein kinase from both cell types was stimulated by cyclic AMP, but the level of MR-4 kinase activity at maximal cyclic AMP concentrations remained considerably lower than F1 kinase activity under the same conditions. Preliminary observations concerning the biological behavior of MR-4 indicate they form tumors earlier and kill the host sooner than the parental F1 cells. We conclude that the biochemical alteration which allows MR-4 cells to replicate in the presence of MSH is a low level of tyrosinase which in turn may be the result of low cyclic AMP dependent protein kinase activity.

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449 CHARACTERIZATION OF INDUCED METASTATIC VARIANTS FROM A CLONED METASTATICALLY HOMOGENEOUS CELL LINE. James E. Talmadge, Jean R. Starkey, William C. Davis, Washington State University, Pullman, WA 99164.

A model of metastasis is presented which utilizes a cloned cell line, metastasizing to the lung, developed from the dimethylnitrosamine transformed syngenic hepatic epithelioid cell line IAR6-1-RT7. We demonstrate that this clonal line exhibits unique specific tumor associated antigens as assayed by antibody-complement and antibody dependent cellular cytotoxicity assays and has a homogeneous metastatic phenotype. We describe the results obtained from utilizing several techniques to reintroduce metastatic variance into the clonal population. Techniques used to alter metastatic phenotype include; induced resistance to antibody-dependent cytotoxicity and adaptation to ascites culture. From the selected variant populations we obtained clones with maximum variance in metastatic expression. The metastatic capabilities of the variant clones were examined in normal and immunologically manipulated animals. Studies on the mechanism of metastasis (trapping, arrest and survival) using ^{125}IUDR labeled cells are also reported. Immunological characteristics of the variant clones are presented. These include; antigen expression, antigen shedding, and cellular sensitivity to various facets of the immune system. Other membrane associated phenomena relevant to metastasis were studied. These include; cell production of enzymes involved in fibrin formation and fibrinolysis, glycosyl transferase production, and concentration of Con A receptors. These characteristics of membrane function are discussed as they relate to the observed metastatic capabilities.

450 IN VITRO SELECTION OF LYMPHOSARCOMA VARIANTS WITH ALTERED MALIGNANCY IN VIVO. Christopher L. Reading, Paula N. Belloni and Garth L. Nicolson. Department of Developmental and Cell Biology, University of California, Irvine, California 92717.

Using immobilized lectins we have selected lectin-attachment variants of malignant RAW117 lymphosarcoma tumor cell lines. The parental RAW117 line grows in suspension and was rapidly bound by immobilized-concanavalin A (Con A), Ricinus communis agglutinin I (RCA_I), peanut agglutinin (PNA) and wheat germ agglutinin (WGA). After attachment, cells remaining in suspension (<1%) were removed and subcultured. This selection process was repeated 7-10 times, and the selected cells tested for malignancy. Parental RAW117 line forms a few (1-10) liver tumors per $5x10^3$ i.v. injected cells after 14 days, while a variant selected on Con A seven times (RAW117/Con A^{-7}) forms >80 liver tumors. RAW117 cells selected 7-10 times on immobilized-WGA, -PNA or -RCA_I show no increase in liver tumor formation. In contrast, RAW117-H10, a variant line selected ten times in vivo for liver colonization (K.W. Brunson and G.L. Nicolson, J. NATL. CANCER INST., in press, 12/78 issue) forms >200 liver tumors. A variant selected ten times on insolubilized-WGA from RAW117-H10 (RAW117-H10/WGA^{-10}) forms only 1-5 liver tumors. Variants of the RAW117-H10 line selected on immobilized-RCA_I, -Con A or -PNA still formed >200 liver tumors. ^{125}I-lectin binding studies indicate decreased lectin binding sites on the selected lines. These studies indicate that alterations in cell surface lectin receptors can modify malignant behavior.

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451 SURFACE CHARACTERISTICS OF METASTATIC AND NONMETASTATIC CELL LINES FROM SINGLE HEPATOCELLULAR CARCINOMAS, Thomas M. Kloppel, D. James Morré, J. Michael Cherry and Linda B. Jacobsen, Purdue University, West Lafayette, IN 47907

In previous investigations, we correlated levels of sialic acid, gangliosides and ganglioside glycosyltransferases with tumorigenesis over a 24 week continuum of growth of hepatocellular neoplasms of the rat induced by the carcinogen N-2-fluorenylacetamide (Morré et al., J. Supramol. Struct., in press; Merritt et al., J. Natl. Cancer Inst. 60, 1313, 1978). However, metastatic tumors developed only rarely and were not analyzed. To investigate surface changes associated with metastasis, well (WDH)- and poorly (PDH)-differentiated hepatocellular carcinomas were transplanted to syngeneic recipients and, after several generations, carried in cell culture. From these initial isolates, metastatic and nonmetastatic lines were obtained and analyzed. As reported previously, the nonmetastatic lines, both WDH and PDH, were characterized by ganglioside patterns depleted in G_{M3} and elevated in the products of the monosialoganglioside pathway, G_{M1} + G_{D1a}. In contrast, metastatic isolates, whether derived from WDH or PDH, exhibited a restoration of G_{M3} and nearer normal levels of G_{M1} + G_{D1a}. The metastatic lines showed altered rates of reduction of exogenously supplied indophenol and ferricyanide and an apparently specific detachment response to exogenous cytochrome c. The findings point to consistent differences in surface glycolipids comparing metastatic and nonmetastatic cell lines derived from single hepatocellular carcinomas and further extend the concept that ganglioside deletions are not causal to tumorigenesis but are the end result of a cascade of events which apparently continues beyond the onset of malignancy.

Supported in part by a grant from the National Institutes of Health CA 18801.

452 TUMOR-ASSOCIATED ANTIGENIC DIFFERENCES BETWEEN THE PRIMARY AND THE DESCENDANT METASTATIC TUMOR CELL POPULATIONS, Eliezer Gorelik, Mina Fogel, Shraga Segal & Michael Feldman, Department of Cell Biology, Weizmann Institute of Science, Rehovot, Israel. We tested whether antigenic differences exist between cell populations of the local growth of the 3LL tumor (L-3LL) and its lung metastases (M-3LL). Normal C57BL/6 spleen cells sensitized in vitro during 5 days against L-3LL monolayers lysed preferentially L-3LL targets but not M-3LL tumor cell targets. Conversely, anti M-3LL sensitized lymphocytes killed M-3LL targets more efficiently than they killed L-3LL targets. Furthermore, spleen cells from mice bearing subcutaneous L-3LL tumors were cytotoxic to L-3LL targets significantly more than to M-3LL targets, and vice versa. M-3LL cells were found also to be more resistant in vitro and in vivo to natural killer cells than L-3LL tumor cells. Anti M-3LL lymphocytes generated both in vitro and in vivo, but not anti L-3LL lymphocytes, admixed with L-3LL or M-3LL tumor cells and inoculated into foot pads of syngeneic recipients suppressed the development of lung metastases These results suggest that metastatic cells are indeed phenotypic variants of the local growing tumor cell populations. Presumably, these variants are selected for their capacity to home to and grow in the lungs, and for their resistance to specific immune effects initially evoked against the local tumor and to nonspecific natural killer cells. These data might be of importance with respect to any rational approach to the problem of immunotherapy.

453 REMOTE EFFECTS OF NEOPLASIA: INHIBITION OF MURINE CEREBRUM CELL AGGREGATION BY SERUM FROM TUMOR-BEARING ANIMALS, Nelson L. Levy and Karen A. Abernethy, Duke University, Durham, N.C., 27710

Tumors have been shown to alter the function of cells and tissues, particularly those of the nervous system, at sites remote from either the primary or metastatic masses. We questioned whether the serum from animals bearing syngeneic tumors could inhibit the basic cell-cell interactions that occur during in vitro aggregation of embryonic murine cerebrum cells. Sera were obtained from C57Bl/6J male mice at intervals after the subcutaneous inoculation of either a methylcholanthrene-induced fibrosarcoma (M10), a melanoma (B16) or a lymphoma (EL4). Cerebrum cells from 14 day C57Bl/6J embryos were enzymatically dissociated, washed first in medium containing normal mouse serum and then in medium alone, and then incubated for 18 hr at 37° on a gyratory shaker in medium containing 2.5% serum, either from the tumor-bearing mice or from normal C57Bl/6J mice. No xenogeneic sera were used in any phase of the system. Aggregation was quantitated both by direct measurement of aggregate size and by coulter counter enumeration of the disappearance of free cells. Aggregates formed in sera from tumor-bearing animals showed a 30 to 70% reduction in volume relative to those formed in normal serum. These sera also had the same inhibitory effect on aggregates formed from allogeneic cerebrum cells. Three lines of evidence argue against these results having been due to an immune reaction to the syngeneic tumors: 1) the sera contained no detectable antibody against embryonic cerebrum cells; 2) serum from mice alloimmunized either with kidney or spleen cells did not inhibit aggregation; 3) inhibitory activity was elaborated into the supernatants of cultured tumor cells.

454 *PLASMA COFACTOR IN PLATELET-PLASMA MEMBRANE VESICLE INTERACTIONS.* Gabriel J. Gasic, James L. Catalfamo, and Tatiana B. Gasic. The University of Pennsylvania, PA. 19104. Plasma membrane vesicles shed by transformed cells interact with blood platelets in vitro and induce platelet aggregation and secretion with unique features (Cancer Res. 38:2950, 1978). This interaction requires a plasma cofactor(s) other than fibrinogen (J. Cell Biol. 79:240a, 1978). Isolation and purification of this cofactor(s) may contribute to an understanding of its role in cell surface interactions in our model, as well as in metastasis. Partial purification of the cofactor(s) has been accomplished by step ammonium sulfate (AS) fractionation (20,40,60,80% saturation) of EDTA anticoagulated rat plasma followed by gel filtration of the active AS precipitate (60%) on either Sephadex G200 or Biogel 0.5 m columns. Fractions were tested in the aggregometer for their capacity to restore reactivity of gel filtered platelets to the aggregating stimulus of purified plasma membrane vesicles shed by 15091A ascites mammary tumor cells. The only active fractions were 36 to 38. Comparison of Coomassie blue stained polypeptide bands of inactive and active fractions on ME-SDS-PAGE, 7.5% slab gels, revealed the presence of 2 bands in the active fractions which were not present in inactive ones. These 2 proteins with similar M.W. appear to be implicated in the activity. The cofactor(s) has an apparent M.W. of 130 K daltons as determined by 7.5% ME-SDS-PAGE and supported by gel chromatography data of the native protein. Preliminary studies have eliminated the possibility of the involvement of LETS subunits or IgG and suggest that some other protein(s), not fully identified, is the responsible cofactor(s) permitting platelet aggregation by tumor vesicles. (Supported by USPHS grant HL 18827).

455 PLATELET ADHESION TO COLLAGEN-COATED SUBSTRATA: ROLE OF FIBRONECTIN, Peter R. Hoffmann and Richard O. Hynes, Massachusetts Institute of Technology, Cambridge, MA 02139.
The clotting reaction can be initiated by the adhesion of platelets to collagen, followed by the subsequent spreading of the attached platelets on the collagen-coated surface, and the release of various agents from granules within the platelets. As the attachment and spreading of cultured cells to various substrata is known to involve fibronectin, we investigated the possible involvement of cold insoluble globulin (CIg) (plasma fibronectin) in the attachment and spreading of platelets on collagen-coated surfaces. Collagen-coated coverslips were prepared by drying acid-soluble collagen onto the coverslip. Platelets were prepared from citrated platelet-rich plasma by gel filtration. CIg was prepared by gelatin-Sepharose affinity chromatography. Platelets suspended in a modified Tyrode's Solution attached relatively poorly to collagen-coated coverslips, and did not flatten or spread on this surface, as seen by phase microscopy or SEM. However, when CIg was added to the platelet suspension, the number of adherent platelets increased, and the platelets spread and in some cases aggregated. By immunofluorescence microscopy, it was seen that the CIg was organized about the spread platelets in fine fibers. We therefore conclude that CIg may be involved in the spreading of platelets on collagen coated surfaces.

456 THE ASSOCIATION OF FIBRONECTIN WITH HUMAN PLATELETS, Mark H. Ginsberg, Charles Birdwell, Richard Painter, Vicky Byers, Lorna Taylor, & Edward Plow, Res. Inst. of Scripps Clinic, La Jolla, CA 92037
Platelets are circulating anucleate cells derived from megakaryocytes. Following stimulation by agents such as thrombin or collagen, platelets adhere firmly to collagen coated surfaces, to polymerizing fibrin, and to each other. Thus platelets represent a potential model for cellular adhesion to surfaces or to other cells. We have evaluated the possible presence of fibronectin related antigen in these cells. Human platelets were washed by a combination of centrifugation and gel filtration and were lysed by detergent and their fibronectin content measured in a radioimmunoassay. An average of 1.8 ug of fibronectin antigen/10^9 cells was detected, less than 5% of which could be attributed to carry-over of plasma fibronectin in the washing procedure. In addition, when the platelets were pelleted by centrifugation, all of the fibronectin antigen was present in the cell pellet. Thus, the fibronectin antigen was cell associated, but the possibility remained that it was bound to another type of cell contaminating the platelet suspension. To examine this possibility, fixed platelets were subjected to immunofluorescent staining by affinity purified anti fibronectin antibody. Virtually every platelet stained usually showing more than 10 pinpoints (~0.2 um diameter) of fluorescent staining per cell. Thus, platelets contain fibronectin antigen, the bulk of which is present in a punctate distribution. The known interaction of fibronectin with collagen, fibrin and its role in cell-cell adhesion suggests the possibility that this material mediates the generation of platelet adhesiveness.

457 INTERACTION OF FIBRINOGEN WITH SURFACE OF HUMAN PLATELETS ALTERED BY PROTEOLYTIC ENZYMES, Stefan Niewiarowski, Andrei Z. Budzynski, Gwendolyn J. Stewart and Thomas Morinelli, Temple University Health Sciences Center, Philadelphia, PA 19140
It is well established that surface membrane glycoproteins are involved in a number of platelet interactions. Suspensions of washed platelets of the human, incubated either with pronase (proteinase V of Streptomyces griseus) or with α chymotrypsin lost residues of membrane glycoproteins that stained with periodic acid-Schiff reagent. On the other hand, residues of α mannose and α glucose were exposed on the surface of these platelets as evidenced by their enhanced interaction (binding and aggregation) with concanavalin A. Intact platelets did not aggregate with fibrinogen and they did not bind ^{125}I-fibrinogen. Platelets treated with pronase or chymotrypsin bound specifically ^{125}I-fibrinogen and aggregated upon addition of this protein. Bivalent cations were required for fibrinogen binding to platelets and for subsequent aggregation. A Steck and Wallach plot indicated one type of high affinity binding sites for fibrinogen on pronase- or chymotrypsin-treated platelets ($K_d \sim 10^{11}$). Electron microscopic observations with ferritin labeled antifibrinogen antibodies showed patchy binding of fibrinogen to chymotrypsin-treated platelets. Experimental evidence suggests that fibrinogen binding sites on the platelet membranes can be made available through the alteration or rearrangement of surface glycoproteins brought about by proteolytic enzymes. Since fibrinogen has a bivalent and symmetrical structure it can be postulated that one molecule of this protein can bind to two platelets thus contributing to the formation of aggregates directly through bridging or indirectly through alteration of surface glycoproteins.

458 DECREASED PLATELET MEMBRANE GLYCOPROTEINS IN GLANZMANN'S THROMBASTHENIA: ASSOCIATION WITH AGGREGATION DEFECT AND WITH CLOT RETRACTION. Dolores M. Peterson, Joel L. Moake, Ronald S. Weinger and Sai-Hung Yu. The University of Texas Medical School at Houston.

Glanzmann's thrombasthenia, a hereditary platelet disorder, is characterized by absent aggregation to platelet aggregating agents except the antibiotic ristocetin in the presence of human von Willebrand's factor (vWF), and absent (or severely decreased) platelet-mediated clot retraction induced by thrombin. There are decreased amounts of membrane glycoproteins IIb and III in thrombasthenic platelets. Separation of platelet proteins using two-dimensional isoelectric focusing and SDS-PAGE show that IIb and III are major platelet membrane proteins and that GPIII of thrombasthenic platelets focuses at a more acidic pH than normal (Circ. 58: II-216, 1978). We report here that thrombasthenic platelets retract fibrin clots provided they are aggregated by ristocetin and vWF. These data indicate that the limited or absent clot retraction exhibited by thrombasthenic platelets reflects defective aggregation in response to thrombin rather than an abnormality either of platelet-fibrin interaction or of the contractile apparatus. Apparently, the attachment of thrombasthenic platelets to the fibrin meshwork occurs although the two major membrane glycoproteins IIb and III are decreased to less than 20% of normal amounts.

459 ISOLATION OF MUTANTS TEMPERATURE-SENSITIVE FOR EXPRESSION OF THE TRANSFORMED STATE FROM CHEMICALLY TRANSFORMED C3H/10T1/2 CELLS, Craig Boreiko, Sukdeb Mondal, and Charles Heidelberger, University of Southern California Comprehensive Cancer Center, 1303 N. Mission Road, Los Angeles, CA 90033.

The non-tumorigenic mouse embryo fibroblast cell line, C3H/10T1/2 Cl 8, can be oncogenically transformed by a variety of chemical and physical agents. Oncogenic transformation occurs without any apparent involvement of RNA tumor viruses. The isolation of cellular mutants temperature-sensitive for the expression of transformation should facilitate our understanding of the mechanisms which underly the chemically-induced oncogenic state. Such mutants have been isolated from N-methyl-N'-nitro-N-nitrosoguanidine-mutagenized populations of C3H/10T1/2 Cl 8 cells previously oncogenically transformed by 3-methylcholanthrene. C3H/10T1/2 Cl 8 cells are not able to grow in confluent monolayers or when suspended in methyl cellulose, making them relatively resistant under these conditions to the toxic effects of high concentrations of FUdR. Selection systems utilizing this resistance were employed to isolate revertant cells which exhibited transformed characteristics at 33^{o} and nontransformed traits at 39.5^{o}C. One class of mutants isolated was temperature-sensitive for the expression of such transformed traits as anchorage independence, high saturation density and transformed morphology. Other classes of mutants were found which exhibited temperature sensitivity only for saturation density and/or morphology. Thus, while the expression of numerous transformed traits sometimes appear to be coupled, we have been able to dissociate expression of these traits from each other. These mutants are being characterized. (Supported in part by Grant #R-80-5208 from the EPA.)

460 EARLY MORPHOLOGICAL CHANGES IN THE CELL SURFACE AND CYTOSKELETON INDUCED BY MALIGNANT TRANSFORMATION WITH RSV TS MUTANTS, C. Bruce Boschek, Institut fur Virologie, Frankfurter Str. 107, 63 GieBen, W.-Germany.

Using a combination of methods it has been possible to correlate various morphological alterations in chick fibroblasts infected with temperature sensitive transformation-defective mutants of Rous Sarcoma Virus. The temporal dynamics of these changes have been analyzed by use of the various techniques at different times following temperature shift. The fine structure of the cytoskeletal stress fibers has been examined by use of the selective mordanting effect of tannic acid on lead staining. Surface ruffles, which appear shortly after shifting from the restrictive to the permissive temperature are visible in the conventional scanning electron microscope and are brightly stained as well by indirect fluorescent-antibody labelling of certain cytoskeletal proteins such as actin. The fluorescent label may be visualized at high resolution by cathode luminescence in a scanning electron microscope equipped with photon detectors. This novel technique allows an exact localization of the stress fibers and their attachment sites in topographical relation to the surface ruffles.

461 ROLE OF THE MICROFIBRILLAR SYSTEM IN KNOB ACTION OF TRANSFORMED CELLS, William D. Meek and Theodore T. Puck. Eleanor Roosevelt Inst. Canc. Res., Denver, Colo. 80262.

Transformed cells characteristically display knobs (or blebs) distributed over their surface. Scanning electron microscopy (EM) and time-lapse cinematography on CHO-K1 cells reveal roughly spherical knobs of 1-4μm in diameter distributed densely around the cell periphery but sparsely in the central nuclear hillock and oscillating in and out of the membrane with a period of 15-60 sec. Cyclic AMP derivatives cause the phenomenon of reverse transformation in which the cell is converted into a fibroblastic morphology with disappearance of the knobs. A model was proposed attributing knob formation to the disorganization of the jointly operating microtubular and microfilamentous structure of the normal fibroblast. Evidence for this model includes the following: (1) either colcemid or cytochalasin B (CB) prevents the knob disappearance normally produced by cAMP, and elicits similar knobs from smooth-surfaced cells, (2) knob removal by cAMP is specific with little effect on microvilli and lamellipodia, (3) immunofluorescence with anti-actin or anti-tubulin sera reveals condensed, amorphous masses directly beneath the membrane of CB-treated cells instead of smooth, parallel fibrous patterns of reverse transformed cells or normal fibroblasts, (4) transmission EM sections show dense, elongated microfilament bundles and microtubules parallel to the long axis of the reverse transformed CHO cell, but sparse, random microtubules throughout the transformed cell and a disordered network of 3-7nm microfilaments beneath the knobs, (5) cell membranes at the end of telophase, when the spindle disorganizes and cleavage is complete, display typical knob activity as expected by this picture,(6) the Porter microtrabeculae demonstrated by high voltage EM with normal human fibroblasts is an intimate network involving both microtubules & microfilaments

462 MEMBRANE ACTIVITY OF MOUSE MAMMARY TUMOR 15091A CELLS IN VIVO: BLEBBING AND SHEDDING OF MEMBRANE VESICLES, Gwendolyn J. Stewart, Gabriel J. Gasic, Tatiana Gasic and James L. Catalfalmo, Temple Medical School and University of Pennsylvania Medical School, Philadelphia, PA 19140.

Under appropriate conditions normal and transformed cells of fibroblastic and epithelial origin exhibit spontaneous or cytochalasin induced blebbing in culture. The two types of blebbing differ from each other with respect to form and stability of blebs and arrangement of microfilaments in blebs. In both cases, the blebs were reported to remain attached and to resorb into the cell under appropriate conditions. Mouse mammary 15091A tumor cells grown in the mouse peritoneal cavity spontaneously form stable, bulbous blebs mounted on stalks (similar to those induced by cytochalasin D). However, the presence of a submembrane cortex of microfilaments resembled spontaneous blebbing. Blebbing was observed on cells in interphase and anaphase, indicating that the stage in the cell cycle was not the major factor inducing blebbing. In contrast to previous studies part of the blebs were shed into the suspending fluid as free membrane vesicles containing ribosomes and a submembrane cortex of microfilaments. Shedding was increased by in vitro incubation of tumor cells in minimal essential medium for one hour. Shed vesicles lost ribosomes and microfilaments during separation on sucrose density centrifugation with EDTA in the buffer. Shed vesicles have at least one biological activity, stimulation of platelet aggregation. This and other activities of vesicles could conceivably participitate in the pathology of malignancy.

463 ULTRASTRUCTURAL ANALYSIS OF THE RETINOID INDUCED REVERSAL OF EPITHELIAL HYPERPLASIA AND METAPLASIA, A. Matter, L. Müller-Salamin, I. Lasnitzki* and W. Bollag, F.Hoffmann-La Roche & Co.Ltd., Basle, Switzerland, *Strangeways Research Laboratory, Cambridge, England

The DMBA-induced skin papilloma of the mouse and the MCA induced hyperplasia and metaplasia in prostate organ cultures were studied in electron microscopy. The two types of tissues both showed a reversal of hyperplasia and metaplasia when treated with retinoids (= vitamin A and analogs), but this reversal was reached by means that are quite characteristic for a given type of tissue: a) in the skin, the DNA-synthetic activity was not influenced by retinoid treatment (Frigg and Torhorst, JNCI 58: 1365, 1977); there were, however, many necroses and an impressive mucous metaplasia (Matter and Bollag, Eur. J. Cancer 13: 831, 1977). The latter might be at least partly responsible for the cell loss, probably through a loss of anchorage in the prickle-cell layer. b) In the prostate, no mucous metaplasia was observed, but an important depression of DNA-synthetic activity (Lasnitzki and Goodman, Cancer Res. 34: 1564, 1974). The secretory apparatus reappeared, together with the microvilli, possibly induced by the slowing down of cell division (Müller-Salamin et al., JNCI, submitted).

464 RETINOID-INDUCED ADHESION OF SPONTANEOUSLY-TRANSFORMED MOUSE FIBROBLASTS (BALB/c 3T12-3CELLS), David Dion, Sergio Adamo, Irene Akalovsky, Pangala V. Bhat, Wlodzmierz Sasak, Carol S. Jones, Gerald J. Chader* and Luigi M. De Luca. National Cancer Institute, and National Eye Institute*. Bethesda, MD 20014.
Vitamin A analogues (retinoids) enhance the adhesion of 3T12 cell monolayers to the surface of the tissue culture flask. The enhanced adhesion is measured by an EDTA-mediated detachment assay and is dose-dependent over the range of 0.5 ug (0.17 uM) to 5 ug (1.17 uM) of retinoid per ml of medium. Of the sixteen retinoids tested the most active were retinol, retinoic acid and their 5,6 epoxyderivatives. The synthetic derivatives of retinoic acid with activity in promoting epithelial differentiation in organ cultures of hamster tracheas were also active in increasing adhesion of 3T12 cells. Retinoids devoid of biological activity in other systems were inactive in inducing adhesion. Cyclic adenosine monophosphate and guanosine monophosphate levels were not affected by retinoid treatment, at least between 6 and 72 hours after treatment. Unaffected was also the level of the 220,000 MW fibronectin as measured by surface iodination, immunofluorescence and metabolic labeling with [2-^{3}H]-mannose. However, retinoids caused a 60% increase in the amount of [2-^{3}H]mannose incorporated into total glycoconjugates and a specific increase in the metabolic labeling of a glycoconjugate of MW 180,000, as shown by SDS-PAGE.

465 CELLULAR DNA SYNTHESIS AND T ANTIGEN QUANTITATION IN SIMIAN VIRUS 40 tsA TRANSFORMED CHINESE HAMSTER EMBRYO CELLS, John M. Lehman, Ph.D., Department of Pathology, University of Colorado Medical Center, Denver, Colorado 80262
When SV_{40} tsA transformed cells are grown at the non-permissive temperature (40.5°C), the cells express a "normal" phenotype; however, at the permissive temperature (33° or 37°C) they express the transformed phenotype. In previous studies with tsA transformed Chinese hamster cells, we showed that at 40.5°C a large percentage of the cells were capable of entry in the S phase and in fact, this percentage was similar at the 33° or 37° temperature. In order to simultaneously quantitate the amount of Tumor (T) antigen and correlate T antigen with the stage of the cell cycle, a double staining procedure was utilized followed by measurement of single cells by flow microfluorometry.

Propidium iodide was utilized to assay cellular DNA and the T antigen was detected by fluorescent isothiocyanate labeled antibodies. Various controls, such as normal serum, normal cells, polyoma transformed cells and singly stained cells demonstrated that this procedure assayed with flow microfluorometry, was capable of quantitating DNA content and T antigen in the same cell. Preliminary data with SV_{40} wild type virus transformed cells demonstrated a similar staining pattern at both temperatures. The G_2 population had twice the green fluorescence (T antigen) as the G_1 cells. Data will be presented concerning the T antigen content of tsA transformed cells at both temperatures correlated with the DNA content of the cells. These studies will be extremely useful in obtaining the role of T antigen in control of the transformed phenotype and cellular DNA synthesis regulation.

466 KINETICS OF $^{3}H_2$DIDS-LABELED GLYCOCALYX RELEASE BY EHRLICH ASCITES TUMOR CELLS, Thomas C. Smith and Charles Levinson, Department of Physiology, The University of Texas Health Science Center, San Antonio, TX 78284
Ehrlich ascites tumor cells spontaneously release cell surface material (glycocalyx) into isotonic saline medium. Exposure of these cells to the tritium-labeled 4,4'-diisothiocyano-1,2-diphenyl ethane-2,2'-disulfonic acid ($^{3}H_2$DIDS) at 4°C leads to preferential labeling of the cell surface coat. We have combined studies of the kinetics of $^{3}H_2$DIDS-label release, the effects of enzymatic treatment and cell electrophoretic mobility to characterize the $^{3}H_2$DIDS-labeled components of the cell surface. Approximately 72% of cell-associated radioactivity is spontaneously released from cells after 5 hr at 22°C. The kinetics of release is consistent with first order loss of two fractions; a slow ($t_{\frac{1}{2}}$=360 min) component representing 33% of the radioactivity, and a fast ($t_{\frac{1}{2}}$=20 min) component representing 39%. Trypsin (1 µg/ml) also removes 72% of the labeled material within 30 min and converts the kinetics of release to that of a single component ($t_{\frac{1}{2}}$=5.2 min). The specific activity (SA) of material released by trypsin immediately after labeling is 90% of the SA of material spontaneously lost in 1 hr. However, trypsinization following a 2 hr period of spontaneous release yields material of reduced (39%) SA. Neither $^{3}H_2$DIDS labeling nor spontaneous loss of labeled material alters cell electrophoretic mobility. However, trypsinization immediately following labeling or after spontaneous release (2 hr) reduces mobility by 32%, suggesting that a fraction of the cell surface coat which contributes to the surface charge is not $^{3}H_2$DIDS labeled.
Supported by grant CA 10917, USPHS, National Cancer Institute.

467 GLYCOPROTEIN RELEASE FROM CELL SURFACES OF INTACT HUMAN ADENOCARCINOMAS. Gerald B. Dermer and Zoltán A. Tőkés, Hospital of the Good Samaritan and USC School of Medicine, LAC/USC Cancer Center, Los Angeles, California 90017.

It is possible to maintain the viability and metabolism of explants of intact human tumors for several days under conditions where three dimensional tissue integrity is conserved. Using such an in vitro organ culture system, we have studied the synthesis of glycoproteins (gp) by surgical specimens of adenocarcinomas and non-malignant epithelium. Gp synthesis was studied by autoradiography of explants pulse-labeled with ^{3}H-glucosamine and maintained in organ culture for up to 72 hours. Data indicate that human adenocarcinomas can be placed in one of two groups which depends on histogenesis. Adenocarcinomas derived from secretory epithelium (breast, prostate, etc.) retain secretory activity and rapidly secrete most newly synthesized gp; those derived from non-secretory epithelium (bronchus, colon, etc.) accumulate newly synthesized gp at luminal cell surfaces. This suggests a residence of the gp within plasma membranes. The difference in the cellular distribution of newly synthesized gp in the two groups provides diagnostic information which reduces the choices of possible primary sites for any adenocarcinoma. Labeled gp are slowly released over a period of several days from surfaces of normal and malignant non-secretory epithelium and enter the culture medium where they can be collected for study. Gp products released from normal secretory epithelium or from secretory adenocarcinomas are also found in spent medium. A heterogeneous collection of 18-22 different released ^{3}H-glucosamine labeled glycoproteins, in various proportions, can be identified using SDS-polyacrylamide slab gel electrophoresis. (Grant No. NCI Ca-14083).

468 TISSUE INTEGRITY DEPENDENT SYNTHESIS AND RELEASE OF GLYCOPROTEINS BY BENIGN AND MALIGNANT HUMAN BREAST EPITHELIAL CELLS. Zoltàn A. Tökés, Juliana Wong, Lawrence Silverman and Gerald Dermer. Dept. of Biochemistry, USC, School of Medicine, LAC/USC Cancer Center, Hospital of the Good Samaritan, Los Angeles, CA 90033.

Previous study of glycoprotein (gp) biosynthesis by human breast and prostate epithelium in organ culture revealed the possibility that cell arrangement markedly effects gp synthesis. (J. Supramol. Structure 7:515-530). Subsequently, a comparison was made of the released gp profiles from benign and malignant breast surgical specimens maintained in organ cultures. Gp synthesis was followed by the incorporation of 3H-glucosamine. Labeled macromolecules released into the culture medium were analyzed by SDS gradient-polyacrylamide slab gels. When tissue integrity and glandular architecture were conserved, gp 48 was released six times more abundantly in adenocarcinomas than benign lesions. The proportion of released large molecular weight gp was higher in benign specimens. These prominant differences were eliminated after destruction of three dimensional tissue integrity by collegenase. 18 labeled and released gp fractions were detected in benign and malignant specimens with no significant differences in their proportions. Established human breast epithelial cell lines in monolayer cultures produce gp which do not resemble the ones produced by intact tissue in organ culture. (Grant NCI CA-14089).

469 PROTEOLYTIC ACTIVITY ASSOCIATED WITH NORMAL AND MALIGNANT HUMAN BREAST EPITHELIAL CELLS. Thaddeus Pullano, Csaba Csipke and Zoltán A. Tökés, Department of Biochemistry, USC School of Medicine. LAC/USC Cancer Center, Los Angeles, CA 90033.

Malignant secretory epithelium is characterized in intact tumors by an altered glandular architecture. We have previously reported glycoprotein (gp) synthesis and release in organ cultures of breast surgical specimens under conditions where three dimensional tissue relationships are conserved, (J. Supramol. Struc. 7:515-530). A marked elevation in the release of gp 48 was observed in breast adenocarcinomas when compared to benign lesions. Also, the relative proportion of large molecular weight gp released from benign specimens was higher. The above observations prompted us to investigate proteolytic activity associated with normal and malignant breast epithelial cells. Two normal human breast epithelial cell lines (BF-724,AT-432) and four malignant lines (MB-175,MB-238,MCF-7,HBL-100) were studied in vitro using substrates supported by latex beads, (Biochm. Biophys. Res. Comm. 73:965-971, 1976). Cells were grown over half the surface area of tilted plastic petri dishes. Latex beads covalently covered with radioiodinated protein substrates were rolled over the viable cell surfaces or on the cell-free area of culture dishes. The rate of iodinated peptide release was proportional to the proteolytic activity. Malignant cell lines exhibited a significant increase in proteolytic activity as compared to normal cells. This increase was also observed when the bead-substrate complex was not in contact with the viable cells. We suggest that the observations of elevated proteolytic activity may be relevant to the altered glandular architecture and to the difference in released gp profiles of intact human breast tumors. (Supported by NCI Ca 14089).

470 SUBCELLULAR DISTRIBUTION OF PLASMINOGEN ACTIVATOR IN 3T3 CELLS, Susan Jaken and Paul H. Black, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114
Previous work from this laboratory by Chou et al (J. Cell Physiol. 91:31-37, 1977) demonstrated that growing and transformed 3T3 cells release plasminogen activator (PA) extracellularly, but that confluent cultures do not. Because the confluent cultures maintain a high intracellular PA level, we reasoned that the difference in release of PA may be related to a difference in the intracellular PA location. Using a discontinuous sucrose-dextran gradient system, we have shown that most of the PA in confluent cells sediments with a heavy membrane fraction, while most of the PA in growing and transformed cells sediments with lighter plasma membrane enriched fractions. Addition of phorbol myristate acetate to confluent cultures results in growth stimulation and an increase in PA activity. It also causes a shift in intracellular distribution of PA from heavy to light membrane fractions. We suggest that the distribution of PA into the plasma membrane enriched fractions is related to extracellular release of PA.

471 AN INCREASE IN INTERCELLULAR ADHESION IS ASSOCIATED WITH GROWTH INHIBITION OF TRANSFORMED CELLS. Raphael J. Mannino, CMDNJ-Rutgers Medical School, Piscataway,N.J.08854 and Kurt Ballmer and Max M. Burger, Biocenter, University of Basel, Basel, Switzerland.

Succinylated concanavalin A (succinyl-conA), a non-toxic, non-agglutinating derivative of the jack bean lectin concanavalin A, inhibits the growth of both 3T3 and SV40 transformed 3T3 cells. Cells whose growth has been inhibited with succinyl-conA accumulate in the G-1 phase of the cell cycle and remain viable. Succinyl-conA induced growth inhibition can be prevented by, or reversed by, α-methyl-D-mannoside, a concanavalin A hapten sugar. Succinyl-conA inhibited SV40 transformed 3T3 cells show a rate of nutrient uptake one-half that of control cells but still higher than that of 3T3 cells. Anchorage independent growth of SV40 transformed 3T3 cells is also inhibited by succinyl-conA.

In addition to its growth inhibitory properties, succinyl-conA also induces an increase in cell-cell adhesion. Scanning electron microscopy demonstrates that cells grown in the presence of succinyl-conA are connected by numerous "microvilli-like" structures. It appears, therefore, that the cell surface-cell surface interactions of cells grown in the presence of succinyl-conA are altered in some way. The functional relevance of the succinyl-conA induced increase in cell-cell adhesion to growth inhibition remains to be determined.

472 POSSIBLE MECHANISM INVOLVED IN LECTIN-INDUCED GROWTH REGULATION OF TISSUE CULTURE CELLS, K. Ballmer and M.M. Burger, Biozentrum der Universität Basel, 4056 Basel, Switzerland

We have shown previously, that the growth of both normal and transformed 3T3 cells is inhibited by the lectin derivative succinyl-con A (SCA). The cells accumulate-transformed cells only partially - in G_o/G_1 of the cell cycle and can reenter S-phase if the cell surface bound lectin is released by adding a lectin specific hapten sugar to the growth medium. To investigate the mechanism of this lectin induced growth inhibition we used Epidermal Growth Factor (EGF) as a model growth promoter and could show, that its specific binding to the cell surface is inhibited by SCA. However the unspecific binding is increased. We will present data obtained in a serum free tissue culture system showing that SCA could act as a growth inhibiting agent by modulating the binding of growth factors to the cell surface.

473 LECTIN AGGLUTINABILITY AND MEMBRANE LIPID FLUIDITY, James R. Lepock, Gord S. Rule and Jack Kruuv, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

The effect of membrane lipid fluidity on Concanavalin A (Con A) induced agglutination was studied by determining the initial rate of agglutination (IRA) of polyoma transformed baby hamster kidney (PyBHK) cells as a function of temperature and butylated hydroxytoluene (BHT), a known lipid perturber, concentration. The IRA was determined by counting, with a cell counter, the remaining number of single cells as a function of time after addition of Con A and extrapolating the rate of change of this quantity to $t = 0$. This is the IRA. The IRA decreased as the temperature was decreased from 37°C to 22°C in an Arrhenius fashion with an activation energy of 12 kcal/mole. Below 22°C there was a sharp decrease to zero in the IRA. The addition of 0.1 mM BHT increased the IRA at all temperatures by about a factor of two and decreased the temperature at which the IRA dropped to zero. The lipid fluidity of the cellular membranes, as determined by the electron spin label probe 2N14, changes by a similar amount upon addition of 0.1 mM BHT. BHT perturbs the packing of the fatty acyl chains of phospholipids and thus increases the lipid fluidity and lowers lipid crystalline to gel phase transitions. Thus the initial rate of Con A induced agglutination of PyBHK cells appears to be directly related to membrane lipid fluidity as determined by spin labelling.

474 FLUIDITY MEASUREMENTS ON PLASMA MEMBRANES OF INTACT 3T3 AND SV101-3T3 CELLS, Nancy Lee and Carl Scandella, State University of New York, Stony Brook, N.Y. 11794.

We synthesized an analog of phosphatidylcholine carrying a 5-doxylstearate chain in the β-position and found conditions which allowed us to incorporate this spin label into intact fibroblasts. The electron spin resonance (ESR) spectra showed that the label was incorporated into membranes. The label was not degraded by the cells as judged by extraction and thin layer chromatography. Isolation of plasma membrane, mitochondria, endoplasmic reticulum and nuclei from spin labeled cells showed that the spin label is mainly localized in the plasma membrane. ESR measurements performed on intact 3T3 and SV101-3T3 cells indicated that plasma membrane fluidity differs in these two cell types. In contrast, a fatty acid spin label, which labels all of the cellular membranes, revealed no difference in fluidity between 3T3 and SV101-3T3 cells (Hatten, Scandella, Horwitz and Burger, J. Biol. Chem. 253, 1972 (1978)).

475 CHOLESTEROL LEVELS IN 3T3 AND SV101-3T3 CELLS, Carl Scandella and James Hayward, State University of New York, Stony Brook, N.Y. 11794.

Cholesterol is a major component of plasma membranes. Hatten and Burger (Biochemistry, in press) observed that 3T3 and SV101-3T3 cells react differently with a cholesterol-specific polyene antibiotic, filipin. Their results prompted us to investigate cholesterol levels in these cells. We found that SV101-3T3 cells have 50-100% higher levels of cholesterol per cell than 3T3 cells under some growth conditions. Furthermore, the variation of cholesterol level with cell density is different for the transformed SV101-3T3 vs 3T3 cells. We starved cells for cholesterol by growth in lipid-depleted medium plus the biosynthetic inhibitor 25- hydroxycholesterol. Under these conditions cholesterol levels in SV101-3T3 cells were depressed by approximately 50% while cholesterol levels in 3T3 cells remained unaltered.

476 ON THE ORIGIN OF HEPATOMA MEMBRANE FATTY ACIDS, M. Waite, R. Morton, J. Hartz, The Bowman Gray School of Medicine, Winston-Salem, NC 27103

We and others have found that the membranous phospholipids isolated from Morris 7777 hepatoma are decreased in their content of polyunsaturated acyl chains, primarily arachidonate and docosohexenoate. Since this change is thought to influence membrane-associated functions, we have investigated the reason for this lack of polyunsaturated fatty acids. First, we examined the capacity of the hepatoma and liver to take up circulation saturated and polyunsaturated fatty acids complexed to albumin. Although the hepatoma took up less total fatty acid than the liver, there was no preferential uptake of fatty acid based on the degree of unsaturation. The metabolic fate of the fatty acid within the two tissues was different: The liver used fatty acids primarily for triglyceride synthesis, whereas the hepatoma incorporated fatty acid into phospholipid or for oxidation. Second, we investigated the activities of the Δ^5, Δ^6, and Δ^9 acyl CoA desaturases of microsomes. Both the Δ^6 and Δ^9 desaturases of the hepatoma were 40% of the control, but the Δ^5 desaturase, key to the production of arachidonate, was only 5% of the control. Studies on the microsomal electron transport system indicated that the alteration in desaturase activity was not the result of the lack of transfer of the electrons to the desaturase. This implies a lack of the Δ^5 desaturase. Third, we have reported on an increased lysophospholipase activity in hepatomas which resulted in an increased free fatty acid pool and a decrease in membranous phospholipid. We conclude that the lack of the Δ^5 desaturase and increased lysophospholipase activity could have far-reaching implications in our understanding of the aberrant membranes of tumors. (supported by AM11799 and CA14318)

477 PHOSPHOLIPID SYNTHESIS IN HERPES SIMPLEX VIRUS TYPE 2 INFECTED HUMAN EMBRYO FIBROBLASTS, L. W. Daniel, R. A. Respess, L. S. Kucera, and B. M. Waite, The Bowman Gray School of Medicine, Winston-Salem, NC 27103

Human embryonic fibroblasts (HEF) are sensitive to infection with herpes simplex virus type 2 (HSV-2), resulting in the production of progeny virus (50-100 infectious virions per cell) and cell death. HEF can synthesize phospholipids by the incorporation of choline and ethanolamine (Waite et al., Lipids 12:698-706, 1976). A double-isotopic tracer technique has been used to study the effects of HSV-2 infection on these metabolic pathways. At the time of infection, the [^{3}H]-precursor was replaced by the precursor with a [^{14}C]-label. In this way we have determined the effect of viral infection on membrane degradation as well as membrane synthesis. Choline was incorporated predominately into phosphatidylcholine and sphingomyelin. In both infected and mock-infected cells the [^{3}H]-choline-labeled phospholipids were slowly degraded and replaced by [^{14}C]-choline-labeled phospholipids at about an equal rate. On the other hand, ethanolamine was incorporated into phosphatidylethanolamine, phosphatidylcholine and the methylated intermediates. The degradation of [^{3}H]-ethanolamine lipids was slightly lower in infected cells. Likewise, the de novo synthesis of [^{14}C]-labeled phospholipids was inhibited by HSV-2 infection. The percent of labeled phosphatidylcholine was decreased by infection, and the methylated phospholipids were increased. The envelope of virions purified from the double-labeled cells was enriched in methylated phospholipids and predominately contained lipids present in the cell before infection. These studies are basic to an ongoing study of the cellular changes induced by HSV transformation of HEF. (supported by CA21146)

478 SURFACE CHARGE CHARACTERISTICS OF CELLS FROM MALIGNANT CELL LINES, Yehuda Marikovsky, The Weizmann Institute of Science, Rehovot, Israel.

B-type human lymphoid cell lines of malignant and normal origin were examined for their surface charge characteristics by means of electron microscopic evaluation of the labeling pattern with cationic ferritin(CF),for their agglutinability with positively charged Poly-L-Lysine,for their electric mobility,and for their sialic acid content. In addition,we have investigated the kinetics of CF-induced redistribution of CF-labeled cell-surface receptors on normal and transformed cells. Two principal findings concerning membrane surface charge characteristics of malignant and normal cell lines are presented in this study: a)Cell membranes of malignant cells labeled with CF prior to fixation with glutaraldehyde exhibit a labeling pattern of clusters and patches,as distinct from a random and even CF labeling pattern of cell membranes of normal cell lines. b)The redistribution of CF-labeled receptor complexes into clusters and patches is a time-dependent process. The time required for the ligand to induce and complete the receptor redistribution varies slightly with different cell types;generally, in all the cell lines that were studied,the redistribution is highly pronounced after 5 minutes of incubation with CF.The differences in the CF labeling pattern observed between cell surfaces of malignant cells and those of normal cells appear to be a surface characteristic of malignant cells,which exhibit an alteration in structural composition and membrane behavior different from that of normal cells.The characteristic redistribution of surface anionic sites with the CF ligand on malignant cells is clearly different from that of normal cells in which the CF does not induce a redistribution of anionic sites. A random and uniform CF labeling pattern was observed with normal cells throughout the 10-minute incubation with CF.

Cell Surface Proteins

479 STRUCTURE AND FUNCTION OF FIBRONECTIN, Denisa D. Wagner and Richard O. Hynes, Massachusetts Institute of Technology, Cambridge MA 02139

Fibronectin is a molecule composed of domains. It is a disulfide bonded dimer composed of two 230K dalton chains with the interchain disulfide bond(s) located within 10K daltons of the C-terminal, as shown by partial digestion with trypsin and carboxypeptidase Y. There is a protease-sensitive, carbohydrate-free segment of 25K daltons, rich in intrachain disulfide bonds, and finally a 200K dalton protease-resistant region containing most or all of the carbohydrate. This major part (87%) of the molecule contains only 8-12 disulfide loops out of the total of 22-24 per intact chain. So the distribution of the intrachain disulfide bonds appears also to be asymmetric. Each chain of the dimeric fibronectin contains a sulfhydryl group. The 200K region contains the gelatin binding site and the free sulfhydryl group(s).

The intact disulfide bonds are necessary for fibronectin to be able to induce more phological changes on transformed cells (Ali and Hynes, BBA 510:140, 1978). They are also necessary for its binding to gelatin and presumably to collagen. The presence of the free sulfhydryl groups makes it possible for fibronectin to be involved in disulfide bonded complexes on the cell surface. On confluent cells, fibronectin dimers are extensively disulfide bonded into higher molecular weight complexes (Hynes and Destree, PNAS 74:2855, 1977). Blocking of the sulfhydryl groups interferes with binding of exogenous fibronectin to the surfaces of hamster fibroblasts, suggesting that formation of intermolecular disulfide bonds may be important in anchoring of fibronectin to the cell surface.

480 COMPARISON OF AMNIOTIC FLUID FIBRONECTIN AND PLASMA COLD-INSOLUBLE GLOBULIN. ISOLATION OF A COLLAGEN-BINDING FRAGMENT COMMON TO THE TWO PROTEINS. Gary Balian, Eva Marie Click, Ed Crouch and Paul Bornstein. Departments of Biochemistry and Medicine, University of Washington, Seattle, WA 98195

Amniotic fluid fibronectin and plasma cold-insoluble globulin (CIG) are two large glycoproteins that are immunologically indistinguishable and show extensive chemical similarities. The intact chain of the dimeric protein, as well as some enzymatically-derived fragments of fibronectin, were heterogeneous on SDS-PAGE, possibly reflecting a variable extent of glycosylation in discrete regions of the molecule. Limited proteolytic cleavage of fibronectin and CIG produced different peptide patterns for the two proteins, pointing to differences either in primary structure or in conformation. A cysteine- and carbohydrate-rich fragment, produced by cleavage with cathepsin D, was found to contain a collagen-binding region which was near the COOH-end of the polypeptide chain in fibronectin. Intrachain disulfide bonds maintained a conformation that was necessary for the interaction of this region with collagen.

481 PROTEOLYTICALLY-DERIVED FRAGMENTS OF HUMAN PLASMA FIBRONECTIN AND THEIR LOCALIZATION WITHIN THE INTACT MOLECULE, Martha B. Furie and Daniel B. Rifkin, The Rockefeller University, New York, NY 10021 and New York University School of Medicine, New York, NY 10016

Plasma fibronectin (FN), also known as cold-insoluble globulin, is similar or identical to FN found on the surfaces of fibroblasts. Plasma FN consists of two polypeptide chains of ∿250,000 daltons joined by disulfide bonds. The NH_2-termini are blocked with pyroglutamic acid. FN isolated from human plasma was cleaved with human α-thrombin (gift of J. W. Fenton), and three major fragments were recovered: two large ones of 235,000 and 230,000 daltons, and a small one of 29,000 daltons. Each consisted of a single polypeptide chain. The small piece was not retained by a gelatin-Sepharose column, while the larger fragments were bound and could be eluted with 2 M guanidine·HCl. No NH_2-terminal residues could be detected in intact plasma FN or in the small fragment by the dansyl chloride technique, while analysis of the pooled large fragments yielded dansyl-alanine. Treatment of the intact molecule and the small piece with pyroglutamyl peptide hydrolase resulted in the appearance of NH_2-terminal alanine. The presence of blocked NH_2-termini in the intact molecule and the small piece was confirmed by automated sequence analysis. Analysis of the large fragments gave an NH_2-terminal sequence of Ala-Ala-Ala-Val-Tyr-Glu-, and pyroglutamyl peptide hydrolase-treated small fragment yielded Ala-Glu-(Glu?)-Met-Val-. The results indicate that the small fragment is located at the NH_2-terminus of plasma FN, while the larger fragments constitute the carboxyl portion. Similar experiments using FN synthesized by cultured human embryo fibroblasts will be described. Characterization of these fragments and assessment of the biological activities which they retain may aid in understanding how FN acts as a bridging, adhesive element in vivo.

482 CHEMICAL STRUCTURE OF GLYCOPEPTIDES ISOLATED FROM HUMAN CIg (COLD INSOLUBLE GLOBULIN) AND PLACENTAL *LETS* (FIBRONECTIN, OPSONIC α_2SB GLYCOPROTEIN), Susan F. Frasch and Roger A. Laine, University of Kentucky, Lexington, KY 40506

CIg, which is electrophoretically and immunologically identical to the LETS glycoprotein, was isolated from human plasma by affinity chromatography on collagen-Sepharose [*Int. J. Cancer 20*, 1 (1977)]. SDS-polyacrylamide gel electrophoresis of the purified glycoprotein gave a double band which migrated near myosin and which stained with Coomassie blue and PAS. Chromatography of the pronase-solubilized CIg glycopeptides on Sephadex G-50 resulted in a single peak which co-eluted with transferrin glycopeptides. Sugar composition analysis of the glycopeptides by GLC of the trimethylsilyl methyl glycosides gave the following molar ratios: sialic acid, 1.6; galactose, 2.0; N-acetylglucosamine, 4.2; and mannose, 3.0. Affinity chromatography of the CIg glycopeptides on Con A-Sepharose resulted in two fractions; one third of the glycopeptides were unbound and two thirds were weakly bound (WB). Methylation analysis of the WB fraction gave mainly 6-linked galactose, 2- and 3,6-linked mannose and 4-linked N-acetylglucosamine. Following digestion with neuraminidase alone or in combination with β-galactosidase, the WB CIg and transferrin glycopeptides co-chromatographed on Sephadex G-50. Methylation analysis of the resulting products suggested the structure of the major CIg and transferrin glycopeptides to be identical. The LETS glycoprotein is a major cell surface component of two types of placental cells [*Conn. Tissue Res. 3*, (1975)]. LETS was isolated from human placentas by urea extraction [*Proc. Nat. Acad. Sci. 72*, 3158 (1975)] and purified as described above. Sugar composition and methylation analysis showed the structure of the pronase-solubilized LETS glycopeptides to be similar to the major CIg glycopeptide.

483 THE GLYCOPEPTIDES OF FIBRONECTIN, Joy E. Koda, Kenneth Keegstra, Stephen Weitzman

The glycopeptides of the cell surface glycoprotein, fibronectin, isolated from BHK 21/13 cells were studied. Since the protein exists in two forms, a surface-associated form and a soluble form present in conditioned media, the glycopeptides from both were analysed and compared. Gel filtration on Biogel P6 of radioactively labeled pronased glycopeptides from both the surface associated and soluble proteins reveals a complex pattern that simplifies into four peaks following digestion with neuraminidase. An additional peak is present at the void volume of the column. Each of these peaks can be metabolically labeled with glucosamine, mannose, galactose, and fucose. A combination of concanavalin A affinity chromatography and digestion with endoglycosidase D from Diplococcus pneumoniae was used to further analyse the carbohydrate present. Results indicate that the four structures present can be fractionated into both Con A binding and non-binding components. The majority of both these components are sensitive to endoase D. A reproducible small fraction of both of these components remain resistant to the enzyme. Ion exchange chromatography on DEAE sephadex of tryptic glycopeptides was used to analyse the attachment sites of the carbohydrate. Results indicate that the soluble protein and the surface protein share at least three attachment sites in common. One structure is present at a unique site on the surface protein that is not shared by the soluble protein.

484 IDENTIFICATION OF PHOSPHATE IN FIBRONECTIN FROM CULTURED CHICKEN EMBRYO FIBROBLASTS, Mei-hui Teng and Daniel B. Rifkin, Department of Cell Biology, New York University, New York, NY 10016

Fibronectins are a class of glycoproteins present in the plasma of vertebrates and on the surfaces of their fibroblasts and some other cell types. We have isolated [32P] fibronectin from normal and Rous sarcoma virus (RSV)-transformed or transformation-defective RSV (td-RSV)-infected chicken embryo fibroblasts. Confluent cell cultures were incubated in the medium containing [32P] orthophosphate and the fibronectin in the conditioned medium was isolated by gelatin-Sepharose column chromatography; the fibronectin on the cell surfaces was isolated by urea extraction, ammonium sulfate precipitation and preparative gel electrophoresis. 32P was stably associated with fibronectin during immunoprecipitation, SDS polyacrylamide gel electrophoresis hot TCA and phospholipid solvent extractions, and acid but not alkaline treatment. After limited acid hydrolysis of fibronectin, both phosphoserine and phosphothreonine were found. Therefore, fibronectin of cultured CEF contains phosphate covalently linked to the hydroxyl side chains of serine and threonine. The specific radioactivities of 32P-labeled fibronectin from the conditioned media of normal and td-RSV-infected CEF were higher than that from the culture of RSV-transformed CEF.

485 FIBRONECTIN-RELEASING POLYPEPTIDES FROM HUMAN FIBROSARCOMA CULTURE MEDIA. Jorma Keski-Oja, Hans Marquardt, Joseph E. De Larco, and George J. Todaro, Laboratory of Viral Carcinogenesis, National Cancer Institute, Bethesda, MD. 20014

A radioimmunoassay specific for human fibronectin has been developed by using purified human plasma fibronectin and rabbit anti-fibronectin antibodies adsorbed with fetal calf serum (Mosher and Vaheri, Exp. Cell, Res. 112: 323, 1978). Cultures of human diploid lung fibroblasts were incubated in serum-free media, and the radioimmunoassay was used to detect differences in fibronectin released from the diploid fibroblasts into the medium after treatment of the cells with factors described below.

Serum-free media concentrated from cultured human fibrosarcoma cell line (8387) was fractionated by gel permeation chromatography using Bio-Gel P-100 column in 1M acetic acid. Different column fractions with mol. wts. between 30,000 and 6,000 daltons were assayed for their ability to release fibronectin from cells. It was found that the column fraction with an apparent mol. wt. of 10,000 daltons contained an activity that released fibronectin from cells. Significant changes could be detected within 30 minutes after treatment of the cells. Several hormonal factors studied, including epidermal growth factor, sarcoma growth factor, insulin and dexamethasone, proved negative in their ability to release fibronectin from human cells. The 10,000 dalton mol. wt. polypeptides may be responsible for maintenance of low levels of surface associated fibronectin in fibrosarcoma cells and may also be involved in their altered growth behavior.

486 CELL SURFACE EXPRESSION OF FIBRONECTIN BY EMBRYONIC GLIAL CELLS, Clifford J. Kavinsky, Lan Bo Chen, and Beatrice B. Garber, University of Chicago, Chicago, IL. and Harvard Medical School, Boston, Mass.

Embryonic brain cell populations dissociated from 10-day chick or 14-day mouse cerebra were separated into homogeneous neuronal and glial cell fractions using procedures involving differential adhesion and selective cytotoxic responses to cytosine-d-arabinoside (Kavinsky et.al., J.Cell Biol.79:99,1978, Wong & Garber, Proc. 9th. Int. Cong. E.M. 3:602,1978). The purity of the fractions was assayed using monospecific antisera directed against glial fibrillary acidic protein (GFA, Dahl and Bignami, Brain Res. 116:150,1976). After reaction with anti-GFA serum, purified glial monolayer cultures exhibited intense flourescence associated with intracellular fibrillar networks as visualized by indirect immunofluorescence microscopy. Furthermore, purified neuronal cultures showed no detectable GFA. Corresponding preparations stained with antisera directed against plasma fibronectin showed a similar distribution: glial cells exhibited intense surface-associated fluorescence which was localized at cell attachment sites; while neurons showed no fluorescent reaction. When the anti-fibronectin serum was tested for reactivity with malignant glial cells grown *in vitro*, no fluorescence was detected. These immunological probes demonstrate fibronectin to be a cell surface marker for embryonic glial cells, and furthermore establish a clear difference between embryonic and neoplastic cell surfaces.

487 TRANSFORMATION-ASSOCIATED AND GROWTH-DEPENDENT CELL SURFACE CHANGES IN LIVER EPITHELIAL CULTURES, Mary S.Rieber, Manuel Rieber, IVIC, Apartado 1827, Caracas, Venezuela

Surface labelling of "normal" non-tumorigenic epithelial cells permitted the detection of a 150,000 dalton component and of another 230,000 dalton molecules with co-migrated with the fibronectin external transformation-sensitive protein of fibroblasts and gave an identical reaction with anti-fibronectin serum. Similar experimental conditions with the tumorigenic epithelial cell counterpart revealed decreased labelling in the 230,000 dalton region and preferential labelling in the 150,000 dalton and 60,000 dalton region. Amino acid limitation but not 0,25% serum starvation was found to decrease proliferation both in normal and malignant epithelial cells, concurrent with a marked decrease in the labelling of epithelial surface fibronectin. No comparable decrease in fibronectin was observed in fibroblasts exposed to a similar amino acid limitation and to neuraminidase treatment, which lead to a marked decrease of the epithelial fibronectin. Amino acid limitation also resulted in the increased expresion of a glycosylated macromolecule larger than fibronectin in "normal" but not in the tumorigenic epithelial cells.

488 SURFACE FIBRONECTIN, ANCHORAGE AND GROWTH BEHAVIOUR IN NORMAL AND NEOPLASTIC CELLS, R. Rajaraman and K. Lonergan, Departments of Medicine and Microbiology, Dalhousie University, Halifax, N.S., Canada.

Normal unestablished cells, established normal cells, virus transformed cells and malignant cells of human and rodent origin have been studied with reference to cell surface and foot-print fibronectin, anchorage and proliferation under conditions favoring (tissue culture plates=TC) and not favoring (bacterial culture plates=BC) cell adhesion. Normal human and rodent cells (unestablished) grew into fibroblastic monolayers on TC plates and obtained saturation density (sd) of 0.73×10^4 and 1.63×10^4 cells/cm^2 with a population doubling time of 75 hr and 28 hr at log phase; these cells showed abundant fibronectin on their foot-prints as well as the cell surface as indicated by immunofluorescence. These cells were unable to grow on BC plates. Established normal BHK-21 cells and all the neoplastic cells were able to grow equally well on both TC and BC plates. When deprived of anchorage, these cells grow as multicellular spheroids (BHK-21; BHK-14B; WI-38-VA13-2RA; WI-26-VA4; SV-T2) or as loose aggregates (Ki3T3; HeLa). These studies indicate that in normal unestablished cells, abundant cell surface fibronectin is accompanied by anchorage dependence of growth; in neoplastic cells, loss of cell surface fibronectin is accompanied by loss of anchorage dependence for growth. Established normal cell line (BHK-21) has gained indefinite division potential and also anchorage independence of growth but still retains surface fibronectin.

489 ADHESION VARIANTS OF CHO CELLS: A POSSIBLE ALTERATION IN THE BINDING OF SERUM PROTEINS. Patricia A. Harper and Rudolph L. Juliano. Research Institute, Hospital for Sick Children, Toronto, and Department of Medical Biophysics, University of Toronto, Toronto, Ontario.

We have isolated variant CHO cells which show a marked reduction in their ability to adhere to protein coated glass, plastic or collagen substrates. Thus after 90 mins. more than 80% of wild type CHO cells will adhere to a serum coated collagen substratum, whereas less than 2% of the variants F11 and F21 will do so. This non-adherent phenotype is not corrected by increasing either the divalent ion concentration or the serum concentration. Purified fibronectin, a serum protein thought to be directly involved in cellular adhesion, promotes the attachment of wild type CHO cells but not the variant F11. However, if an alternative ligand is used for attachment such as plant lectins or polylysine, the variants will attach. The F11 cells attach to a concanavalin A coated plastic substrate with the same kinetics as wild type cells and thereafter the variant cells spread and attain a normal morphology. This evidence implies that the variants such as F11 are in some manner unable to recognise or interact with the serum component(s) such as fibronectin, that are necessary for the normal adhesion process.

490 THE FIBRONEXUS: A TRANSMEMBRANE ASSOCIATION OF FIBRONECTIN AND ACTIN AT THE FIBROBLAST SURFACE, Irwin I. Singer, Institute for Medical Research of Bennington, 110 Hospital Drive, Bennington, Vermont 05201.

Transmission electron microscope studies of hamster fibroblasts transformed by a temperature sensitive mutant of SV40 (A28), and human WI-38 and MRC-5 cells, have demonstrated an apparent attachment between external fibronectin fibers and cytoplasmic 50 Å actin microfilaments at the surface membrane. This association between fibronectin and actin fibers occurs at a dense submembranous area denoted the fibronexus. LETS antigens were localized on the external fibers with immunoferritin, and the 50 Å microfilaments were continuous with conspicuous intracellular microfilament bundles. These two sets of fibers remained colinear during thin-section tilting through an angle of 40° around the fibrillar long axis, demonstrating that their association is not an artifact due to image superimposition. Fibronexuses remain after expression of the transformed phenotype, despite reductions in fibronectin matrix and alterations of microfilament bundles.

491 CROSSLINKING OF FIBRONECTIN TO PROTEOGLYCANS AT THE CELL SURFACE, Margaret E. Perkins, Tae H. Ji, and Richard O. Hynes, Massachusetts Institute of Technology, Cambridge, MA 02139

Fibronectin added to normal and transformed cells binds to the surface and in the latter case, effects profound changes in surface ultrastructure, cytoskeleton and cell morphology. The nature of the interaction of fibronectin with the cell surface and the subsequent changes in the arrangement of the cytoskeletal fibrils is unknown. We have initiated a study into this problem using heterobifunctional crosslinkers of the type, methyl 3(4 azidophenyl)dithiopropiomidate. Fibronectin reacted with the imidoester end is added to the cells. Following a suitable incubation time, the azide end of the crosslinker is activated by UV light. The resultant crosslinked complexes are isolated and the components crosslinked to fibronectin are identified on polyacrylamide gels. We find that under these conditions the predominant interaction of fibronectin with both normal and transformed cells is with the sulfated proteoglycans.

Several characteristics of binding and crosslinking of fibronectin in transformed cells differ from those in normal cells, and these may reflect differences in the population of proteoglycans in these two cell types.

492 CROSS-LINKING OF FIBRONECTIN TO FIBRIN AND COLLAGEN BY FACTOR $XIII_a$, Deane F. Mosher, Peter E. Schad, and Hynda K. Kleinman, University of Wisconsin, Madison, WI 53706, and National Institute of Dental Research, Bethesda, MD 20014

Soluble fibronectin is found in body fluids and media of cultured adherent cells. Insoluble fibronectin is found in tissue stroma and matrices around cultured cells. Fibronectin is a substrate for Factor $XIII_a$ (plasma transglutaminase) and binds to fibrin and collagen. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was used to study Factor $XIII_a$-mediated cross-linking of fibronectin. Fibronectin could be cross-linked to the $\alpha 1(I)$ chain of Type I collagen and to iodinated cyanogen bromide fragment 7 (^{125}I-CB7) of the $\alpha 1(I)$ chain. CB-7 blocked cross-linking of fibronectin and fibrin. Conversely, fibrin blocked cross-linking of fibronectin and ^{125}I-CB7. Fibronectin-$\alpha 1(I)$ and fibronectin-fibrin cross-linking were blocked by amidination of lysines on fibronectin and $\alpha 1(I)$ but not by amidination of fibronectin. These studies suggest that cross-linking is between a common Factor $XIII_a$-susceptible glutaminyl residue on fibronectin and lysyl residues on fibrin and collagen. Cross-linking of fibronectin may be a mechanism for immobilizing fibronectin in clots and tissue matrices and may be important for normal wound healing.

493 IN VITRO EVOLUTION OF AN EXTRACELLULAR FIBRONECTIN AND COLLAGEN MATRIX ON NORMAL HUMAN FIBROBLASTS, Leo T. Furcht, Dean F. Mosher, Gwen Wendelschafer-Crabb, Jean M. Foidart, Univ. of Minn., Univ. of Wisc., National Institute of Dental Research.

In these studies we use the peroxidase anti-peroxidase method for the immunocytochemical, ultrastructural localization of fibronectin and type I procollagen at various times after subculture of human skin fibroblasts. In very low density nonconfluent cultures, fibronectin is distributed in non-filamentous patches on the membranes of cells, while procollagen type I is present in an extremely sparse distribution on the membrane, seen by electron, but not by light microscopy. As cell density increases fibronectin and procollagen I staining increases in a diffuse form on the plasma membrane and short filaments then appear. As cells reach confluence an extracellular matrix of nonperiodic fibrils develops. The nonperiodic fibrils are 15-25 nm in diameter and stain for both fibronectin and procollagen and are seen in young confluent cultures. With time at confluence, or following ascorbate treatment periodic fibrils develop. The periodic fibrils stain intensely for procollagen I and have the 67 nm axial periodicity typical of mature type I collagen fibrils. Fibronectin is distributed along periodic fibrils in bands which have the same 67 nm axial periodicity. Subsequent to the development of periodic collagen fibrils in old or ascorbate treated cultures there is a different pattern in response to collagenase and trypsin degradation compared to the non-periodic fibrils seen in young confluent cultures. These observations suggest that non-periodic fibrils of fibronectin and procollagen may form by condensation of cell surface associated fibronectin and procollagen and may then serve as a precursor of the definitive periodic collagen fibril which fibronectin then associates with.

494 CO-DISTRIBUTION OF FIBRONECTIN AND COLLAGEN ON THE SURFACE OF HUMAN HYBRID CELLS AND THEIR RELATIONSHIP TO TUMORIGENICITY. C.J.Der and E.J.Stanbridge, Dept. of Medical Microbiology, University of California, Irvine, College of Medicine, Irvine, CA 92717

Cell surface fibronectin (FN) expression was evaluated on a series of human cell hybrids derived from tumorigenic and normal parental cells. These hybrids continued to express many of the in vitro transformed properties of their respective tumorigenic parent, but were non-tumorigenic when examined in nude mice. From three independent nontumorigenic hybrids malignant segregant subpopulations were isolated which formed progressive tumors in nude mice. A qualitative and quantitative comparison of the FN expression, using both surface iodination and specific immunofluorescent staining, was done for each malignant segregant cell line and its corresponding nontumorigenic parental hybrid cell line. A radical alteration in the organizational pattern of cell surface FN was observed in the malignant segregants. The non-tumorigenic cell hybrids expressed an incomplete, branched fibrillar network at regions of cell-cell contact which also extended partially across the cell surface. In all the tumorigenic subpopulations FN was expressed as short, predominantly unbranched fibrils localized exclusively at regions of cell-cell contact. By immunofluorescence it was found that FN and collagen showed extensive codistribution and organizational similarities. In addition, both collagen and FN showed a coordinate shift in organizational patterns associated with the acquisition of tumorigenic potential in the malignant segregants. This parallel alteration in collagen and FN expression in the extracellular matrix may represent an important property required for the in vivo growth potential of these malignant segregant cell lines.

495 EXTRACELLULAR MATRIX OF VASCULAR ENDOTHELIAL CELLS Charles R. Birdwell, Scripps Clinic and Research Foundation, La Jolla, California

I have been working with a cloned line of adult bovine aortic endothelial cells. These cells secrete a thick layer of extracellular matrix, similar in appearance to a basement membrane in vivo. This matrix is only deposited underneath the cell monolayer, and a major component of the matrix is fibronectin (Birdwell et al, Proc. Natl. Acad. Sci. USA, July, 1978); no fibronectin is found on the upper surface of these endothelial cells as detected by immunofluorescence. I am presently conducting ultrastructural studies of the extracellular matrix and studying the distribution of fibronectin in the extracellular matrix and on the lower cell surface using immuno-electron microscopic techniques. I am also studying the function of fibronectin in the extracellular matrix, and the ability of the matrix to support cell growth.

In collaboration with Dr. Garth L. Nicolson, the interaction of metastatic tumor cells with vascular endothelial cells is being studied, with particular attention given to the fate of endothelial fibronectin after tumor cells are plated onto monolayers of endothelial cells.

496 ENDOGENOUS AND EXOGENOUS FIBRONECTINS AS BUILDING BLOCKS OF EXTRACELLULAR MATRIX, Edward G. Hayman and Erkki Ruoslahti, Division of Immunology, City of Hope Medical Center, Duarte, CA 91010

Fibronectin, a 450,000 dalton glycoprotein is present in an insoluble form on the surface of many normal cells and in the surrounding extracellular matrix where it can be visualized as a three-dimensional fibrillar matrix. Fibronectin is also found in a soluble form in plasma and serum. The function of the circulating fibronectin is not known. We have found that fetal bovine serum fibronectin can be incorporated into pericellular matrix of cultured rat fibroblasts.

Normal rat kidney cells were cultured in medium supplemented with usual normal fetal bovine serum or serum depleted of fibronectin. The cell surface fibronectin of these cultures was visualized by indirect immunofluorescence using species specific antisera for either rat fibronectin or bovine fibronectin. Anti-rat fibronectin revealed fibrillar structures on the cells grown in either normal medium or fibronectin depleted medium. Anti-bovine fibronectin revealed fibrillar networks only on the cells grown in medium containing bovine fibronectin. Staining in both cases was abolished by absorption with homologous antigens. It appears that exogenous fibronectin was incorporated into the same structures as endogenous fibronectin. This finding suggests that circulating fibronectin may serve as a building block for the assembly of extracellular matrix, possibly by cells which are incapable of synthesizing it.

497 FIBRONECTIN IN THE EXTRACELLULAR MATRIX, K. Alitalo, M. Vuento, M. Kurkinen, K. Hedman, A. Vaheri, University of Helsinki, Helsinki, Findland

The pericellular substrate-attached matrix has been isolated from cultures of human fibroblasts. The matrix forms a structural entity and contains mainly fibronectin, procollagens, heparan sulfate and hyaluronic acid. The fine structure is filamentous.

Fibronectin has been purified from human plasma by a double step affinity procedure in nondenaturing conditions. In polyacrylamide gel electrophoresis, in defined conditions, two subunits with different mobilities are seen. Fibronectins purified from plasma, from the media of normal and transformed cells show distinct patterns of proteolytic peptides.

Polymerisation of purified plasma fibronectin can be induced by changes in ionic strength, and by the pesence of certain charged molecules. The polymerised fibronectin has a filamentous fine structure similar to that found in the fibroblast matrix.

498 IN VITRO BASAL LAMINA FORMATION BY NORMAL AND TRANSFORMED EPITHELIAL CELLS. Guido David and Merton R. Bernfield, Stanford University, Stanford, CA 94305.

Destruction of the basal lamina (BL) is thought to be important for invasion and metastasis of carcinomas. The mouse mammary epithelial BL, similar to the BL of certain other epithelia, is glycosaminoglycan (GAG)-rich. Low passage (LP) secondary cultures of Namru mouse mammary epithelial cells produce a heparan sulfate-rich BL when maintained on type I collagen gels, but no BL is formed on plastic substrates (David & Bernfield, PNAS, in press). This in vitro BL formation correlates with and may be due to a collagen-mediated reduction in degradation rate of the cell-associated GAG. To determine whether absence of a BL at sites of tumor invasion in vivo could be duplicated in vitro , we investigated the effect of transformation on the in vitro production of a BL by these cells.

Benign cystadenomas surrounded by a continuous BL are formed by LP cells injected into *nude* mice, whereas high passage (HP) cultures of the same origin form adenocarcinomas showing interrupted or absent BL in their extensive invasive regions. In contrast to LP cells, these transformed HP cells do not deposit a BL in vitro in response to a collagen substrate. HP cells show no collagen mediated reduction in their rate of GAG degradation, supporting the idea that BL formation and reduced degradation are causally related. HP cells have less immunoreactive fibronectin (LETS, CSP) at their surfaces. This normal matrix constituent may be involved in the organization and stabilization of the extracellular materials in a BL through its interaction with collagen. (Supported by NCI contract NO1-CB53903; NIH grant HD06763 and NIH Fellowship 1F05 TW02548; GD is a Fellow of the Damon Runyon-Walter Winchell Cancer Fund (DRG-243-F)).

499 CELL SURFACE ASSOCIATED GLYCOSAMINOGLYCANS OF HUMAN NORMAL AND MALIGNANT GLIAL CELLS. B. Glimelius,B. Norling,B. Westermark and A. Wasteson, The Wallenberg Laboratory and The Institute of Med. and Physiol. Chemistry, University of Uppsala, Uppsala, Sweden.

Cell surface associated glycosaminoglycans (GAG) of human, normal glial and malignant glioma cells in culture were studied using ^{35}S-sulphate and ^{3}H-glucosamine as markers for GAG. The amount, distribution, composition and turnover were similar in all of the four tested normal cell lines, but showed great variability in the nine malignant cell lines. The GAG of material liberated by trypsin from the intact cell layer (pericellular pool), from EDTA-detached cells (membrane fraction) and from the substrate (SAM) of the normal cells consisted mainly of heparan sulphate (HS) with only minor amounts of the other GAG. The analogous material of most glioma cells showed hyaluronic acid as the major GAG. No HS was present in SAM of any glioma cell line. The sulphated GAG of normal cells showed heterogeneous turnover rates and most of the GAG were internalized and degraded. A considerable portion of pericellular GAG, including SAM, showed a slow turnover rate with a half life time of several weeks. In the glioma cell lines the turnover rates were usually more rapid and most of the GAG were released in macromolecular form to the culture medium. Several glioma cell lines showed no fraction, except SAM, with the long half life time. When present, it usually consisted of more chondroitin sulphate than HS in contrast to the normal cells. The results indicate that HS-proteoglycans participate in the intercellular matrix of cultured glial cells. The presence of cell surface associated HS with the slow turnover rate also correlates with the presence of other matrix components. The glioma cells show various abnormalities, including those of the GAG, of the cell surface and in the organization and composition of the intercellular matrix.

500 SULFATED GLYCOSAMINOGLYCANS ARE TRUE COMPONENTS OF THE EXTERNAL MEMBRANE SURFACE OF HUMAN FIBROBLASTS IN CULTURE, Kathryn G. Vogel, Department of Biology, The University of New Mexico, Albuquerque, New Mexico 87131

Cultures of human embryo fibroblasts (IMR90) were allowed to incorporate ^{3}H-glucosamine and $Na_2{}^{35}SO_4$ for 4 or 24 hours. Following their release from the cell surface glycosaminoglycans were quantitated by filtration on Sephadex G-25 to obtain labeled macromolecular material and then fractionation as either the carbohydrate moiety of glycoprotein (1% CPC soluble), hyaluronic acid (soluble in 0.5N HCl), or sulfated glycosaminoglycan (CPC insoluble).

Attempts were made to release glycosaminoglycans from the surface of intact fibroblasts by changes in pH and ionic strength of the surrounding buffer. No additional macromolecular material was released by such 5-min treatments and 4M GuHCl reptured the cells, while trypsin (0.1 mg/ml) released nearly 50X the amount naturally shed. This suggests a covalent attachment of glycosaminoglycans to the cell membrane.

The amount of labeled macromolecular material released naturally by cells incubated in Earle's Balanced Salt Solution at 37° for 30 min was compared with the amount released by treatment of the same cells with trypsin. The glycopeptide and sulfated glycosaminoglycan fractions of naturally-shed samples showed a 2.5X increased in the quantity of radioactivity between 4 and 24 hours of labelling, while these trypsin fractions showed a 4.5X increase. This suggests that these components are accumulating at the cell surface prior to their release from the cell. The hyaluronic acid fractions of both buffer and trypsin-released samples were increased 4.5X between 4 and 24 hours, suggesting that hyaluronic acid may be directly secreted from the cell. (NIH AG 00191)

502 ISOLATION OF MEMBRANE PROTEINS AFTER DERIVITIZATION WITH TRINITROBENZENE SULFONIC ACID. G. Kaplan and J. C. Unkeless. The Rockefeller University, New York 10021

A simple reproducable method for isolating externally disposed plasma membrane proteins from cultured cells has been developed. Cells biosynthetically labeled with ^{35}S-methionine or other precursors were incubated with trinitrobenzene sulfonic acid (TNBS) which derivatized free amino groups on the cell surface with the trinitrophenyl (TNP) moiety. Under the conditions used (10mM TNBS in PBS, pH 7.9, 4°C for 30 min) the TNBS did not penetrate the cells. The TNP-derivatized cells were then incubated with rabbit anti-DNP IgG at 4°C, washed, lysed with NP-40 and the anti-DNP IgG-TNP protein complexes isolated by absorption with fixed Staphylococcus aureus for analysis by polyacrylamide gel electrophoresis.

This method was used to investigate the composition, biogenesis, and turnover of macrophage membrane proteins. In a series of pulse-chase experiments, we found that a variety of glycosylated surface proteins first appeared on the cell surface about 60-90 min after a 15 min pulse with ^{35}S-methionine. The method was also used to investigate the surface membranes of the J774 macrophage cell line and a series of variants lacking the Fc receptor for IgG2b aggregates (FcRII). The electrophoretic profiles of radiolabeled proteins isolated from the variants were significantly different from the parent cell line and were consistent with processing and/or glycosylation defects.

503 RESOLUTION AND CHARACTERIZATION OF PLASMA MEMBRANE GLYCOPROTEINS OF NOVIKOFF TUMOR CELLS, John R. Glenney, Jr. and Earl F. Walborg, Jr., The University of Texas System Cancer Center, Science Park-Research Division, Smithville, Texas 78957.

The saccharide moieties of glycoproteins present at the surface of Novikoff ascites hepatocellular carcinoma cells were radiolabeled by sequential oxidation (galactose oxidase or periodate) and reductive tritiation (NaB^3H_4). Plasma membrane glycoproteins could be labeled by galactose oxidase/NaB^3H_4 only after prior treatment of the cells with neuraminidase, indicating the exposure of Gal and/or GalNAc residues by neuraminidase digestion. Treatment of the cells with periodate/NaB^3H_4 produced efficient labeling of cell-surface sialoglycoproteins. The radiolabeled plasma membrane glycoproteins were solubilized in nonionic detergent (0.5% NP-40), resolved by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, and visualized by fluorography. Affinity chromatography of the glycoproteins on Sepharose-bound lectins, e.g. Ricinus communis agglutinins I and II and soybean agglutinin, allowed further resolution of the glycoproteins based on the structure of their heterosaccharide moieties. Using this methodology several classes of glycoproteins were characterized: those in which all of the glycoprotein bound to the affinity support, those demonstrating saccharide heterogeneity in which only a fraction of the glycoprotein bound to the affinity support and those glycoproteins that only bound to the affinity support after prior treatment of the cells with neuraminidase. These studies suggest a methodology for investigating differences in the plasma membrane glycoproteins of normal and malignant cells. Supported by grants from Natl. Cancer Inst. (CA-18829), Paul and Mary Haas Fdn. and George and Mary Josephine Hamman Fdn. J.R.G. was the recipient of a J.S. Abercrombie Fdn. Predoctoral Fellowship.

504 MEMBRANE GLYCOPROTEINS POSSIBLY INVOLVED IN CELL-SUBSTRATE AHESION. Clayton A. Buck, Patricia Rao, Karen Knudsen and Caroline H. Damsky, Wistar Institute, Philadelphia, Pa. 19104.

In an attempt to identify the integral membrane molecules involved in cell substrate adhesion, we developed a series of immunoglobulins which reversibly and in a non-toxic manner cause hamster cells to round and detach from tissue culture plate. Differential immunoprecipitation of NP-40 extractable material from these cells implicate a group of 140K M.W. glycoproteins as being involved in the regulation of cell adhesion and morphology (Wylie et al, J. Cell Biol., in press). To confirm these results we have developed an assay in which NP-40 extracts of hamster cell membranes are used to block immunoglobulin-induced rounding and detachment of the cells from the substratum. The blocking activity in the NP-40 extracts has been partially purified by ion exchange chromatography on Affi-Gel 102 columns by a modification of the procedure of Steck et al (personal communications). Upon fractionation of 14C-glucosamine-labeled NP-40 extracts, the blocking activity was found restricted to one fraction. The active fraction was co-electrophoresed on SDS-PAGE with 3H-glucosamine labeled glycoproteins from immunoprecipitates previously thought to be important to adhesion. The radioactive profiles were virtually superimposable lending further support to the contention that a glycoprotein or group of glycoproteins with an apparent subunit M.W. of 140K are responsible in some manner for controlling cell adhesion and/or morphology. Supported by USPHS Grants CA-19144, CA-10815, CA-21124 and CA-09171.

505 THE ROLE OF BAND 1 PROTEIN IN NEURAL TUMOR SUBSTRATE ADHESION, Richard Akeson, The Children's Hospital Research Foundation, Cincinnati, Ohio 45229

The characteristic morphology of cultured fibroblasts is dependent, in part, on a large surface glycoprotein (LETS, CSP, or fibronectin). Cultured neuronal tumors synthesize relatively small amounts of collagen and LETS and do not adhere strongly to the culture dish. We have recently described a nervous system specific cell surface glycoprotein on murine neuroblastoma cells which shares some properties with LETS but appears to be an independent polypeptide (Akeson, R. and Hsu, W-C., Exp. Cell Res. 115,367-377,1978). For many cultured neuronal cells, strong cell-dish interaction results in the extension of long processes resembling axons. Clone N18 cells in serum-free media and clone NB2a cells treated with Bu_2cAMP extend processes. Under these conditions the amount of band 1 per cell increases 3-4 fold. After removal of the cells with EGTA, a component which comigrates with band 1 on SDS polyacrylamide gels is enriched in the polypeptides left on the dish relative to total cellular protein. The proportion of band 1 in such substrate attached material is somewhat increased in cells extending processes compared to non-axonating controls. These experiments all provide circumstantial evidence for the role of band 1 in mediating cell-substrate adhesion. More direct evidence for such a role is currently being sought using antibodies to band 1. The results of these experiments and others examining the interaction of band 1 with more biological growth substrates will also be presented.

506 DYNAMICS OF SURFACE PROTEINS ON THE NRK CELL AND ITS VIRAL TRANSFORMANTS, William W. Carley* and William M. Mitchell+, Department of Biochemistry, Cornell University*, Ithica, N.Y. 14853 and Department of Pathology, Vanderbilt University+, Nashville, TN. 37232

Cell surface proteins of 250 x 10^3, 190 x 10^3, 77 x 10^3, and 67 x 10^3 molecular weight (MW) resolved by SDS-PAGE are readily labeled by the ^{125}I-lactoperoxidase method on intact normal rat kidney (NRK) cells in culture. NRK cells transformed by the Kirsten sarcoma virus (K-NRK) are devoid of the 250 x 10^3 and 67 x 10^3 MW proteins and have reduced marking of the 190 x 10^3 MW membrane component. In addition to the 77 x 10^3 MW protein, a new major component at 52 x 10^3 MW is observed on K-NRK. These changes in cell surface proteins on K-NRK correspond to marked alterations of morphology and intracellular cAMP content (R.A. Carchman, G.S. Johnson, I. Pastan, E.M. Scolnick, Cell 1:59, 1974). NRK cells are contact inhibited and have an epitheloid appearance with a flattened surface free of microvilli. Transformation results in a loss of contact inhibition, vastly decreased intracellular cAMP content, and a rounded cell contour with abundant surface microvilli (W.W. Carley, H.L. Moses, W.M. Mitchell, J. Supramol. Structure 5:309, 1976). Cells transformed by temperature sensitive (ts) mutants of the Kirsten sarcoma virus, in which contact inhibition, intracellular cAMP content, and surface microvilli can be modulated by growth temperatures, exhibit 250 x 10^3 and 190 x 10^3 MW surface proteins at restrictive temperatures. The 250 x 10^3 MW surface protein decreases at permissive temperatures concomitant with the expression of the transformed phenotype.

507 IRON DEPLETION INDUCES TWO TRANSFORMATION-SENSITIVE MEMBRANE PROTEINS IN NORMAL CELLS. J.A. Fernandez-Pol, VA Medical Center and St. Louis University, St. Louis, MO 63125.

Rapid depletion of iron from the growth medium specifically induces the synthesis of two membrane proteins of MW 160,000 and 130,000 in cultured normal rat kidney (NRK) cells. These proteins were termed iron-regulated proteins, IRP-160 and IRP-130, respectively. We estimated the turnover rate of the proteins and show that it is slower than that of control NRK cells. We find that IRP-160 and IRP-130 are the underglycosylated forms of two membrane glycoproteins of MW 163,000 and 132,000, respectively. Thus, glycosylation appears to have an essential role in the turnover of IRP-160 and IRP-130. Simian virus 40-transformed (SV-NRK) and Kirsten sarcoma virus-transformed (K-NRK) cells showed a defective response to iron deprivation manifested by greatly reduced levels of both IRP-160 and IRP-130 and altered time-sequence of the induction. In a SV40-transformed BALB/3T3 mutant cell line, that release siderophore-like growth factor (Cell 14:489, 1978), IRP-160 and IRP-130 are absent. In NRK cells IRP-160 is readily labeled by lactoperoxidase-mediated iodination. High specific activity iodination of SV-NRK and K-NRK cells showed greatly reduced labeling of IRP-160. IRP-130 was inaccessible to iodination in all cell lines. To further characterize IRP-160 and IRP-130 they have been isolated from plasma membranes of NRK cells. Antibodies generated against these proteins provide a means of determining its function. Experiments have indicated that IRP-160 may constitute a subunit of the transferrin receptor. The major alteration in the induction of IRP-160 and IRP-130 in transformed cells strongly suggests that these proteins may have an instrumental role in loss of growth control of transformed cells. (Supported by VA and NIH research funds)

508 ABERRANT CELL CYCLE - CYCLIC AMP-DEPENDENT PLASMA MEMBRANE PHOSPHORYLATION IN TRANSFORMED CELLS, Robert E. Scott, Dept. of Pathology, Mayo Clinic, Rochester, MN 55901

Purified plasma membrane preparations were isolated from rapidly growing 3T3, methylcholanthrene transformed and Simian virus 40 transformed 3T3 cells by the vesiculation technique (Scott, *Science 194*:743, 1976). The ability of these specimens to phosphorylate exogenous and endogenous substrates in the presence and absence of cyclic AMP was tested. The membranes of all three cell types showed phosphorylation of exogenously added histone which was stimulated 3 to 6 fold by cyclic AMP. By contrast, when endogenous membrane proteins were used as the substrate for the endogenous cyclic AMP-dependent membrane protein kinase, 3T3 membranes were exceeding efficient phosphate receptors relative to SV3T3 or MCA3T3 plasma membranes which showed significantly reduced or no cyclic AMP-dependent plasma membrane phosphorylation, respectively. These data suggest that the relative inability of plasma membrane proteins to undergo endogenous cyclic AMP-dependent phosphorylation may be a unique characteristic of transformed cells. Preliminary results suggest that the ability of the plasma membrane to show modulation of cyclic AMP-dependent phosphorylation during the cell cycle may be an even more useful method to distinguish nontransformed from transformed cells. In studies on the plasma membranes of synchronized cell populations, we found that 3T3 cells down modulate cyclic AMP-dependent endogenous membrane phosphorylation during the late G_1 phase of the cell cycle. By contrast, in SV3T3 and MCA3T3 plasma membranes the level of cyclic AMP-dependent plasma membrane phosphorylation remained fixed during the G_1 and S phases of the cell cycle. We propose that the inability of transformed cells to modulate plasma membrane phosphorylation during the cell cycle may account at least in part for their loss of growth control.

509 THE INTERACTION OF 5'-NUCLEOTIDASE WITH ACTIN AND ACTIN'DNAase I COMPLEX H.G. Mannherz, M.Magener and G.Rohr, Max-Planck-Institut für medizinische Forschung, Jahnstr. 29, 6900 Heidelberg

The integral plasma membrane protein 5'-nucleotidase is able to reverse the inhibitory action of actin on pancreatic DNAase I. It also accelerates the rate of actin polymerisation, indicating direct interaction with actin. The formed actin polymer, however, does not have the normal structural appearance of thin filaments, instead a filamentous network can be visualized after negative staining.resembling Cytochalasin B treated F-actin. Evidence will be presented indicating that 5'-nucleotidase might act as an anchorage protein of submembranous actin and that its inhibition will lead to an inhibition of its ability to interact with actin.

510 IDENTIFICATION OF THE BINDING PROTEIN(S), NECTIN, FOR HUMAN ERYTHROCYTE SPECTRIN. John Yu* and Steve R. Goodman+. Department of Cellular and Developmental Immunology, Scripps Clinic and Research Foundation, La Jolla, CA 92037 and The Biological Laboratories, Harvard University, Cambridge, MA 02138+

Spectrin comprises 25-30% of total erythrocyte membrane protein and appears to play a key role in the maintenance of discoid shape and in the restriction of lateral mobility of membrane macromolecules. The protein(s) which provide the binding site for spectrin is now identified using two dimensional peptide mapping technique. Analysis based on this mapping technique showed that all the major polypeptides of human erythrocyte membrane display characteristic peptide maps; however, band 2.1 and several polypeptides below this band exhibit similar tryptic and chymotryptic maps and thus appear to be a family of closely related proteins or degraded products. Furthermore, they all contain a subset of peptides which are accounted for by the peptides from two known spectrin-binding fragments. One of the fragments, the 72,000 dalton fragment, had been further purified and its role in spectrin binding confirmed. The other fragment, 3', was found to appear with a concurrent loss of band 2.1 upon aging of ghost preparations. It was therefore concluded that both fragments derived from 2.1-related proteins and 2.1 and its related proteins, which we name "nectin," bind spectrin and connect it to erythrocyte membrane. [Supported by grant HL 21845-01 from NIH, an Established Investigatorship (to J.Y.) from the American Heart Association and a postdoctoral fellowship (to S.R. Goodman) from NIH.]

511 LYMPHOCYTE PLASMA MEMBRANE PROTEINS ON THE CYTOPLASMIC SURFACE. Alan P. Johnstone and Michael J. Crumpton. National Institute for Medical Research, Mill Hill, London.

Plasma membrane proteins have been implicated in regulating various aspects of cell behaviour, such as cell-cell recognition and interaction, cell growth and differentiation as well as response to hormones and drugs. In addition, some differences between normal and neoplastic cells have been ascribed to altered membrane proteins or membrane-associated proteins (e.g. cytoskeletal elements). These phenomena are mediated by transmembrane signals involving, in part, proteins on the cytoplasmic side of the plasma membrane; thus, it is important to determine the molecular structure of the membrane inner surface. Our approach to this study is the biochemical characterization of lymphocyte plasma membrane and sealed membrane vesicles with an "inside-out" orientation, as well as using antisera against the inner surface to probe the nature and arrangement of the exposed proteins. Sealed membrane vesicles were isolated by dextran gradient centrifugation of plasma membrane and were fractionated by chromatography on ConA-Sepharose columns. This fractionation apparently does not reflect differences in protein composition of the vesicles but rather differences in orientation of the faces of the membrane and therefore it can be used to isolate "inside-out" vesicles. Antisera against the membrane inner surface recognised actin, albumin and and several other proteins. The relevance of these proteins to the structure of the membrane inner surface will be discussed.

512 LIGATIN FROM EMBRYONIC CHICK NEURAL RETINA, Emma R. Jakoi, and Richard B. Marchase, *Duke University, Durham, N.C. 27710.*

Ligatin, a filamentous protein previously found in suckling rat ileum, has been purified from plasma membranes of embryonic chick neural retina. The isolated plasma membranes are covered in part by 4.5 nm filaments that can be released from the membranes by treatment with Ca^{++}. Subsequent dialysis against EGTA followed by sieve chromatography results in purification of the 10,000 dalton ligatin monomer. Upon readdition of Ca^{++}, purified ligatin monomers polymerize to form filaments 3 nm in diameter. However, in both retina and ileum, the filaments observed on plasma membranes are larger than 3 nm in diameter. In ileum, this enlargement results from ligatin's function as a baseplate for the attachment of β-N-acetylhexosaminidase to the cell surface. In retina, a corresponding difference in diameter between filaments seen in vivo and those formed from repolymerized ligatin alone and the co-solubilization of other proteins with ligatin suggest that ligatin may also function there as a baseplate for other cell-surface proteins. The proteins associated with ligatin in retina differ morphologically from β-N-acetylhexosaminidase and do not possess this enzymatic activity. Purified retinal ligatin does, however, inhibit the reassociation of trypsinized retinal cells, suggesting that in retina ligatin may function as a baseplate for cell-surface molecules responsible for intercellular adhesion.

513 LECTIN-2 FROM EMBRYONIC CHICK MUSCLE INTERACTS WITH GLYCOSAMINOGLYCANS FROM EXTRA-CELLULAR MATRIX, Paula J. Shadle, David Kobiler, and Samuel H. Barondes, Univ. of California at San Diego, La Jolla, CA 92093

Embryonic chick muscle contains two developmentally regulated lectins which may be involved in cell interactions. Lectin-1 interacts with fixed trypsinized rabbit erythrocytes, is markedly inhibited by lactose, and has been purified by affinity chromatography (Nowak, et al. JBC: 252, 6026-6030, 1977). Lectin-2 (Mir-Lechaire and Barondes, Nature 272: 256-258, 1978) reacts with fixed trypsinized rabbit erythrocytes only after they have undergone a modification with prolonged aging or treatment with ethanol; is insensitive to lactose; and can be partially purified by centrifugation at 100,000 x g for 12 hrs., subsequent dissociation with high salt and gel filtration. This partially purified material contains no fibronectin by SDS PAGE and does not react with antiserum to fibronectin. Lectin-2 is markedly inhibited by 0.1 N NaOH extracts of substrate attached material (SAM) from embryonic chick muscle cultures. The inhibitor from SAM is insensitive to boiling, trypsin, pronase, hyaluronidase and chondroitinase AC, but 90% is destroyed by chondroitinase ABC. It binds to DEAE-cellulose and elutes in two peaks,one at 0.5 M NaCl and one at 0.1 N NaOH. Some purified glycosaminoglycans also inhibit lectin-2. Heparin and dermatan sulphate are especially potent, heparin sulphate less so and hyaluronic acid and chondroitin sulphates (A and C) are inactive. These results raise the possibility that lectin-2 functions by interacting with extracellular glycosaminoglycans, either in the matrix or on adjacent cells. Supported by Grants from the USPHS and McKnight Foundation. Paula J. Shadle is supported by a NSF Predoctoral Fellowship and David Kobiler by a Weizmann Fellowship.

514 LECTIN-2 ACTIVITY IN MEDIA OF POST-FUSION MYOBLAST CULTURES, Howard Ceri, David Kobiler and Samuel H. Barondes, Univ. of California at San Diego, La Jolla, CA 92093

Media and cells from primary chick myoblast cultures obtained from 11 day old chick embryos and containing 90% myoblasts were assayed for lectin-1 and lectin-2 activity as described in Shadle et al. (this meeting). Extracts were made by homogenizing cells in buffer containing 0.1 M lactose and 0.1M N-acetyl galactosamine and assays were done after dialysis of the extract. Extracts of myoblasts were rich in both lectin-1 and lectin-2 which remained at fairly constant levels from the time of plating to termination of the experiment about 2 weeks later. Media from the cultures contained neither lectin activity until about 8 or 9 days after culture at which time considerable myoblast fusion had occurred. Lectin-2 activity then rose dramatically in the medium such that there was approximately an equal amount in the medium and in the cells. No lectin-1 activity was ever detected in the medium. Lectin-2 in the medium was inhibited by glycosaminoglycans and substrate attached material (SAM) extracted as in Shadle et al. It appears identical to partially purified lectin-2 extracted from embryonic muscle that was shown to be unrelated to fibronectin. Unlike fibronectin it does not bind to collagen and is fully active as an agglutinin in the presence of EDTA. Fibroblast and cardiac muscle cultures contained considerable lectin-2 but none was ever detected in the medium. A function of extracellular lectin-2 from myoblasts could be to interact with extracellular glycosaminoglycans like those detected in SAM, or known to be associated with some cell surfaces. Supported by Grants from the USPHS and McKnight Foundation. Howard Ceri is supported by the Medical Research Council, Canada, David Kobiler is supported by a Weizmann Fellowship.

515 BIOSYNTHESIS AND MATURATION OF CELLULAR MEMBRANE GLYCOPROTEINS. Lawrence A. Hunt, Dept. Microbiology, Univ. Kansas Medical Center, Kansas City, Kansas 66103

Previous studies of the asparagine-linked oligosaccharide chains of cellular glycoproteins from cultured fibroblasts have demonstrated both a heterogeneous array of oligomannosyl core sizes with acidic and neutral glycopeptides, and a growth dependent difference in the oligomannosyl core size distribution (Muramatsu et al., J. Biol. Chem. 251:4673 (1976)). In order to examine the intracellular biosynthesis of such oligosaccharides, cellular membrane glycoproteins were isolated from BHK21 and human diploid fibroblasts after pulse and pulse-chase labeling with (2-^{3}H) mannose, and pronase-digested glycopeptides were characterized by gel filtration and glycosidase treatment. Newly synthesized glycoproteins contained a relatively homogeneous population of neutral oligosaccharides (8-9 mannoses and 2 N-acetylglucosamines), which were smaller than the lipid-linked oligosaccharides from the cell and apparently devoid of terminal glucose. After pulse-chase or long labeling periods, a significant fraction of the large oligomannosyl cores were processed by addition of branch sugars and/or removal of mannose units, resulting in a heterogeneous array of core sizes with 5-9 mannoses in neutral structures and 3 or 5 mannoses in complex acidic structures. This heterogeneity is similar to that reported for the oligosaccharides of avian RNA tumor virus glycoproteins (Hunt et al., J. Virol. (in press, Jan. 1979)), and the processing is analogous to that of the vesicular stomatitis virus glycoprotein in the same cell line (Hunt et al., Proc. Nat. Acad. Sci. 75: 754 (1978)).

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516 GLUCOSIDASES INVOLVED IN THE PROCESSING OF CELLULAR GLYCOPROTEINS, Lynn S. Grinna and Phillips W. Robbins, Massachusetts Institute of Technology, Cambridge, Mass. 02139

The carbohydrate portions of cellular glycoproteins undergo extensive modification or processing during transit to the cell surface. Glycoproteins are glycosylated as nascent polypeptide chains by the en bloc transfer of the oligosaccharide $Glc_3Man_9GlcNAc_2$ from polyisoprenoid lipid carrier to the polypeptide. Immediately after transfer, processing of the oligosaccharide begins by the sequential removal of the three glucose residues and 0-6 mannose residues and the addition of carbohydrate residues which are characteristic of the mature cellular glycoprotein.

We have identified glucosidase activities which catalyze removal of glucose from the oligosaccharide $Glc_3Man_9GlcNAc_2$, $Glc_2Man_9GlcNAc_2$ and $Glc_1Man_9GlcNAc_2$. The enzymes, which are integral membrane proteins, are localized in the rough and smooth endoplasmic reticulum and show asymmetric spatial orientation, with the active sites of the enzymes on the cisternal surface of the reticulum. Detergent solubilization studies, inhibitor studies, and temperature studies indicate that at least two distinct glucosidases are present in the membranes. One of these glucosidases has been solubilized and partially purified. These glucosidases are suggested to be of biological importance in catalyzing the initial events in the post glycosylation processing of cellular glycoproteins.

517 SPECIFIC ALTERATIONS IN THE STRUCTURE AND PROCESSING OF GLYCOPROTEIN OLIGOSACCHARIDES OF TWO LECTIN-RESISTANT CHINESE HAMSTER OVARY CELL LINES, James R. Etchison and D.F. Summers, U. of Utah, SLC, UT. 84132

A clonal line of Chinese hamster ovary cells selected for resistance to the cytotoxicity of phytohemagglutinin (PHA; clone B2p^{1}1 = Pha^{RI}) and a second line isolated from the Pha^{RI} line after selection for resistance to Con A (clone Bp^{1}c^{II}3B = Pha^{RI}ConA^{RII}) exhibit alterations in glycoprotein oligosaccharide structure. The vesicular stomatitis virus glycoprotein, used as a probe to isolate and purifiy cell oligosaccharide structures, has been shown to lack NeuNAc-Gal-GlcNAc branches and to contain altered oligomannosyl core structures when virus is grown in these cell lines.* The sequence of these altered oligomannosyl cores has been determined:

ex Pha^{RI}: Manα1-6(Manα1-3)Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-peptide

ex Pha^{RI}ConA^{RII}: Manα1-3Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-peptide

Hence, the resistance to Con A cytoxicity in the double mutant was accompanied by the loss of a specific α-mannose residue. The lipid-linked oligosaccharide precursors to these altered glycoprotein oligosaccharides have also been characterized.

*M.A. Robertson et al., Cell 13, 515 (1978).

518 BIOSYNTHESIS OF MANNOSYL-N-ACETYL-GLUCOSAMINYL PYROPHOSPHORYL POLYPRENYLS BY RETINA, Edward L. Kean, Case Western Reserve University, Cleveland, Ohio 44106

Previous studies from this laboratory have described the biosynthesis by the retina of the embryonic chick of dolichyl-β-mannosyl phosphate and mannosyl-oligosaccharide lipids. This report is concerned with the formation of N-acetyl-glucosaminyl-mannosyl pyrophosphoryl polyprenols. When homogenates of the retina were incubated,under conditions approximating initial rates, with Mn^{2+} (3.3 mM), TES buffer, pH 7.0, TX-100 (0.19%) and dolichyl phosphate (23 μM), the presence of GDP-mannose (67 μM) resulted in a 10 fold stimulation in the rate of transfer of radioactivity from UDP-(^{3}H)-GlcNAc (14 μM) to material extractable into chloroform : methanol (2:1). The stimulation was specific for GDP-mannose. The GlcNAc-containing lipids had the characteristics of pyrophosphates on DEAE cellulose acetate. When examined by TLC, three (^{3}H)-GlcNAc areas were detected: (A),a slow mobility material (Rf=0.06); (B), $(GlcNAc)_2$-lipid; (C), GlcNAc-lipid. In the presence of GDP-(^{14}C)-mannose, after removal of dol-P-mannose by DEAE chromatography, only component (A) contained (^{14}C). Although stimulated by GDP-mannose, the major GlcNAc labeled material was (B), with the ratio of labeling from UDP-(^{3}H)-GlcNAc of 1 : 4 : 2, for A, B, and C, respectively. The rate of incorporation of label from GDP-(^{14}C)-mannose into the GlcNAc-lipids was about 2% of that into dolichyl-phos-mannose. The formation of the GlcNAc-lipids was almost completly inhibited by tunicamycin, slightly by bacitracin, and unaffected by streptozotocin. Although labeled UDP-glucose served as a substrate for the lipid-intermediate pathway , it did not stimulate the labeling of the GlcNAc-lipids in the presence or absence of GDP-mannose.

519 EFFECTS OF BRDURD ON ENZYMES INVOLVED IN CELL MEMBRANE BIOSYNTHESIS, Irene M. Evans, David Hockenberry, and H. Bruce Bosmann, The University of Rochester, Rochester, New York, 14642

Bromodeoxyuridine (BrdUrd), a thymidine analogue, has various effects on biological systems including suppression of the synthesis of differentiative products and reduction of the tumorigenicity of transplantable tumor cells cultured in its presence. Previous studies have demonstrated cell surface membrane alterations in cultured melanoma cells grown in BrdUrd (Evans et al, 1977, FEBS Lett. 78:109; Rosenthal et al, 1978, Exp. Cell Res., 112:419). In order to determine whether BrdUrd might affect nucleoside sugar reactions which are involved in membrane biosynthesis, measurements were made of the activity of glycosyltransferase and neuraminidase enzymes in cell extracts from BrdUrd, thymidine, or untreated cells. Our experiments detected no changes in the activity of either UDP-galactose:glycoprotein glycosyltransferase or CMP-N-acetylneuraminic acid glycoprotein sialyltransferase enzymes. Neuraminidase activity was 3x higher in the BrdUrd treated cells.

520 ANALYSIS OF MEMBRANE GLYCOPROTEIN OLIGOSACCHARIDES FROM MOUSE EMBRYONAL CARCINOMA CELL LINES, J.R. Etchison, C. Georgopoulos, and D.F. Summers, Univ. of Utah Medical Center, Salt Lake City, UT 84132

Recent studies have shown that mouse embryonal carcinoma (EC) cells contain a class of large oligosaccharide structures isolated after digestion of membrane glycoproteins with Pronase.* These structures were absent in differentiated cell lines derived from multipotent EC cell lines.* Recent studies on a "quasi"-nullipotent EC cell line indicated a predominance of "high-mannose"-type oligosaccharides and little, if any, complex-type oligosaccharides.** It was suggested that these cells may be deficient in oligosaccharide processing. We have used replication of vesicular stomatitis virus (VSV) to probe oligosaccharide biosynthesis in three EC cell lines and two differentiated cell lines derived from EC cells. We found that VSV glycoprotein produced in the EC cells contains neither the large, differentiation-sensitive (LDS) oligosaccharides nor unprocessed high mannose oligosaccharides but contains complex type oligosaccharides typical of other mammalian cell lines. Analysis of uninfected EC cell glycoproteins confirmed that the complex-type oligosaccharides were a small percentage of the total structures. In addition, we have found that the EC cells incorporate more fucose into their glycoproteins than do the differentiated cell lines derived from the EC cells.

*Muramatsu et al., PNAS 75, 2315 (1978)

**Muramatsu et al., in press.

521 COMPARATIVE ANALYSIS OF THE OLIGOSACCHARIDE MOIETIES OF XENOTROPIC AND ECOTROPIC MURINE LEUKEMIA VIRUSES (MuLV), Maurice C. Kemp, Sukla Basak and Richard W. Compans, Department of Microbiology, Univ. of Alabama Med. Ctr., Birmingham, Al. 35294.

The oligosaccharide moieties of a xenotropic MuLV (AKR-MuLV) 69X9 and those of Raucher and AKR-MuLV ecotropic murine leukemia viruses were analyzed by gel filtration on Bio-Gel P6 or P10 following exhaustive digestion with pronase and treatment with neuraminidase. Four different glycopeptide size classes having molecular weights of approximately 5100, 2900, 2700 and 1500 daltons (G_1, G_2, G_3 and G_4), respectively, were shown to be associated with ecotropic Rauscher-MuLV (R-MuLV) virions grown in JLS-V9 cells. The two larger glycopeptides G_1 and G_2 were shown to be sulfated complex (type I) galactose-containing glycopeptides. The two smaller glycopeptide size classes, G_3 and G_4 were shown to be mannose rich glycopeptides. G_4 was more sensitive to digestion with endoglycosidase H than G_3, suggesting that the mannose core of G_3 may contain fewer mannose residues. A comparison of the oligosaccharide moieties of ecotropic R-MuLV and AKR-MuLV, both grown in III6A cells, showed that they possessed glycopeptides similar to those of R-MuLV grown in JLS-V9 cells including the large glycopeptide G_1. Analysis of the glycopeptides of the cell associated gp70 isolated from xenotropic AKR-MuLV 69X9-infected CCL64 mink lung cells showed that the 69X9 virus gp70 did not possess G_1. In addition, the mannose labeled R-MuLV glycopeptides were present in ratios of 13:1:7 (G_2:G_3:G_4), whereas the mannose labeled 69X9 glycopeptides were present in ratios of 13:10:1. The observed differences in the glycopeptide composition between the ecotropic and xenotropic MuLV may be due to host-dependent variations in the glycosylation pattern. However, differences in the primary structure of the viral glycoproteins have been noted and it may be expected that such changes may cause differential glycosylation of the viral glycoproteins.

522 GLYCOCONJUGATES OF MOUSE TERATOCARCINOMA CELLS. Marie-Hélène Buc-Caron. Institut Pasteur, Unité de Génétique cellulaire, 25 rue du Dr. Roux, 75015 Paris.

We are currently trying to correlate the presence of specific cell surface molecules of totipotent mouse teratocarcinomas with events occuring during differentiation. This tumor was chosen as a model for the study of the early development of the mouse embryo. It has been shown, using serological and biochemical techniques, that specific glycopeptides and glycolipids are expressed in large amounts on the undifferentiated primitive cells and that these are greatly reduced or not present on differentiated derivatives of the tumor, whereas other glycoconjugates appear.

523 STRUCTURAL DETERMINANTS OF RCA_I AND RCA_{II} OLIGOSACCHARIDE SPECIFICITY. Jacques U. Baenziger and Dorothy Fiete, Washington University, St. Louis, MO 63110

Glycopeptides radiolabeled with iodinated 3-(4-hydroxyphenyl) propionic acid N-hydroxysuccinimide ester ($50-200 \times 10^3$ cpm/pmole) have been utilized to examine the structural determinants affecting glycopeptide interaction with the saccharide binding site of lectins. Scatchard plot analysis of saturation curves obtained with RCA_I and RCA_{II} isolated from Ricinus communis yields association constants (ka) for immunoglobulin and fetuin complex oligosaccharides which are generally 2-fold higher for RCA_I than RCA_{II} and range from 2 to 17×10^6 M^{-1}. Complex oligosaccharides with 1,2, or 3 residues of terminal galactose have a progressive increase in their ka from 10 to 17×10^6 M^{-1} for RCA_I and from 3 to 14×10^6 M^{-1} for RCA_{II}. In both cases the presence of sialic acid results in a significant (generally 2-fold) reduction in the ka for complex oligosaccharides. RCA_I and RCA_{II} do not display demonstrable binding to the Gal β1,3 GalNAc α-ser/thr oligosaccharides of fetuin which bear sialic acid. RCA_I and RCA_{II} differ markedly in their binding of the O-glycosidic units of IgA_1 yielding ka's of 6×10^6 and 47×10^6 M^{-1} respectively. Detailed knowledge of lectin specificity for oligosaccharides should aid in the interpretation of cell surface changes following transformation which are monitored by lectin binding. This information has also been useful for the establishment of separation procedures for glycopeptides based on lectin-sepharose affinity chromatography.

Tumor Antigens as Molecules

524 MONOCLONAL ANTIBODIES AGAINST MURINE FIBROBLAST CELL SURFACE ANTIGENS. Edward N. Hughes and J. Thomas August, Department of Pharmacology and Experimental Therapeutics, Johns Hopkins University School of Medicine.

An analysis of the protein composition of the plasma membrane of mouse fibroblasts is being carried out through the use of hybrid cell lines secreting specific monoclonal antibodies. Sprague-Dawley rats were immunized with NIH 3T3 fibroblasts. Spleen cells of these rats were fused to the murine myeloma P3-X63Ag8 (Galfre, G. et al., Nature 266, 1977). Of 121 initial hybridoma cultures analyzed, antibodies against NIH 3T3 cell surface determinants were detected in 36 culture supernatants by a cell binding assay utilizing ^{125}I goat IgG anti-rat IgG (Williams, A.F. et al., Cell 12, 1977). The distribution of these determinants was analyzed using a variety of different cells. Each of these antigenic sites was present on NIH 3T3 cells transformed by Harvey sarcoma virus. Most noteworthy was the finding that all of the major antigens thus detected on murine fibroblasts were also found on thymocytes of a variety of mouse strains. In contrast, these determinants were undectable on murine erythrocytes and absent or significantly reduced on fibroblasts of other species. The specificity of these monoclonal antibodies has been analyzed by immunoprecipitation and SDS-PAGE of ^{35}S-methionine labeled polypeptides. One principal reactive component was a protein of 65,000 daltons immunoprecipitated by antibody from four of the thirty-six positive hybridoma cell lines.

525 PRODUCTION OF MONOCLONAL ANTIBODIES AGAINST CONCANAVALIN A RECEPTORS ISOLATED FROM CHINESE HAMSTER OVARY CELL PLASMA MEMBRANES, Kenneth D. Noonan and James J. Starling, Department of Biochemistry, University of Florida, Gainesville, Florida 32610.
Plasma membranes isolated from the $H\text{-}7_w$ subclone of CHO cells were solubilized in 50mM sodium borate containing 0.5% Triton X-100 (pH 7.4) and applied to a Con A-Sepharose affinity column. The Triton-soluble membrane components which did not bind to the Con A-Sepharose affinity column exhibited a coomasie blue staining profile on SDS-PAGE which was significantly different from the hapten-released, Con A binding fraction. [^{125}I] Con A overlays of the SDS-PAGE demonstrated that there were virtually no Con A receptors associated with the unretarded peak whereas the material which was bound and specifically eluted from the Con A-Sepharose column contained approximately 15 different bands which bound [^{125}I] Con A. In order to produce monoclonal antibodies to the various Con A receptors, cell fusions were performed using spleen cells obtained from Balb/c mice immunized with the Triton soluble Con A receptor fraction and mouse myeloma P3-X63-Ag8 cells. The supernatants derived from the fused hybridoma cells were screened for antibodies which could bind to live and glutaraldehyde-fixed CHO cells as well as to the soluble Con A receptor glycoproteins. Several hybridoma clones were subsequently isolated. The clones secreted antibody which exhibited preferential binding to both CHO cells and the Triton-soluble Con A receptor glycoproteins. On-going experiments are aimed at identifying the individual Con A receptor(s) which is(are) the target for the clonal antibody(ies) so that these antibodies may be used to address questions concerning the role of individual Con A receptors in the phenomena of Con A-induced receptor site capping as well as Con A-initiated cell agglutination.

526 HEAT SENSITIVITY OF $P60^{SRC}$ KINASE FROM TRANSFORMATION DEFECTIVE TS MUTANTS OF AVIAN SARCOMA VIRUS, Helga Rübsamen, Robert R. Friis and Heinz Bauer, Institut für Virologie, Frankfurter Str. 107, 63 Gießen, W.-Germany.
Sera have been made in SR-D tumor-bearing rabbits which recognize a phosphorprotein of Mw 60,000 in cells transformed by four different strains of avian sarcoma virus (ASV) but not in normal cells, or cells infected by Rous-associated viruses. All characteristics of the protein detected by our sera agree with the findings of Erikson and coworkers (1,2), we therefore have called it $p60^{src}$. In the immunoprecipitates from all ASV transformed cells a protein kinase activity was observed which phosphorylated the heavy chain of IgG. The activity of this kinase may be controlled by phosphorylation since concomitant to the loss of kinase activity $p60^{src}$ looses 70 % of its PO_4^{-3} content. The protein kinase was found to be more heat-sensitive in vitro than the kinase from wild-type transformed cells when it was derived from cells transformed by GI 251, a transformation-defective temperature-sensitive (T class) mutant of RSV. This finding suggests that it is coded for by the mutated src gene of GI 251 and that therefore $p60^{src}$ is a protein kinase. The amount, the degree of phosphorylation and the kinase activity of $p60^{src}$ have been studied in a set of 5 T class mutants of PrA and were correlated with the biological characteristics of the mutant infected cells at permissive and restrictive temperature.

References: 1) Brugge, J.S. and Erikson, R. L. (1977) Nature 269, 346-348.
2) Brugge, J.S., Erikson, E., Collett, M. S. and Erikson, R. L. (1978) J. Virol. 26, 773 - 782.

527 TRANSFORMED MAMMALIAN CELLS SECRETE SPECIFIC PROTEINS AND PHOSPHOPROTEINS, Donald R. Senger, Dyann F. Wirth, and Richard O. Hynes, M.I.T., Cambridge, MA 02139

Transformed cell lines from several mammalian species secrete transformation-specific proteins of molecular weight 58,000 daltons. The 58K secretory proteins of transformed hamster, rat, and mouse cell lines are all antigenically related. Secretion of the 58K proteins occurs with either RNA or DNA virus transformation. Cells transformed by RNA and DNA tumor viruses also secrete major phosphoproteins of similar size but these are immunologically distinct from the 58K secreted proteins. The sizes of the phosphoproteins are species-specific and unrelated to the transforming virus. Incubation of transformed cell conditioned media with γ ^{32}P-ATP labels phosphoproteins which are apparently identical to the major secreted phosphoproteins labeled in vivo. This indicates that the media contain both protein kinase and substrate. All three properties (58K protein, phosphoprotein, in vitro phosphorylation) are closely correlated with transformation in cells transformed by temperature-sensitive viruses.

528 MURINE LEUKEMIA VIRUS FUSION ACTIVITY, David A. Zarling and Ilana Keshet, Univ. of Wisconsin, Madison, 53706.

The fusion activity associated with many strains of murine leukemia virus (MLV) is commonly used to measure MLV infectivity. Other strains of MLV lack fusion activity and some of these can produce variants which have fusion activity. The SC-1/UV-XC fusion assay measures the infectivity of MLV grown in SC-1 cells by the XC cell fusion activity of the progeny virus. The fusion activity of parental MLV in XC cells can be measured directly within a few hours after infection. No new macromolecular synthesis is required for the fusion process. Treatment of MLV virions with proteolytic enzymes destroys their ability to fuse XC cells. The infectivity of MLV is much more susceptible to UV-irradiation than viral fusion activity and non-infectious (UV-irradiated) MLV can cause XC cell fusion. When MLV virions are disrupted with NP-40 detergent and the viral cores removed, the fusion activity can be restored after dialysis of the detergent and reassembly of (non-infectious) MLV envelope-like particles. The fusion activity of MLV envelope-like particles is inhibited by antiserum against MLV gp70. Thus, the fusion activity of MLV is associated with the virion envelope protein. MLV fusion activity appears to be essential for MLV infectivity and fusion activity may also play an essential role in cell-mediated immunity against virus infected or transformed cells.

529 MODULATION OF CELL FUSION INDUCED BY HERPES SIMPLEX VIRUS (HSV), Gloria T. Lee and Patricia G. Spear, Committee on Virology, University of Chicago, Chicago, IL 60637.

HSV infection results in cell fusion under specific conditions. Previous studies with viral mutants have revealed that one of the HSV membrane glycoproteins, designated B_2, plays an essential role in inducing cell fusion whereas another viral gene product, possibly the HSV glycoprotein designated C_2, appears to modulate or inhibit fusion-inducing activity. The existence of this "modulator" explains the findings that wild-type HSV usually does not induce cell fusion whereas mutation can result in the syncytial phenotype. We have conducted experiments designed to determine which constellations of HSV membrane glycoproteins must be present in the membranes of neighboring cells in order for fusion to occur. The approach is to quantitate the fusion of cells from one population with those of another whose membranes differ with respect to cellular or viral constituents, under conditions that prevent the spread of virus between cells and that allow identification of nuclei from the two different cell populations. Our results indicate that the modulator of fusion can prevent a cell from initiating fusion only if the inducer(s) and modulator are expressed by the same cell but cannot prevent a cell from being recruited into a polykaryocyte. Also, cells expressing the fusion inducer(s) only can fuse with uninfected cells, suggesting that the inducer(s) interact with cellular constituents in order to initiate fusion. Because cells expressing the modulator can be recruited into polykaryocytes, the possibility that the modulator inhibits fusion by affecting cellular constituents is ruled out. We have also noted that uninfected cells of different types differ in the efficiency with which they are recruited into polykaryocytes. Thus our studies indicate that HSV-induced cell fusion is influenced not only by multiple viral gene products but also by the nature of cell-determined surface components.

530 CELLULAR ORGANELLES AS TARGETS OF TUMOR-INDUCING PROTEINS: STUDIES OF CELL SURFACE AND CHROMATIN ALTERATIONS DURING CELLULAR TRANSFORMATION, Reinhard Kurth, Adolfine Huesgen, Gert Huesgen and Johannes Löwer, Friedrich Miescher-Laboratorium, Max Planck-Institutes, 74 Tübingen, West Germany.

We had previously defined by immunological means a tumor-specific cell surface antigen (TSSA) which is invariably expressed on Rous sarcoma virus transformed cells of a variety of different species. Genetic studies had furthermore implicated a close correlation between TSSA expression and the presence of a functional src-gene of Rous sarcoma virus, which is needed for virus-induced fibroblast transformation. The recent characterization of the fibroblast-transforming protein ($p60_{src}$) of the Rous sarcoma virus by Erickson and his collaborators prompted our studies to look for a possible relationship between TSSA and $p60_{src}$. Preliminary data indicate that the cytoplasmic $p60_{src}$ does not appear on the cell surface in an immunologically recognizable form. However, it cannot be excluded at present that the cytoplasmic $p60_{src}$ appears on the tumor cell surface as a potentially immunogenic molecule altered by proteolytic cleavage and/or glycosylation. We have therefore initiated the biochemical isolation of Rousvirus-induced cell surface antigen.

For the study of another cellular organelle as potential primary target for $p60_{src}$, nuclear histone and non-histone proteins have been purified from normal and virus-transformed cells. These proteins are presently being investigated as possible substrates for the known phosphokinase activity of $p60_{src}$.

531 A CELL SURFACE PROTEIN SPECIFIED BY MUTANT HAPLOTYPES OF THE MOUSE T/t COMPLEX
Lee M. Silver, Karen Artzt, and Dorothea Bennett
Sloan-Kettering Institute, New York, New York 10021.

Mutant haplotypes of the mouse T/t complex have profound effects on embryonic development, chromosomal recombination, gene transmission frequencies, and spermatogenesis. A combination of histological and serological data suggest that genes in the T/t complex define cell surface antigens expressed during embryogenesis and spermatogenesis. It has been hypothesized that the T/t complex is an embryonic analogue of the major histocompatibility complex.

Two-dimensional gel electrophoresis has been used in conjunction with other techniques to identify a major cell surface protein--p63/6.9--which is specified by a gene in the T/t complex. All mutant t-haplotypes analyzed cause the expression of a form of p63/6.9 which is different from the wild-type form. The $\underline{T^{Hp}}$ haplotype is thought to represent an extensive deletion over a region of the T/t complex. Molecular analysis demonstrates that the $\underline{T^{Hp}}$ haplotype does not allow expression of either form of p63/6.9. Three alternate alleles of the T/t complex "p63/6.9 gene" have been defined--a wild-type allele, a mutant t-haplotype, and a $\underline{T^{Hp}}$ *null allele.*

532 IMMUNOPRECIPITATION OF POLYSOMES SPECIFYING THYMUS-LEUKEMIA ANTIGENS*, Ryszard Slomski and Edward P. Cohen, La Rabida-University of Chicago Institute, Chicago, IL 60649

Thymus-Leukemia (TL) antigens are associated with the surface membranes of leukemia cells and immature thymocytes of certain mouse strains. mRNAs for TL antigens were obtained from immunoprecipitated polysomes of TL (+) cells using specifically purified mouse TL antibodies and rabbit anti-mouse immunoglobulins (RAM-Ig). ASL-1 cells, a TL (+) leukemia cell line of A/J mice was converted to $\underline{\text{in vitro}}$ growth by hybridization with LM(TK)$^-$ cells. Hybrid cells formed approximately one-half the quantities of TL antigens as their ASL-1 parents. Polysomes were prepared by sucrose gradient centrifugation from 5×10^9 hybrid cells, lysed previously with 1.5% Triton X100 and 0.5% DOC. The gamma globulin fractions of both RAM-Ig and mouse anti TL 1,3 sera, were prepared by $(NH_4)SO_4$ precipitation. RNase activity was removed by DEAE-cellulose chromatography. Approximately 8 A_{260} units of polysomes were incubated at 0° with 1.0 mg/ml TL antiserum for 30 min followed by incubation at 0° for an additional 30 min with an immunologic equivalent of RAM-Ig. 1.2 percent of the polysomes present precipitated. mRNA was isolated from immunoprecipitated polysomes by phenol-detergent extraction and two passages through an oligo(dT)-cellulose column. The poly(A)-RNA obtained migrated as a single peak in a sucrose gradient. 0.5 µg of the RNA stimulated in the wheat germ system the incorporation of 190,000 cpm into TCA precipitable material, a 200-fold increase over endogenous levels. Approximately 10% of the cpm obtained precipitated with TL antiserum; less than 0.4% of the cpm obtained under similar conditions with globin mRNA precipitated with TL antiserum. *Supported by USPHS grant number CA-19265-03.

533 THE HUMAN i BLOOD GROUP ANTIGEN AS A MODEL FOR CROSS-REACTING ONCOFETAL ANTIGENS. Zoltán A. Tökés, Department of Biochemistry, USC School of Medicine, LAC/USC Cancer Center, Los Angeles, CA 90033.

Human fetal erythrocytes express the i blood group antigen which gradually disappears after birth as the I blood group antigens appear. With several conditions the i antigen can be reexpressed on adult erythrocytes. This antigenic modulation and the availability of well defined monoclonal antibodies against these antigens create an ideal model to investigate the molecular events related to oncofetal antigen expression and recognition. The disappearance of i antigen from erythrocytes is independent of the change from fetal to adult type hemoglobins. Enzymatic modification of adult (i-) erythrocyte surfaces slowly uncovers the i antigen when sialoglycopeptides are gradually removed. Disruption of erythrocyte membrane integrity reveals the presence of this antigen but at only half the concentration found in fetal erythrocyte stroma. Cell types such as lymphocytes and liver cells which can relocate cell surface bound macromolecules always express i antigen throughout adult life. Increased capability to undergo patching increases the probability for multiple binding of monoclonal IgM and enhances the detection of i antigen. Quantitation of molecular fractions with I and i blood group antigen from cord and adult erythrocytes suggests that the expression of i- phenotype can be explained by the topographic distribution of molecules at the cell surface. (NCI CA-14089).

534 TUMOR CELL SURFACE AND NATURAL SURVEILLANCE, D.A. Chow and A.H. Greenberg, University of Manitoba, Winnipeg, Canada R3E 0V9

The rejection of a small subcutaneous inoculum of syngeneic tumor cells is being studied as a model for the natural surveillance of spontaneous tumors. The effector mechanism has been demonstrated in both normal and immunodeficient (ATxBM) mice and is macrophage dependent and NK cell independent. In addition, it can be blocked by the IV injection of soluble autologous but not allogeneic tumor membrane preparations, suggesting the participation of a specific receptor molecule. Cloning of tumors used in this study yielded cell lines which exhibited differences in tumor frequencies. The phenotype of the cells, defined by the tumor frequency following a small subcutaneous inoculum, was quite stable for clones maintained in in vitro culture. In addition, recloning yielded cell lines which expressed the same susceptibility to surveillance as did the parent clones, providing further evidence for phenotype stability in vitro. Tumor cell membrane characteristics were sought which would relate to the differences observed in these tumor frequencies. To date only susceptibility of clones to complement mediated lysis via natural antibody in normal mouse serum has been shown to correlate with a low tumor frequency in the in vivo model. Furthermore, pretreatment of mice with proteose peptone, which increased the levels of natural antibody in serum, resulted in a decrease in tumor frequency. These observations suggest that natural antibody binding may define a tumor phenotype which is susceptible to natural surveillance.

535 CHARACTERIZATION OF THE MAJOR SIALOGLYCOPROTEIN, A PEANUT LECTIN RECEPTOR, OF TWO ASCITES SUBLINES OF THE 13762 RAT MAMMARY ADENOCARCINOMA. Anne P. Sherblom, Robert L. Buck and Kermit L. Carraway, Biochem. Dept., Oklahoma State Univ., Stillwater OK 74074.

The MAT-B1 and MAT-C1 sublines of the 13762 rat mammary adenocarcinoma exhibit striking differences in agglutinability, sialic acid content and xenotransplantability. Chemical labeling of cells with (^{3}H)-$NaBH_4$ following periodate oxidation or metabolic labeling with (^{3}H)-glucosamine reveals a sialoglycoprotein (ASGP-1) with apparent MW >300 K by SDS gel electrophoresis as the major labeled component for both sublines. ASGP-1 from each subline is readily purified from a crude plasma membrane preparation of ascites cells by centrifugation in a CsCl gradient containing 4 M guanidine•HCl (GuHCl). ASGP-1 from the MAT-C1 subline, which is transplantable into mice, appears slightly larger and more heterogeneous than the MAT-B1 glycoprotein both by sedimentation velocity in 4 M GuHCl and by gel filtration in SDS. MAT-C1 ASGP-1 also appears to have larger oligosaccharide components, as indicated by chromatography of products from alkaline borohydride elimination. Isopycnic centrifugation in CsCl reveals a higher density for the MAT-C1 glycoprotein compared with MAT-B1 ASGP-1 both in the absence and presence of GuHCl. The amino acid compositions for the ASGP-1 molecules from the two cell types are quite similar, but they differ substantially in carbohydrate compositions with MAT-C1 having much larger amounts of galactose, glucosamine, fucose and sialic acid. Differences in composition and physical properties of these glycoproteins may correlate with dramatic differences in cell surface properties which are important to tumor survival.

536 OCCURRENCE OF PEANUT LECTIN RECEPTOR (BLOOD GROUP T-ANTIGEN) IN ASCITES BUT NOT SOLID AND CULTURED FORMS OF THE 13762 RAT MAMMARY ADENOCARCINOMA. Susan P. Howard, Tim P. Trenbeath, John W. Huggins and Kermit L. Carraway, Biochem. Dept., Oklahoma State Univ., Stillwater, OK 74074

The Thomsen-Friedrich antigen (blood group T-antigen), a precursor of M and N blood group substances, is found associated with malignant, but not benign, human mammary lesions. It can be detected by its cross-reactivity with peanut lectin (PNA). We have investigated the presence of this antigen in various forms of the metastatic 13762 rat mammary adenocarcinoma by its interaction with ^{125}I-PNA after electrophoresis of the cells or tissues on polyacrylamide gels in SDS. Five different 13762 ascites sublines have PNA receptor associated almost exclusively with a major sialoglycoprotein (ASGP-1) of apparently high molecular weight (>300 K). The solid tumor from which the ascites forms were derived and a variant maintained in cell culture (MR) do not have the PNA receptor in significant amounts. A solid tumor obtained by transplanting MR into rats does not have the PNA receptor, but the ascites form obtained by transplanting MR does have the receptor, again associated with the ASGP-1. In solid tumors derived by transplantation from the various ascites forms, the PNA receptor was greatly reduced or absent. Our results suggest that the expression of PNA receptor is related to the environment in which the tumor is grown. We suggest that the sialoglycoprotein bearing PNA receptor contributes to the survival of the tumor in an ascites form and may be important to the ability of the tumor to metastasize.

537 REDISTRIBUTION AND SHEDDING OF CONCANAVALIN A AND PEANUT LECTIN RECEPTOR OF ASCITES SUBLINES OF THE 13762 RAT MAMMARY ADENOCARCINOMA. John W. Huggins, Tim P. Trenbeath and Kermit L. Carraway, Biochem. Dept., Oklahoma State Univ., Stillwater, OK 74074.

Because the shedding of cell surface components has been implicated in the escape of mammary tumors from immune surveillance, we have investigated cell surface receptor redistribution and shedding in two ascites sublines of the metastatic 13762 rat mammary adenocarcinoma. The sublines differ substantially in cell surface labeling profiles, lectin agglutinability, morphology and xenotransplantability. MAT-C1 has a highly irregular surface with branched microvilli extending from the cell body. It is transplantable into mice. MAT-B1 has a more normal surface architecture and is not xenotransplantable. Concanavalin A receptors on MAT-B1 are mobile and form caps in the presence of Con A. Colchicine treatment in the presence of Con A induces prolific shedding of Con A receptors. The MAT-C1 Con A receptors appear immobile, even in the presence of cytoskeletal perturbants such as colchicine, cytochalasin B and dibucaine. However, hypotonic swelling of MAT-C1 cells followed by Con A treatment will cause reorganization and shedding of the branched microvilli without apparent mobility of the receptors on the microvilli. The results suggest a stabilization of cell surface organization of the microvilli. Treatment of either MAT-B1 or MAT-C1 cells with peanut lectin (PNA), which reacts almost exclusively with the major sialoglycoprotein (ASGP-1) of these cell surfaces, causes copious shedding of the PNA receptors on membrane fragments, including microvilli. These results indicate that specific cell surface interactions are critical to shedding processes, which may contribute to the survival of metastatic tumors.

538 CHARACTERIZATION OF IMMUNE COMPLEXES SHED FROM MOUSE ASCITES TUMOR CELLS, Richard L. Rader[1], Harry G. Rittenhouse[2], Matthew D. Lynn[2], Diane Ar[2] and Michael M. Moon[1],

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Rittenhouse et al. (Biochemistry 17:829-837, 1978) have demonstrated that Ehrlich ascites tumor cells, obtained from the peritoneal cavity of Swiss-Webster mice will spontaneously release cell surface components upon incubation in isotonic buffer for 1h at 4°. A portion of the shed material occurs as high molecular weight (MW) aggregates (cell coat particle) which contain host-derived tumor-associated immunoglobulin G (TAIg) and a complement component (C3). The release of such immune complexes has been implicated in the ability of tumor cells to escape host immune mechanisms. In order to distinguish tumor-derived glycoproteins from host components (e.g.,TIAg or complement), cells were metabolically labeled in vivo by injecting ^{14}C-glucosamine i.p. into tumor-bearing mice. After 4h the cells were harvested and the cell coat particle was isolated by ultracentrifugation of the shed material.Autoradiographs obtained after SDS-PAGE revealed numerous glycoproteins ranging in MW from approximately 25K-200K. The predominantly labeled glycopolypeptide had an apparent MW of 60K. Specific immunoprecipitation of the shed glycoproteins with rabbit anti-mouse IgG revealed that only one major glycopolypeptide (45K) was tightly bound by TAIg. It is possible that the spontaneous shedding of tumor cell surface glycoproteins may be influenced by the binding of host antibodies directed to the tumor cell surfaces. We are presently studying the kinetics of antibody-mediated release of cell surface glycoproteins from EL4 lymphoma cells maintained in syngeneic C57BL/6 mice. Supported by CA18900 (RLR) and USPHS S-07RR05383 (HGR).

539 REDUCED TUMORIGENICITY OF LYMPHOMA CELLS LACKING A NORMAL DIFFERENTIATION ANTIGEN, J. Buxbaum & R. Basch, Depts. of Medicine and Pathology, New York University Medical Center; Dept. of Medicine, Manhattan V.A. Hospital, New York, New York

Cloned, cultured cells from the Thy-1.2(θ)+ BALB/c lymphoma RL$^{\delta}$1 were immunoselected to obtain Thy-1.2(-) variants in a single step procedure. Analysis of a prototype (-) clone suggested that the defect was due to a point mutation at the structural locus for Thy-1.2 (Somatic Cell Genetics 3:1,1977). The single demonstrable difference between the (-) clone and its Thy-1.2 (+) sibling was associated with a marked reduction in tumorigenicity. 10^6 cells from the (+) clone killed 82% of recipients with a mean death time (MDT) of 46 days. An identical cell dose obtained from the Thy-1.2(-) clones killed 25% of the recipients with a MDT of 62 days. The two clones had similar in vitro generation times and were serologically identical in other surface antigens, including lymphocyte differentiation and MuLV-associated antigens. The reduction in tumorigenicity appeared to be related to an alteration in the host response to the tumor, since (+) and (-) cells produced tumors in irradiated recipients at the same rate. Animals surviving challenge with the (-) cells were specifically protected against re-challenge with the (+) cells (mortality 30% vs 85% in untreated animals). The protection was associated with the appearance of cytotoxic antibody to the inoculated cells. The role of cell-mediated immunity is not yet clear. The experiments suggest that the genetic removal of a normal cell surface antigen results in the exposure of molecules not normally strongly immunogenic in the host. These then become effective tumor specific transplantation antigens, recognizable and attackable by the host immune system. Studies in other systems, including Lewis lung carcinoma, teratocarcinoma and other murine lymphomas suggest that this may be a relatively common event in tumor biology.

540 MODIFICATION OF MALIGNANT CELL SURFACES BY VIBRIO CHOLERAE NEURAMINIDASE AND EMITINE. Angelyn Rios. Department of Developmental and Cell Biology, University of California, Irvine, California 92717.

Modification of malignant cell surfaces has been shown to alter tumor growth in vivo when treated cells are injected into syngeneic tumor bearing animals (M.D. Prager and F.S. Baechtel, METH. CANCER RES. 9: 339-400, 1973). Tumor growth can be strongly inhibited when mice bearing established transplantable syngeneic tumors are challenged with tumor cells which have been treated in vitro with Vibrio cholerae neuraminidase (VCN) plus mitomycin-C (A.M. Rios and R.L. Simmons, J. NATL. CANCER INST. 51 : 637-644 , 1973; A.M. Rios and R.L. Simmons, ANN. N.Y. ACAD. SCI. 276: 45-60, 1976). The present study examines effects of combining VCN with Emetine for in vitro cell treatment. VCN acts on the cell surface to remove terminal sialic acid residues. Emetine is a protein synthesis inhibitor which inhibits regeneration of sialic acid (C.E. Brinckezhoff and M. Lubin, CANCER RES. 38: 3668-3672, 1978). Mean survival time of mice which has received VCN- and Emetine-treated tumor cells was enhanced (76.7±7.1 days) as compared to VCN plus mitomycin-C treated cells (70.5±5.2 days) or animals which had received mitomycin-C treated cells alone (65.1±6.3 days). Tumor growth was also inhibited. The results suggest that combining in vivo VCN cell treatment with Emetine enhances the in vitro immunotherapeutic effect of VCN-treated tumor cells to prolong tumor-bearing animal survival time.

541 CHARACTERIZATION OF CROSS-REACTING ANTIGENS ON THE EPSTEIN-BARR VIRUS ENVELOPE AND PLASMA MEMBRANES OF PRODUCER CELLS, David A. Thorley-Lawson, Sidney Farber Cancer Institute, Harvard Medical School, 44 Binney St., Boston, Ma. 02115

A rabbit antiserum has been prepared against the B95-8 transforming strain of EBV. The antiserum has a high virus neutralizing titer (approx, 1:1000) against both the marmoset B95-8 EBV and the human P3HR-1 EBV. The neutralizing antibodies may be absorbed completely with EBV producer cell lines, but, not with non-producer cell lines or producer cell lines treated with phosphonoacetic acid (PAA) so as to be non-producer. After repeated absorption with PAA treated B95-8 the serum remains reactive with the membranes of producer cell lines as judged by immunofluorescence or the ^{125}I Staphylococcal protein A radioimmunoassay. Thus the neutralizing antigens are expressed on the membranes of producer cell line and may be purified from this source using the serum and ^{125}I Staph A binding as an assay. The ability of the serum to differentiate producer and non-producer cells by means of cell surface determinants has been exploited to achieve a separation of these two populations from the same culture. Immunoprecipitation by the protein A technique shows that the serum recognizes two polypeptides from producer cells of approximate molecular weights 150,000 and 75,000.

542 BIOCHEMICAL STUDY OF THE MOLONEY LEUKEMIA VIRUS-DETERMINED CELL SURFACE ANTIGEN (MCSA) IN RELATION TO IMMUNORESISTANCE, F. A. Troy and E. M. Fenyö, Univ. of Calif. Sch. of Medicine, Davis, CA 95616 and Dept. of Tumor Biology, Karolinska Inst., Stockholm, Sweden

An experimental procedure involving SDS-PAGE of Triton X-100 solubilized cell surface components has recently been developed which can detect a large number of surface antigens as separate molecular entities (Troy, F.A. et al: PNAS 74, 5270 (1977). The capacity to recover individual proteins from the gels and test them in specific cytotoxic assays has permitted the assignment of specific antigenic determinants to distinct mol. wt. species. Application of this technique to Moloney Leukemia Virus (MLV) induced mouse lymphoma cells of strain A mice (YAC) which express on their membranes an MLV-directed cell surface antigen (MCSA) in addition to the type C viral proteins has shown that MCSA is distinct in size and antigenic determinants from the known ML virion proteins or the histocompatibility antigen, H-2^a.

The surface expression, mol. wt. distribution and quantitation of MCSA in an immunoresistant subline of YAC, YAC-IR, has been examined and found to be distinctly different from the parental line. In this relatively antigen deficient IR line, MCSA is associated with a different mol. wt. species. Since MCSA is the antigen recognized by the syngeneic host, these results suggest that the altered surface expression of MCSA on YAC-IR may relate to the inability of this line to elicit an immune rejection reaction. Whether the surface alteration of MCSA represents a general mechanism of escape from immune surveillance and how commonly IR sublines alter their CSA when faced with becoming IR again is presently being studied by examining the surface expression of MCSA on independently selected IR lines.

543 SURFACE ANTIGENS OF KNRK CELLS INFECTED WITH HL23V VIRUS DETECTED BY ANTIBODIES IN NORMAL HUMAN SERA. J.A. Hope, E. Cardoso, The Human Tumour Immunology Group, University College Hospital Medical School, University Street, London WC1E 6JJ.

We are investigating the binding of human sera previously shown to be capable of precipitating disrupted HL23V virus, to the surfaces of KNRK cells infected with this virus. Using a sensitive solid phase radioimmune assay we have detected antibody binding to the cell surface. This binding activity can be blocked by bovine sera (foetal calf, calf, agammaglobulinemic calf, and adult), and sera from other species. We will present results on the nature of the blocking antigen(s) and their relationship with viral antigens presented on the cell surface.

544 BIOCHEMICAL AND IMMUNOCHEMICAL CHARACTERISTICS OF TUMOR SPECIFIC ANTIGENS ON CHEMICALLY INDUCED RAT TUMORS, R. W. Baldwin, J. G. Bowen, M. J. Embleton and M. R. Price, Cancer Research Campaign Laboratories, The University, Nottingham, U.K.

Sarcomas induced by 3-methylcholanthrene (MCA) and hepatomas induced by 4-dimethylaminoazobenzene in the rat express individually distinct tumor-specific antigens which are associated with integral plasma membrane components. Conventional biochemical separative procedures, lectin affinity and specific immunoadsorption techniques have indicated that the antigenic determinants are expressed upon papain released glycopeptides (molecular weight 55,000) although activity may be solubilized with other agents such as 3M KCl. In the hepatoma studies it was further established that the tumor-specific antigens are related to normal alloantigens since they could be isolated by binding to alloantibody-containing immunoadsorbents. But they are probably not modified products of the major histocompatibility complex (RT-1) since hepatoma D23-specific antigen did not bind to immunoadsorbents prepared from antisera to allogeneic erythrocytes and lacks association with rat β_2-microglobulin. These findings have been interpreted to suggest that the tumor-specific antigens arise following carcinogen induced modification of the genes coding for normal cell antigens or that there is a derepression of genetic information which is not normally expressed in the adult host. This latter point is emphasized by related investigations showing that neoantigens with the same specificity as the tumor-specific antigens on MCA-induced sarcomas can be induced on rat embryo cells following a short exposure to 3-methylcholanthrene.

545 ANTIGENIC ANALYSIS OF A MURINE LYMPHOMA L-5178Y, Lionel A. Manson and Neomi Moav, The Wistar Institute, Philadelphia, PA 19104

The L-5178Y cell line is a methylcholanthrene-induced transplantable thymoma of H-2^d origin. The L-5178Y is highly compatible with its host of origin, DBA/2; 10 cells inoculated i.p. will kill more than 90% of the inoculated mice. Yet the tumor line has a TSTA(s), in that mitomycin C-treated cells or cell-free membranes (MLP) will sensitize DBA/2 mice to reject a challenge of 100 live cells. Once immunized, immune DBA/2 mice will reject 10^6 live cell challenges. The MLP is also a complete H-2 immunogen, in that it will sensitize allogeneic mice to reject skin grafts at an accelerated rate as well as induce anti-H-2 cytolytic antibodies. The cell line is known to be Ia negative. We have been studying the antigen composition of L-5178Y in order to identify the TSTA(s). The initial experiments were carried out with NP40 lysates of ^{3}H-glucosamine-raised cells. Using a C57BL/6 anti-L-5178Y serum (cytolytic titer with DBA/2 spleen cells is 1:10,000), immunoprecipitates showed in SDS-PAGE peaks that had apparent molecular weights of 7,000, 27,000, 47,000, 52,000 and 70,000 daltons. Using an NP40 extract of MLP made from labeled cells, similar peaks were observed. This serum brings down only one peak from ^{3}H-fucose-labeled DBA/2 spleen cells, with a molecular weight of 45,000 daltons. Standard NIH anti-H-2^d sera have been used, D-4, D-8, D-13, D-28, and D-31, and all show the same 5 peaks with the L-5178Y extracts. Further studies are being carried out to determine what are the relationships, if any, between the TSTA(s), the additional peaks seen in SDS-PAGE, and the H-2 gene products. (Supported by NCI Grants CA 10815 and CA 07973).

546 Immune Reactants Associated With Human Solid Tumors, J.M. MacSween, Dept. of Medicine, Dalhousie University and Camp Hill Hospital, Halifax, Nova Scotia, Canada

Immunoglobulins were eluted with acidic buffers from washed cell suspensions obtained from freshly resected solid tumors and adjacent normal tissue. Elution of antibody to the tumor was suggested in some cases by increased concentration of IgG in acidic eluates compared to preceding eluates obtained at pH 7.4. Rebinding of immunoglobulins in the acidic eluates to the tumor cells as an indicator of antibody activity was studied with the use of ^{125}I staphylococcal protein A. This was compared to the binding of eluates from adjacent normal tissue which had been equalized for protein content. In seven of fifteen tumors, there was clearly greater binding of ^{125}I protein A to cells exposed to tumor eluates than to eluates obtained from corresponding normal tissue. Two of thirteen acidic eluates from other lung tumors showed increased binding to cells obtained from a lung carcinoma. Recovery of putative tumor antigen in 3M KCl extracts from the tumor cells was investigated by determining their inhibition of binding of immunoglobulins in the acidic eluates. Three of four KCl eluates from different lung tumors showed greater than 40% inhibition of binding of an acidic eluate to autologous lung tumor cells. KCl eluates from four colonic tumors failed to inhibit in this system whereas two of five KCl eluates from breast tumors were inhibitory. Antigenic material in a KCl eluate from a gastric carcinoma was purified by immune adsorption with insolubilized acidic eluate from the same tumor. After labelling with ^{125}I, there was evidence of autoantigenic activity on the basis of primary binding by the acidic eluate. The major molecular species in this case was of less than 13,700 daltons molecular weight.

547 CYTOTOXIC T CELLS AGAINST A METASTASIZING MURINE TUMOR VARIANT: SPECIFICITY AND PROTECTIVE CAPACITY IN VIVO. Volker Schirrmacher and Klaus Bosslet. Institute of Immunology and Genetics, Deutsches Krebsforschungszentrum, Heidelberg, FRG.

A model system is described that allows the study of interactions between cells of the immune system and tumor metastases. The model consists of a non-metastasizing chemically induced lymphoma (Eb) of DBA/2 mice and a spontaneous variant thereof (ESb) with pronounced metastasizing capacity. Syngeneic cytotoxic T lymphocytes (CTL's), induced in vivo by immunization with Eb or ESb and restimulated in vitro for 5 days with mitomycin C-treated autologous tumor cells, show high specific anti-tumor cytotoxic activity in a 4 h ^{51}Cr release assay.

Anti-tumor CTL's were highly specific: (i) Anti ESb CTL's lyzed ESb but did not lyze anyone of fourteen syngeneic or allogeneic murine tumors. (ii) Anti Eb CTL's lyzed Eb and cross-reacted with one of the fourteen tumors above (RL$^{\delta}$1). (iii) Surprisingly, no cross-reactivity was observed on the level of specific CTL's between Eb and ESb.

Thus, CTL's were able to recognize unequivocally deviations between the parental tumor and its metastasizing variant. Similar antigenic differences could be demonstrated in vivo in protection experiments.

These results suggest that in our system CTL's recognize the individually specific tumor-associated transplantation antigens (TATA), which have hitherto been only demonstrable in vivo.

548 ASSOCIATION OF MELANOMA TUMOR ANTIGEN ACTIVITY WITH B_2-MICROGLOBULIN, Denis R. Burger, R. Mark Vetto, Arthur Vandenbark, Arthur Malley, Veterans Administration Medical Center, Portland, OR 97201 and Oregon Regional Primate Research Center, Beaverton, OR

This report describes experiments evaluating the relationship between the B_2-macroglobulin (B_2-μ) and melanoma tumor antigen (MTA) present in the KCl extracts of freshly excised tumor tissue. The MTA activity was measured in vivo by dermal testing and in vitro by the leukocyte adherence inhibition (LAI) test. Analysis of our experimental data suggesting that MTA activity present in the KCl extracts might be associated with B_2-μ is summarized below. The majority of melanoma tumor antigen activity present in melanoma extracts derived from fresh tumor tissue binds to a Sepharose-anti B_2-microglobulin adsorbent. Removal of HLA antigens from the extracts of melanoma tissue using a KBr flotation technique did not reduce either the tumor antigen activity of the extracts or the binding of melanoma tumor antigen (MTA) activity to the Sepharose-anti B_2microglobulin adsorbent. The complete blocking of MTA activity by pretreating the anti B_2-microglobulin adsorbent with B_2-microglobulin and the lack of detectable MTA binding to a Sepharose anti-normal human serum adsorbent demonstrated the specificity of the binding of MTA to the anti B_2-microglobulin adsorbent.

549 CYTOFLUOROMETRIC ENUMERATION OF TUMOR-SPECIFIC ROSETTE-FORMING CELLS IN CANCER PATIENTS, R. Mark Vetto, William Kraybill, Alex Tong, Arthur Vandenbark, Denis R. Burger, Veterans Administration Medical Center, Portland, OR 97201.

This report describes the use of a cytofluorometric technique (utilizing a Bio/Physics Cytofluorograf) to enumerate the rosette-forming cells in human blood. Cells forming rosettes with antigen-conjugated erythrocytes (Ag-RBCs) are differentiated electronically from unrosetted mononuclear cells by their increased integral size. Using automated counting of rosetted populations, one can accurately determine the percent rosettes (ABCs) in a population of 100,000 cells in a short period of time (10 min/determination) avoiding the subjectivity of visual determinations. We have used this rosette counting technique to quantitate tumor antigen binding cells in patients with newly diagnosed squamous cell cancer of the head and neck. Patients without evidence of malignant disease did not exhibit tumor specific rosettes.when background rosettes to HSA or KLH were compared to squamous antigen rosettes. In contrast, nine of seventeen patients with untreated, squamous cell carcinoma of the head and neck had squamous antigen binding mononuclear cells. It was noted that with increasing tumor burden in these patients (TNM tumor stage classification) the percentage of patients with squamous antigen binding cells increased ($p<0.05$, $R_3 = 0.94$)

550 EXPRESSION OF HUMAN PLACENTAL CELL SURFACE ANTIGENS ON NORMAL AND NEOPLASTIC CELLS IN CULTURE, Thomas A. Hamilton, H. Garrett Wada, and Howard H. Sussman, Stanford University School of Medicine, Stanford, CA 94305

Trophoblastic and neoplastic cells share some significant behavioral properties including invasiveness, escape from immunosurveillance, and cell surface charge properties. In order to examine this relationship on a molecular level, the expression of a defined set of human placental brush border glycoprotein antigens was studied in a series of cultured human cell lines. These lines included two independently derived gestational choriocarcinomas (BeWo and JEG-3), a transformed non-trophoblastic cell (Chang liver cells), and two "normal" fibroblastic cell lines (WI-38, GM-38). Fourteen components were resolved by immunoprecipitation with anti-placental brush border antiserum and two-dimensional electrophoresis. Ten of these were expressed in all cells examined. The expression of the other four antigens (including placental alkaline phosphatase) was variable. Two of the 10 commonly expressed glycoproteins were not seen in adult human kidney or liver tissue. Their appearance in both normal and neoplastic cultured cells suggests that these antigens may represent functions lost during tissue differentiation which are re-expressed as a result of adaptation to autonomous growth in vitro.

551 INFLUENCE OF AN INCREASED NA SUPPLY ON THE ACTIVITY OF THE NA-K PUMP IN CULTURED ANIMAL CELLS, Jeffrey B. Smith and Enrique Rozengurt, Imperial Cancer Research Fund, London, WC2A, England, and Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14853

The Na-K pump in quiescent fibroblasts derived from mice is not fully active due to the limited supply of internal Na. Hence certain ionophores, (e.g. gramicidin and monensin) that increase the supply of cell Na markedly enhance the activity of the Na-K pump in the intact fibroblasts (Smith, J.B., and Rozengurt, E. (1978) PNAS, in press). Moreover, the stimulation of the pump in quiescent 3T3 cells by serum and growth factors appears to be mediated by an increase in the Na permeability of the plasma membrane. The activity of the Na-K pump in a variety of cultured animal cells has been found to be stimulated to varying degrees by monensin. In 3T3 cells transformed by SV40, the Na-K pump is stimulated less by monensin than the pump in 3T3 cells implying that the supply of Na to the pump in the transformed cells is nearly sufficient for maximal pump activity.

552 ROLE OF INTRACELLULAR Na^+ AND K^+ IN THE EXPRESSION OF THE TRANSFORMED PHENOTYPE Robert F. Garry and Marilynn R. F. Waite, The University of Texas at Austin, Austin, Texas 78712.

Chick cells transformed with the Bryan or Schmidt-Ruppin strains of Rous sarcoma virus have intracellular concentrations of Na^+ and K^+ which differ slightly from those found in normal cells. The alterations require a functional sarc gene product and the concentrations differ depending on the strain of the transforming virus. Changes in the intracellular monovalent cation concentrations similar to those caused by each virus can be induced by appropriate manipulations of the NaCl concentration of the medium. The cells rapidly come to resemble the RSV-transformed culture with similar intracellular concentrations of monovalent cations. The treated cells also exhibit loss of contact inhibition and grow to higher saturation densities; they have reduced levels of LETS and of succinic dehydrogenase; they produce excess amounts of lactate and pyruvate; and they transport increased amounts of hexose. They do not, however, grow in soft agar or in low serum medium. These results indicate that there might be only two functions for the sarc protein, one of which changes the anchorage and serum requirements for growth, and one of which changes the intracellular concentration of monovalent cations. The other phenotypic changes characteristic of transformed cells appear to result from the cation changes.

553 ENDOGENOUS cAMP DOES NOT MODULATE TRANSPORT OF NUTRIENTS IN CHINESE HAMSTER OVARY CELLS, R. M. Wohlhueter and P. G. W. Plagemann, University of Minnesota, Minneapolis, MN 55455

Decreased uptake of nucleosides, hexoses and nucleic acid bases has been reported in some cell lines when intracellular cAMP has been elevated, or was presumed to have been elevated, by treatment with dibutyryl-cAMP, phosphodiesterase inhibitors or ß-adrenergic hormones. The decreased uptake has been implicated in cAMP-mediated regulation of cell proliferation. Uptake of these nutrients into cells involves, minimally, two processes operating in tandem - transport across the membrane and intracellular phosphorylation. We have examined the question of whether the first of these processes - transport - is influenced by cellular cAMP levels. Initially, we screened several cell lines, grown both in suspension and monolayer, for their cAMP response to prostaglandin E_1, papaverine and isoproterenol, and selected prostaglandin E_1 - treatment of Chinese hamster ovary cells as a system giving the largest and most consistent elevation of cAMP. Then we employed rapid kinetic techniques to measure transport of adenosine, adenine and 3-O-methylglucose in cells incapable of metabolizing the transport substrate (for example, in cells selected for deficiency in adenosine kinase and blocked in adenosine deaminase by deoxycoformycin). In no case did a substantial change in nutrient transport rate accompany the elevation - generally 5- to 50-fold - in cellular cAMP concentration.

554 6-DEOXY-D-GLUCOSE AND D-XYLOSE: ANALOGS FOR THE STUDY OF HEXOSE TRANSPORT IN 3T3 AND SV40 TRANSFORMED 3T3 CELLS. Nancy D. Connell and Antonio H. Romano, University of Connecticut, Storrs, Connecticut 06268.

Studies on glucose transport in animal cells using 2-deoxy-D-glucose (2-DOG) are ambiguous, because 2-DOG is phosphorylated by intracellular kinases. 3-O-methyl-D-glucose (3-OMG) is not phosphorylated, but has other limitations: it shows significant passive diffusion into cells, and less specificity than 2-DOG for the glucose transport system.

We have investigated the use of 6-deoxy-D-glucose and D-xylose as alternative non-phosphorylatable analogs for transport by 3T3 cells and their SV40 transformant (SV101), and found that they show advantages over 3-OMG. As shown in the table, 6-DOG in particular is a more efficient inhibitor of 2-DOG uptake, and 6-DOG uptake is more effectively inhibited by D-glucose than is 3-OMG. Thus, a greater specificity is indicated.

We have measured the transport of ^{3}H-6-DOG and ^{14}C-xylose by 3T3 and SV101 cells grown to confluency (48 hr), and by 48 hr 3T3 cells that received fresh medium with 20% serum 10 hr before transport measurement. SV101 and serum-stimulated 3T3 cells transported 6-DOG at similar rates, significantly greater than 48 hr 3T3 cells. Thus, the enhanced rate of glucose transport attributed to transformed cells appears not to be a specific consequence of transformation, but a reflection of the growth rate and physiological state of the cell.

Substrate	Inhibitor	K_m(mM)	K_i(mM)
2-DOG	Glucose	1.7	1.8
2-DOG	3-O-MG	1.7	15.0
2-DOG	6-DOG	1.7	5.0
2-DOG	Xylose	1.7	10.0
3-O-MG	Glucose	5.6	12.0
6-DOG	Glucose	5.0	2.0
Xylose	Glucose	6.6	2.0

555 ACTIVE CARRIER-MEDIATED TRANSPORT OF MELPHALAN BY TWO SEPARATE AMINO ACID TRANSPORT SYSTEMS IN LPC-1 PLASMACYTOMA CELLS IN VITRO, Gerald J. Goldenberg, Hing-Yat P. Lam, Asher Begleiter, University of Manitoba and the Manitoba Institute of Cell Biology, Winnipeg, Manitoba, Canada. Previous studies have shown that uptake of several alkylating agents occurs by independent transport mechanisms. Evidence suggesting that melphalan uptake is by an active process is that uptake of free intact melphalan proceeds uphill against a concentration gradient of approximately 11-fold, is temperature and sodium dependent and is inhibited by several metabolic antagonists. Other findings supporting the concept that melphalan uptake is carrier-mediated is that uptake followed biphasic Michaelis-Menten kinetics, is chemically specific and is mediated by at least 2 separate amino acid transport systems. Melphalan uptake was strongly inhibited by β-2-aminobicyclo[2,2,1] heptane-2-carboxylic acid a specific inhibitor of the L amino acid transport system but not by 2 aminoisobutyric acid or 2-(methylamino) isobutyric acid specific inhibitors of the A amino acid system. Under conditions in which systems L and A were saturated by their respective inhibitors drug uptake was inhibited by alanine, serine, cysteine and other amino acids known to be transported by the ASC transport system. In reciprocal experiments with leucine as substrate melphalan inhibited uptake of the amino acid by both system L and a second system resembling ASC. Carrier-mediated uptake of melphalan could be accounted for entirely by transport on these two amino acid transport systems.

556 MEMBRANE POTENTIAL-INDUCED CHANGES IN KINETIC PARAMETERS OF AMINO ACID TRANSPORT IN EHRLICH CELLS, Rose M. Johnstone and Philip C. Laris, McGill University, Montreal, Quebec, Canada, H3G 1Y6, University of California, Santa Barbara, Calif. 93106. Cellular amino acid pools can have multiple effects on influx of amino acids. In addition to the phenomenon of exchange diffusion and trans inhibition, cellular pools of amino acids may increase influx indirectly by hyperpolarization of the cell membrane. Efflux of cellular amino acids by the Na^+-dependent route causes hyperpolarization of the cell. The increased membrane potential, in turn, enhances influx of labelled amino acids from the medium. Phenomenologically, the latter event appears similar to exchange diffusion, but is in fact quite different since there is no obligatory stoichiometric relationship between the flows in potential-induced transport changes in contrast with true exchange diffusion. This observation may account for the demonstration in the published reports that under some conditions "exchange diffusion" is Na^+ dependent. Kinetic analyses of the effects of Na^+ per se and changes in membrane potentials per se on amino acid transport have been executed. The results showed that with $\Delta\tilde{\mu}_{Na} = 0$, Na^+ primarily affects the K_m for the amino acid, a decreased K_m being observed with increased Na^+. In contrast, an increase in membrane potential (inside more negative) mainly increased J_{max}. At high cellular levels of amino acid, exodus concomitant with hyperpolarization may mask a trans inhibition of influx of amino acids by the cellular amino acid pool.

557 PURIFICATION OF A PLASMA MEMBRANE GLYCOPROTEIN APPARENTLY UNIQUE TO COLCHICINE-RESISTANT PERMEABILITY MUTANTS OF CHINESE HAMSTER OVARY CELLS, John R. Riordan† and Victor Ling*, Research Institute, Hospital for Sick Children and Dept. of Clin. Biochem., University of Toronto; *Dept. of Medical Biophysics and Ontario Cancer Institute, Toronto.

Isolated plasma membrane vesicles from colchicine resistant mutants of Chinese hamster ovary cells contain a glycoprotein with a molecular weight of about 170,000 (P-glycoprotein) not present in vesicles from drug sensitive wild type or revertant cells (Riordan and Ling, J. Cell Biol. 79, 225a, 1978). Since we have been unable to find any other differences in either the lipid or polypeptide compositions of vesicles from resistant and sensitive cells, it is suspected that the P-glycoprotein may be responsible for the restricted permeability to drugs. Rigorous testing of this postulate will require purified glycoprotein.

Initial attempts to dissociate the molecule from the membrane provided evidence that it was an integral membrane protein. The following steps which were quantitated using ^{125}I labelled membranes resulted in successful purification. (1) 35 mM lithium diiodosalycilate dissociated 45% of the total membrane protein; (2) 0.5% N-dodecylsarcosinate dissolved 90-95% of the remaining protein; (3) the P-glycoprotein and some other minor glycoproteins which together accounted for about 30% of the total membrane protein were separated from other detergent soluble proteins using a Ricinus communis I-agarose column; (4) the minor glycoproteins were separated from the P-glycoprotein using a Lens culinaris-agarose or other lectin affinity columns; (5) N-dodecylsarcosinate was effectively removed from the purified product by dialysis. (Supported by the MRC of Canada)

558 ALTERATION OF CELL BEHAVIOUR BY THE RECONSTITUTION OF THE BACTERIAL DICARBOXYLATE TRANSPORT SYSTEM INTO EUKARYOTIC CELLS. Vincent Duronio, Karen Tippett, Debra Sorbara and Theodore Lo, Department of Biochemistry, University of Western Ontario, London, Canada N6A 5C1

At least two membrane transport proteins (SBP 1 and SBP 2) are involved in the translocation of dicarboxylic acids across the cytoplasmic membrane of E. coli. We have been able to isolate biologically active SBP 1 and SBP 2 through the use of aspartate-coupled Sepharose 4B. Using ^{3}H-Triton X-100 to solubilize the transport components, it is found that less than 0.00008% (v/v) of Triton X-100 is associated with 1 µg of SBP 1 or SBP 2. Rat myoblasts or mouse L-cells by themselves cannot transport succinate. The addition of either one of the two transport components does not stimulate succinate transport; however, the addition of both SBP 1 and SBP 2 confers to the cells the ability to take up succinate. This newly acquired transport system has an apparent Km of 20 µM for succinate, and is responsible for the uptake of succinate, fumarate and malate --- similar to the kinetic properties of the bacterial transport system. The above findings suggest that SBP 1 and SBP 2 are indeed inserted through the membrane matrix. The "reconsituted" cells are peculiar in that they are altered in their cell morphology, they have lost their ability to anchor to the substratum, and their membrane surface components are changed. Furthermore, the myoblasts are now unable to carry out myogenic differentiation.

559 PYRIMIDINE BIOSYNTHESIS IN NORMAL AND TRANSFORMED CELLS, Mayo Uziel and J. K. Selkirk, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830

We have developed procedures for sensitive measurement of excreted pyrimidine nucleoside specific radioactivities from cells in culture. The changes in the observed values reflect dilution of the added label through de novo biosynthesis of nonradioactive pyrimidine nucleosides or by shifting and equilibration of other nucleotide pools into the free uridine pool. It is thus possible to monitor uridine biosynthesis occurring in intact cells without destroying or disrupting the cell population. On comparing a series of normal and transformed lines, we have observed several patterns of change in specific activity. The normal hamster embryo fibroblast, an epithelial cell line derived from rat liver (IAR20), and a transformed epithelial cell line (IAR28) capable of inducing cancer cease making Urd by de novo synthesis, although each turnoff occurs at different stages of growth. However, the hamster V79 cells and malignant IAR19 cell lines do not stop diluting the Urd at any time, but the relative rate of dilution is 13- to 40-fold less compared to the initial rate. This method is one additional aid in recognizing and differentiating transformed cells in culture that do not exhibit the transformed fibroblast phenotype. (Research sponsored by the Division of Biomedical and Environmental Research, U. S. Department of Energy, under contract W-7405-eng-26 with the Union Carbide Corporation.)

560 ALTERATION OF TUMOR CELL MEMBRANE FUNCTIONS BY CHROMOSOME MEDIATED GENE TRANSFER, Peter N. Gray and Razia S. Muneer, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190

BOT-2 cells have an impaired ability to utilize exogenous thymidine (Tdr). Previous studies revealed that the cells are deficient in transport of pyrimidine nucleosides. In cell free extracts, thymidine kinase (TK), is present and active. Chromosome mediated gene transfer (CMGT) is a valuable system for transferring genetic information between eucaryotic cells in culture. BOT-2 recipient cells were incubated with isolated sized HeLa-65 metaphase donor chromosomes as lipochromes or with poly-L-ornithine. Transferent clones were isolated in HAT selective media at a frequency of 10^{-5} and 4×10^{-5}, respectively. Clones MGP-1, MGL-1 and MGX-100 were characterized on the bases of purine and pyrimidine nucleoside uptake, methotrexate resistance and relative activities of TK, alkaline phosphatase (AP) and dihydrofolate reductase (DHFR). MGP-1 and MGL-1 are stable transferrents. MGX-100 is unstable and loses its methotrexate resistance when grown in non-selective medium for several generations. The DHFR in the stable clones is 2-4 times more active than BOT-2, and MGX-100. All transferents contained adequate TK activity. However, alkaline phosphatase activity of MGP-1 is 6 times that of BOT-2 and MGX-100. Donor HeLa cells and MGL-1 have extremely low AP activity. The nucleoside uptake experiments demonstrate that MGX-100 and MGL-1 have limited Tdr uptake similar to BOT-2 whereas, MGP-1 takes up 3 times more Tdr and incorporates it into DNA. We have separated a thymidine permease defect from TK and can correct it via CMGT. In addition, we have altered methotrexate uptake and regulation of alkaline phosphatase.

Hormones, Growth Factors and Receptors

561 DIRECT IDENTIFICATION OF EGF RECEPTORS ON MOUSE AND HUMAN CELLS LABELED BY SURFACE SPECIFIC IODINATION, Michael Wrann & C. Fred Fox, Molecular Biology Institute and Department of Microbiology, University of California, Los Angeles, CA 90024

The human epidermoid carcinoma cell line A431 has an unusually high number of specific saturable receptor sites for the polypeptide hormone, epidermal growth factor (EGF). When exposed to unlabeled EGF in the culture medium, these cells lose approximately 50% of their EGF specific binding activity within a few hours (receptor down regulation). By comparing the surface proteins of untreated and down regulated cells by surface iodination and gel electrophoresis, we have identified the EGF receptor on this cell line as a protein of apparent M_r = 175,000. In contrast to normal mouse and human fibroblasts, the EGF receptor on A431 cells is one of the most heavily labeled proteins following surface specific labeling by the chloroglycoluril procedure (Biochemistry, 1978, 17:4807-4817). By the same procedure, the EGF receptor of human and mouse fibroblasts could not be visualized. This was accomplished when cells were surface labeled under rigorously defined conditions of trypsin treatment. An apparent M_r = 170,000 was obtained for the receptor on mouse fibroblasts using gradient gels. The molecular identity of both human and mouse EGF receptor was confirmed with direct labeling studies. We have previously reported that a small portion of cell bound labeled EGF becomes attached to its receptor. Gel electrophoresis of solubilized human and mouse cells that had been incubated with ^{125}I-EGF revealed radioactive bands which migrated slightly slower than the EGF receptor. This difference can be accounted for by the increased M_r of EGF receptor after attachment of ^{125}I-EGF. Supported by grants from USPHS and the Muscular Dystrophy Association of America. M.W. is a Max Kade Foundation fellow.

562 DOWN REGULATION OF THE MITOGENIC RESPONSE TO EPIDERMAL GROWTH FACTOR, B. Westermark, A. Wasteson and C. Heldin, Department of Molecular, Cellular & Developmental Biology, University of Colorado, Boulder, CO 80309 and University of Uppsala, Uppsala, Sweden.

Edidermal growth factor (EGF) binds with high affinity and saturation kinetics to a cell surface receptor and is subsequently internalized and degraded. The binding reaction leads to a major loss of EGF receptors (down regulation). The objective of the present investigation was to see if the down regulation is operational in the modulation of the cellular response to the factor. We took advantage of the fact that human glia-like cells require serum albumin to be responsive to the mitogenic action of EGF, whereas the down regulation of the receptors is albumin independent. We therefore could denude the cells of receptors almost completely without inducing cell multiplication by pretreating the cells with 1 µg of EGF per ml for 24 h in albumin-free medium. Pretreated cells and controls were then exposed to various concentrations of EGF in permissive medium, i.e., containing 0.1% albumin. Induction of DNA synthesis was measured as % labelled cells in autoradiograms after a 48 h exposure to ^{3}H-thymidine. The dose-response curves (EGF conc. vs. % labelled cells) clearly showed that the loss of receptors induced by pretreatment with EGF was accompanied by a reduction in the fraction of labelled cells. We therefore conclude that the down regulation mechanism can modify the response to a mitogenic factor and may constitute an important mechanism in the regulation of the hormonal response, at the target cell level.

563 DIRECT LINKAGE OF EPIDERMAL GROWTH FACTOR TO ITS RECEPTOR, Peter S. Linsley, Cindy Blifeld & C. Fred Fox. Mol. Biol. Inst. & Dept. Microbiol., UCLA, Los Angeles 90024
Epidermal growth factor (EGF) is a polypeptide hormone that initiates a potent mitogenic response upon binding to its specific receptor on the surface of target cells. A small fraction of the ^{125}I-EGF specifically bound to cultured murine 3T3 cells or human A431 cells (an epidermoid carcinoma) becomes directly complexed to a 185,000 dalton protein. This complex is resistant to boiling in solutions containing 3% sodium dodecyl sulfate and 2-mercaptoethanol and contains a single immunoreactive EGF molecule which permits its precipitation with antiserum specific to EGF. The complex has the biological and biochemical properties expected of the EGF-receptor complex: its formation has a saturation profile nearly identical to that for physiological EGF binding. Complex formation is reduced as a result of EGF mediated down regulation of the EGF receptor. When cells containing this complex are incubated at 37°, the complex is degraded to yield three major products similar to those reported for the photoaffinity linked EGF-receptor complex by Das and Fox (1978) Proc. Natl. Acad. Sci. USA 75:2644. The complex arises in a step subsequent to binding, and its rate of formation is more sensitive to temperature than is EGF binding. Preliminary studies with chemically modified EGF species suggest that complex formation may be inhibited with no concommitant alteration of total EGF specific binding. This strategy is being exploited further to explore the possible biological significance of formation of this stable, and possibly covalent, complex. Supported by grants from the Muscular Dystrophy Association and the American Cancer Society. P.S.L. is supported by NIRSA CA 09056.

564 THROMBIN AND EGF BECOME LINKED TO THEIR CELL SURFACE RECEPTORS, J.B. Baker, R.L. Simmer, K.C. Glenn, and D.D. Cunningham, University of California, Irvine, CA 92717
Studies with the mitogens epidermal growth factor (EGF) and thrombin (Th) revealed that a significant fraction of the growth factor molecules that bound to cell surface receptors became linked to their receptors. Six to nine % of the ^{125}I-EGF molecules bound to receptors at 37° became linked in an EGF-receptor complex (EGF-R) of about 180K daltons. EGF-R survived heating in 3% SDS and 1% 2-mercaptoethanol, and treatment with 6M guanidine·HCl and 0.1M 2-mercaptoethanol, suggesting the linkage of EGF to its receptor was covalent. EGF-R was formed within 10 min and was absent when ^{125}I-EGF binding to receptors was competitively inhibited by a vast excess of unlabelled EGF. EGF-R was formed at the cell surface but was internalized within 1 hr, and was degraded to fragments of about 70K, 50K, and 40K daltons. Similarly, incubation of cells with ^{125}I-Th for 9 min at 37° resulted in formation of a Th-receptor complex (Th-R) of about 70K daltons that survived heating in 3% SDS and 1% 2-mercaptoethanol. Th-R accounted for most of the cellular binding of ^{125}I-Th at ^{125}I-Th concentrations below 4 ng/ml. At higher levels of ^{125}I-Th the amount of Th-R remained constant, whereas the amount of bound but unlinked ^{125}I-Th continued to increase and comprised a majority of the total specifically bound ^{125}I-Th at ^{125}I-Th concentrations about 100 ng/ml. Th-R was absent when ^{125}I-Th binding to cell receptors was inhibited by a vast excess of unlabelled Th. Like EGF-R, Th-R was formed at the cell surface and then entered the cells within 90 min. Unlike EGF-R, Th-R was not degraded to well-defined fragments. EGF-R and Th-R were not caused by transfer of just ^{125}I. Chymotryptic digests showed that each contained a significant part of the growth factor molecule. (Supported by NIH grant CA 12306).

565 PHOTOAFFINITY LABELING OF THE THROMBIN RECEPTOR ON MOUSE EMBRYO FIBROBLASTS. D.H. Carney, K.G. Glenn and D.D. Cunningham, Dept. of Medical Microbiology, Univ. of Calif, Irvine 92717, M. Das and C.F. Fox, Molecular Biology Institute, Univ. of Calif., Los Angeles, 90024.
Thrombin initiates division of fibroblast-like cells in serum-free medium. Recent studies have shown that thrombin action at the cell surface is sufficient for initiation (Cell 14:811, 1978), and that initiation appears to require thrombin binding to specific cell surface receptors (Cell, Dec. 1978, in press). Using photoaffinity procedures, we have labeled the thrombin-receptor complex to identify the thrombin receptor. N-(4-azido-nitrophenyl) ethylene diamine (NAPEDE) was linked to ^{125}I-thrombin by carbodiimide condensation. After incubating mouse embryo cells for 2½ hr at 22° in the dark with 50 ng/ml ^{125}I-thrombin-NAPEDE, the NAPEDE crosslinker was activated by exposure to U.V. light. The cells were then rinsed, dissolved in SDS, and samples were analyzed by SDS PAGE. Gel profiles revealed only two radioactive peaks, one in the 35,000 molecular weight range (about 25% of the total radioactivity) and a second at about 80,000 (approximately 60% of the total radioactivity). If excess unlabeled thrombin (4 µg/ml) was present during incubation there was only one peak at 35,000, thus the 80,000 molecular weight peak represented specific binding of thrombin to its receptor. NAPEDE thrombin alone (with or without photoactivation) migrated with the lower molecular weight peak, indicating that the 80,000 molecular weight peak was not an aggregate of NAPEDE thrombin. These results indicate that the 80,000 molecular weight peak represents a complex between ^{125}I-thrombin (35,000) and its receptor which has an apparent molecular weight of about 45,000 daltons. (Supported by Grant CA-12306 from NCI.)

566 THROMBIN-STIMULATED CELL DIVISION INVOLVES PROTEOLYSIS OF A CELL SURFACE RECEPTOR. Kevin C. Glenn and Dennis D. Cunningham, Dept. of Medical Microbiology, University of California, Irvine, Irvine, CA 92717

Previous studies have shown that cell surface action of thrombin (TH) is sufficient to initiate division of chick embryo (CE) cells under serum free culture conditions. It has also been shown that both the proteolytic activity of TH and its binding to a specific cell surface receptor are required for mitogenic stimulation. To probe the question of cause and effect relationship, we conducted studies on secondary CE cells that divide after TH treatment and on 4 different isolates of CE cells at population doubling 25 that do not divide after TH treatment but remain fully responsive to the mitogenic action of serum. Cell surface proteins were labeled using lactoperoxidase and $^{125}I^-$. Following SDS-PAGE and autoradiography, we found a 43,000 dalton cell surface protein (43K) on both the responsive and unresponsive CE cells. However, 43K was cleaved by TH only on the responsive cells; it was TH insensitive on the 4 unresponsive populations. Two other kinds of experiments correlated cleavage of 43K with the mitogenic action of TH. First, there was a close correspondence between cleavage of 43K and initiation of cell division as a function of TH concentration; importantly, submitogenic concentrations of TH did not remove 43K. Second, there was a correspondence between duration of removal of 43K and amount of cell division as a result of varying the intervals of TH treatment. The TH receptor was labeled using a photoreactive crosslinking derivative of ^{125}I-TH (NAPEDE-^{125}-TH). The molecular weight of the receptor was 43,000, identical to the molecular weight of 43K discussed above. These separate approaches suggest that a 43,000 dalton cell surface protein produces a mitogenic signal by binding TH and undergoing TH-mediated proteolysis.

567 THROMBIN-CATALYZED HYDROLYSIS OF SPECIFIC MEMBRANE GLYCOPROTEIN; PROTEOLYTIC RECEPTORS ON HUMAN PLATELET PLASMA MEMBRANES. D.R. Phillips, Dept. Biochemistry, St. Jude Children's Research Hospital, Memphis, TN 38101

Thrombin is a highly specific protease which can produce different responses in different cells (e.g., mitosis in cells grown in culture; aggregation of platelets). In this study, we attempted to determine if any platelet membrane protein was hydrolyzed by thrombin and if hydrolysis was responsible for thrombin-induced platelet aggregation. The glycoproteins on washed, human platelets were labeled using neuraminidase/galactose oxidase/(^{3}H)-$NaBH_4$. Various concentrations of thrombin were incubated with platelets for 10 min. The lowest concentration yielding complete hydrolysis of glycoprotein V was 10^{-9} M. The glycopeptide hydrolytic product (Mr = 70,000) produced from this event was released into solution. The kinetics of glycoprotein V hydrolysis using 2 x 10^{-9} M thrombin were comparable to those of thrombin-induced aggregation: 70% of glycoprotein V hydrolysis occurred within the first 15 sec. incubation; aggregation occurred 10 to 15 sec. following this proteolytic event. Thrombin inactivated with phenylmethylsulfonylfloride was found to inhibit thrombin-induced aggregation and the hydrolysis of glycoprotein V. Trypsin and thermolysin, which also initiate aggregation of platelets, were also found to catalyze the hydrolysis of this glycoprotein. However, glycoprotein V hydrolysis was not observed when platelets were aggregated by collagen, ADP, or the Ca^{++} ionophore A-23187. The data indicate that glycoprotein V is a thrombin receptor and that cleavage of this glycoprotein is a prerequisite for thrombin-induced aggregation.

568 INSULIN-INDUCED PHOSPHORYLATION OF A POSSIBLE CYTOSKELETAL PROTEIN, Dominick L. Pucci and William B. Benjamin, SUNY at Stony Brook, Stony Brook, NY 11794

Hormone-induced change in phosphoprotein phosphorylations were studied in isolated rat fat cells and in subcellular fractions derived from fat cells. Insulin action increased the phosphorylation of two proteins, designated 2 and 6b (subunit M_r 130,000 and 62,000) while isoproterenol treatment decreased the phosphorylation of protein-6b and markedly increased the phosphorylation of protein-5 (subunit M_r 60,000). Subcellular fraction of ^{32}P-labeled fat cells showed that protein-2 was found with the cytosol while protein-6b was not extracted by standard buffers and was found with the "fat cake". Nonidet P40 containing buffer extraction of the "fat cake" released 6b. Protein-6b was now associated with a new pellet composed of filaments and short membrane segments. To determine whether protein-6b was found with actin-associated proteins, ^{32}P-labeled fat cells were extracted with 0.6 M KCl buffers and crude actin-containing fractions prepared. Protein-6b was not found with the actin-associated proteins. In addition, buffers containing 1% Trition X-100 and ribonuclease treatment did not solubilize protein-6b. Two-dimensional gel electrophoresis of fractions rich in phosphoprotein-6b separated it from all other ^{32}P-labeled proteins. Since the phosphorylation of protein-6b is increased by insulin action and because it is associated with a filamentous fraction which is consistent with intermediate filaments of the cytoskeleton, phosphoprotein-6b is a likely canidate to be involved in the transduction of insulin action.

569 INSULIN AND ILAs BINDING TO SPONTANEOUSLY TRANSFORMED AND UNTRANSFORMED C3H T10½ CELLS AS A FUNCTION OF CULTURE DENSITY, James F. Perdue and Iwan Susanto, Lady Davis Institute, Jewish General Hospital, Montreal, Canada H3T 1E2.

Alterations in the binding constant or the number of plasma membrane receptors which bind serum mitogens as a consequence of transformation may be a mechanism for controlling cell replication. This hypothesis was evaluated by measuring ^{125}I-insulin and ^{125}I-insulin-like activity (ILAs) binding to C3H T10½ and spontaneously transformed mouse cells. ^{125}I-Insulin bound optimally at pH 7.4 and was maximum by 60 min of incubation at 22°C. ILAs had an optimum pH for binding between 7.8 and 8.2 and required 2 hours of incubation at 22°C. Competitive binding experiments indicated that the C3H cells have unique receptors for each of the mitogens. Binding of ILAs and insulin was determined for normal and spontaneously transformed cells plated at densities of 0.25, 0.5, 1 and 1.5 x 10^5 and cultured for 3 days in media containing 10% serum. Insulin binding to normal C3H cells displayed a cell density-dependent decrease from 164±20 fmoles/mg for cells plated at the lowest density to 107±12 for those plated at 150,000. Spontaneously transformed cells bound greater quantities, e.g. those plated at 25,000 bound 274±74 fmoles/mg protein, and displayed only slight changes as a function of culture density. ILAs binding to both normal and spontaneously transformed C3H cells decreased as a function of culture density from about 0.7-0.6 to 0.17 μU/mg protein. These results suggest that cell-cell interactions or a differential in the utilization of serum mitogens during cell culture modulates the availability of receptors which specifically bind insulin whereas transformation is accompanied by an increased availability of these receptors.

This work was supported by grant GM23763 from the NIH.

570 GENETICS OF CELL SURFACE RECEPTORS FOR BIOACTIVE POLYPEPTIDES: A VARIANT OF MOUSE BALBc/3T3 CELLS POSSESSING ALTERED INSULIN BINDING ABILITY, N. Shimizu and Y. Shimizu, Dept. Cell. & Dev. Biol., Univ. of Arizona, Tucson, AZ 85721.

A number of human and rodent established cell lines were characterized in terms of insulin binding ability. Among those, mouse BALBc/3T3 fibroblasts possessing a high binding ability and contact-inhibited property was mutagenized with ethylmethane sulfonate under conditions sufficient to kill 90% of cell populations. After allowing a period for expression of the mutant phenotype the surviving cells were plated in monolayer using DME-medium containing 10% fetal calf serum and selected for mutation resulting in non-responsiveness to mitogenic action of insulin. By simultaneously treating non-dividing cells with insulin (5 x 10^{-11} M), vinblastine sulfate (1.0 μg/ml) and BSA (1% w/v), we were able to eliminate those cells that were stimulated by insulin to enter mitosis. Four surviving colonies were found and isolated after 3 cycles of this selection procedure. One of those primary clonal lines (IN-2) exhibited no binding ability at 0°C under which condition wildtype 3T3 cells showed normal saturable binding kinetics. The insulin binding ability of IN-2, however, was recovered substantially at 15°C and increased with higher temperature. The susceptibility of IN-2 to vinblastine sulfate seemed normal. Since this is a novel phenotype for insulin binding ability the IN-2 cells are being further characterized in order to determine whether such a phenotype is derived from mutation in the structural gene(s) for insulin receptors or from other epigenetic mechanisms. (Supported by Grants from NIH, GM24375 and ACS, JFRA-9.)

571 STIMULATION OF HUMAN VASCULAR ENDOTHELIAL CELL GROWTH BY A PLATELET-DERIVED GROWTH FACTOR AND ITS POTENTIATION BY THROMBIN, Bruce R. Zetter*and Harry N. Antoniades+, *Children's Hospital Medical Center, Harvard Medical School, and +Center for Blood Research and Harvard Universtiy School of Public Health, Boston, Ma. 02115

Repair of a vascular wound is mediated by migration and subsequent replication of the endothelial cells that form the inner lining of blood vessels. We have measured the growth response of human umbilical vein endothelial (HuE) cells to two growth promoting agents that are transiently produced in high concentrations at the site of a wound; the platelet-derived growth factor (PDGF) and the protease thrombin. When 10^4 HuE cells are seeded as a dense island (2 mm diameter) in the center of a 2 cm tissue culture well in medium containing 20% human platelet-poor plasma, no increase in cell number or colony size is observed. With the addition of 0.5 ng/ml of highly purified human PDGF, colony size increases and the number of cells after 6 days is 3.8 x 10^4. When human thrombin (2 ug/ml) is added along with the PDGF, the cell number rises to 7.2 x 10^4. Since thrombin alone stimulates no increase is cell number, it is likely that the enzyme acts to potentiate the cellular response to the PDGF. The ability of PDGF to stimulate endothelial cells appears to be species dependent since human PDGF fails to stimulate the growth of bovine endothelial cells derived from aorta, umbilical vein or adrenal capillaries. It is of interest that the mitogenic response of HuE cells to these growth factors is observed when the cells are plated as a dense island with room to outgrow but not when the same number of cells are seeded sparsely in the same 2 cm well suggesting that cell density or cell-cell interactions may play a positive role in human endothelial cell proliferation.

572 POTENTIATION OF SERUM DERIVED MITOGENIC ACTIVITY IN MEDIUM CONDITIONED BY NORMAL AND CARCINOGEN-ALTERED RAT LIVER CELL LINES BY THROMBOGENIC FACTOR Xa, Jean R. Starkey, Howard L. Hosick, James E. Talmadge and David F. Counts, Washington State University, Pullman, WA 99164.

Partial characterization of growth factors in complete medium conditioned by normal and carcinogen-altered rat liver cell lines indicates that a mitogenic factor(s) is generated from the large molecular weight fraction of serum, is proteinaceous in nature, and is susceptible to inhibition of activity by the protease inhibitor, epsilon-amino-N-caproic acid. Production of growth factor activity is markedly enhanced by factor Xa and depressed by the serum protease inhibitor of Xa and thrombin, antithrombin III. The concurrent cellular production of a small molecular weight inhibitor of growth is documented. Further studies on the mitogenic activity of factors from the fibrinolytic and the thrombogenic pathways towards these liver cell lines are described. The relative sensitivity of the normal and the carcinogen-altered cellular derivatives to these mitogens is documented as are studies on the production of prothrombin, proconvertin, factor X, thromboplastin and cancer procoagulant A. The possible involvement of thrombogenic factors in tumor embolus trapping, maintenance of the transformed cell phenotype and as tumor cell mitogens is discussed.

573 REGULATION OF THE CELL CYCLE BY HORMONAL GROWTH FACTORS OR SV40. C.D. Scher, W.J. Pledger, H.N. Antoniades, J.J. Van Wyk and C.D. Stiles, Harvard Medical School and Harvard School of Public Health, Boston, MA, 02115; University of North Carolina Medical School, Chapel Hill, NC, 27514

Serum contains two sets of factors which function synergistically to stimulate growth of Balb/c-3T3 cells. A platelet derived growth factor (PDGF), stored in platelet α-granules, is released into serum during clotting. PDGF, which has been purified to homogeneity, has a pI of 9.8 and a M.W. of 13,000 on reduced SDS-PAGE gels. Like other polypeptide hormones, PDGF is active at 10^{-10}M; about 5000 molecules per cell stimulate a round of DNA synthesis. PDGF induces the initial event in the replicative response, "competence." Plasma induces a later event, "progression", which allows competent (but not incompetent) cells to pass through G_0/G_1 and enter the S phase. Plasma from hypox. rats is deficient in progression activity and does not allow competent cells to synthesize DNA. The addition of somatomedin C to hypox. rat plasma stimulates competent cells to synthesize DNA demonstrating that somatomedin C has progression activity. A variety of growth factors such as multiplication stimulating activity (MSA) and fibroblast growth factor (FGF) were assayed for competence and progression activity. FGF, $Ca_3(PO_4)_2$ and "wounding" induce competence: DNA synthesis occurs after the addition of plasma.[3] Somatomedins A or C, MSA, or high concentrations of insulin allow progression: addition to competent cells in hypox. rat plasma stimulates DNA synthesis. None of these agents induce both competence and progression. Unlike hormonal stimulated DNA synthesis, SV40 induces DNA synthesis in the absence of PDGF or normal plasma. Thus, SV40 overrides the requirement for both competence and progression factors.

574 HUMAN FIBROBLAST GROWTH-PROMOTING FACTOR "HFGPF" AND CELL PROLIFERATION. Anwar A.Hakim. Department of Surgery and Oral Diagnosis. University of Illinois Medical Center.Chicago Illinois 60680.

A growth promoting agent was isolated from cultures of normal human fibroblasts(HNF) and designated as "HFGPF". It was purified as described by Hakim (Experientia-In Press). The final preparation yielded one single band on polyacrylamide disc gel electrophoresis at pH 8.5. The biological activity of HFGPF was measured by stimulation of DNA synthesis in bovine vascular endothelial cells (BVEC), canine cardiac cells (CCC) and Human normal epithelial mammary (HNMEC) and mammary carcinoma (HMC) cells.HFGPF did not bind to HNF.The binding of HFGPF to BVEC averaged 35 fold greater than the binding capacity to CCC.Using four different HMC cell lines, the binding capacity of HFGPF varied from one cell line to another, and averaged 30 fold greater than the binding capacity to HNMEC. The time course of binding of HFGPF reached maximal at 37°C after 30 min for HMC cells and 95 min for the HNMEC. When the binding of HFGPF to HMC and HNMEC was compared to its mitogenic effect on these cultures, it was observed that although HFGPF did bind to HNMEC, it had no mitogenic effect upon them, as shown by the absence on increase in cell number. By contrast, HFGPF was a strong mitogen for HMC cell culture and gave a maximal increase 15 to 22 fold in cell number and thymidine uptake at concentration as low as 0.3 ng/ml and a half maximal effect at 17 pg/ml. Trypsin decreased, causing the cells to round up, whereas collagenase and neuraminidase enhanced the growth-promoting effects of HFGPF. The growth-promoting agent increased the adhessiveness of trypsinized BVEC, CCC and HNMEC. It is suggested that HFGPF binds at the cell surface and possess angiogenic effects. Also, the presence of HFGPF binding sites is, by itself not sufficient for the mitogenic effect and induction of cell proliferation by HFGPF.

575 CORTICOSTEROIDS INDUCE PROLIFERATION OF HEMOPOIETIC STEM CELLS AND ADIPOCYTES AND STIMULATE LEUKEMOGENESIS IN LONG-TERM BONE MARROW CULTURE, Joel S. Greenberger, Joint Center for Radiation Therapy, Department of Radiation Therapy, Harvard Medical School, and Sidney Farber Cancer Institute, Boston, MA 02115

Maintenance of a hemopoietic microenvironment with proliferation of granulocyte-macrophage progenitor cells and pluripotent hemopoietic stem cells is required for in vitro leukemogenesis by viruses with long latent periods. In the absence of free corticosteroid, mouse marrow cultures grown in 25% horse, fetal calf or other sera failed to maintain proliferation of adipocytes, macrophages, and endothelial cells required as stroma for hematopoietic cells. Following addition of 10^{-7} M hydrocortisone sodium hemisuccinate, or other 17-hydroxycorticosteroids, two separable effects were observed. In horse serum corticosteroid stimulated preadipocyte-proliferation and accumulation of intracellular oil-red-O positive neutral fat. Stromal cell proliferation without lipid accumulation was seen in corticosteroid-supplemented calf serum; however, hematopoiesis was maintained for 14-21 weeks. Thus, corticosteroid-stimulated proliferation of pluripotent hematopoietic stem cells and granulocyte-macrophage progenitor cells is separable from lipogenesis. In corticosteroid-supplemented cultures induction of endogenous ecotropic type-C virus was detected in marrow from high leukemia-incidence mouse strains associated with high frequency generation of preleukemic morphologic alteration in granulocytes. Myeloid leukemogenesis in this system suggests that corticosteroid associated removal of lymphocytes may facilitate virus induction and transformation of rapidly dividing granulocyte precursors.

576 GLUCOCORTICOID MEDIATED INDUCTION OF TYROSINE HYDROXYLASE IN A CLONAL CELL LINE. Rosanne Goodman and Harvey R. Herschman, UCLA, Laboratory of Nuclear Medicine and Radiation Biology, 900 Veteran Avenue, Los Angeles, CA 90024

Glucocorticoids have been shown to modulate in vivo the phenomena known as transsynaptic enzyme induction. In organ cultures of superior cervical ganglia the glucocorticoids enhance the Nerve Growth Factor mediated induction of tyrosine hydroxylase. In addition, in primary explants of a rat pheochromocytoma tumor, glucocorticoids also induce tyrosine hydroxylase activity. In order to examine the molecular basis for this glucocorticoid induction of the adrenergic enzyme tyrosine hydroxylase, we have isolated from a rat pheochromocytoma tumor, a clonal cell line in which tyrosine hydroxylase specific activity is increased 6 - fold in the presence of the glucocorticoid dexamethasone. Data on dose response, time course, and specificity of this response will be presented.

577 ANIMAL MODEL SYSTEMS FOR MALIGNANT MELANOMA: STEROID HORMONE RECEPTOR STUDIES. Francis S. Markland and Diane Horn. Dept. of Biochemistry, LAC/USC Cancer Center, USC School of Medicine, and Dept. of Biological Sciences, USC, Los Angeles, CA.

Recent evidence suggests that hormonal factors may influence the incidence and growth characteristics of a certain percentage of human malignant melanomas. Recently we initiated studies to determine if steroid hormone receptors are present in animal melanomas and if the presence of receptors correlates with growth characteristics of the tumors. For these studies we used the B16 melanotic melanoma and the Syrian hamster melanoma cell line, 3460. Studies with B16 melanoma removed 10-14 days after transplantation indicated that a cytoplasmic estrogen receptor (ER) is present. There is no evidence for androgen, progestin or glucocorticoid receptors. Sucrose density gradient centrifugation of cytosol incubated with ^{3}H-estradiol (^{3}H-E_2) revealed the presence of an 8S peak that is suppressed by 100-fold molar excess radioinert diethylstibestrol (DES) or E_2. Binding varied from 5-35 fmoles per mg cytosol protein. Titration of cytosol from the B16 melanoma with ^{3}H-E_2 and subsequent Scatchard analysis gave a linear plot indicating a single class of high affinity binding sites. The dissociation constant is approximately 6 X 10^{-10}M. The receptor molecule is estrogen specific since ^{3}H-E_2 binding is inhibited by radioinert E_2 and DES but not by glucocorticoids, androgens or progestins. C-57 mouse plasma and skin contain no ^{3}H-E_2 binding. Additional studies on nuclear translocation of ER and hormonal sensitivity of the tumor are in progress. In contrast to B-16, no ER is found in the Syrian hamster melanoma line, 3460. Paradoxically, tamoxifen, an anti-estrogen, inhibits growth of 3460 in culture. These cells also respond to glucocorticoid since dexamethasone causes both an inhibition of growth and a decrease in final cell density.

578 PHOSPHOLIPID METHYLATION AND BETA-ADRENERGIC RECEPTORS IN HELA CELLS, R.C. Henneberry J.F. Tallman, F. Hirata, and J. Axelrod, NINCDS and NIMH, NIH, Bethesda, MD 20014
Numerous biochemical changes can be induced in HeLa cells by including certain short-chain saturated fatty acids in the culture medium. We have previously shown that HeLa cells have functional beta-adrenergic receptors on their surface and that the number of receptors per cell, but not the cellular content of adenylate cyclase (AC), increases markedly during exposure of cells to butyrate. Receptors can be induced in 2 forms, either "coupled" to or "uncoupled" from AC; this relationship can subsequently be altered by manipulation of the culture medium contents. This system now proves to be of value for studies on the role of phospholipid methylation in receptor function. HeLa cells contain 2 asymmetrically distributed membrane-bound enzymes which catalyze the synthesis of phosphatidylcholine (PC) from phosphatidylethanolamine (PE) by sucessive N-terminal methylations. Synthesis of PC by these reactions is stimulated by beta-adrenergic agonists while antagonists such as propanolol block the stimulation; alpha-adrenergic antagonists such as phentolamine are inactive. The increased rate of methyl group incorporation into PC is due to occupancy of receptor rather than to stimulation of AC; cholera toxin, under conditions which maximally activate AC, has no effect on phospholipid methylation. When inhibitors of methylation such as 4-deazaadenosine are included in the medium, phospholipid methylation is specifically inhibited. Concurrently, the number of beta-adrenergic receptors on the cell surface decreases as does isoproterenol-stimulated cyclic AMP production. It appears that phospholipid methylation affects the exposure of the beta-adrenergic receptor on the HeLa cell surface, as well as the ability of the receptor to activate AC when occupied by beta-hydroxylated catecholamines.

579 HORMONAL REGULATION OF GROWTH AND DOME FORMATION BY KIDNEY EPITHELIAL CELLS IN SERUM FREE MEDIUM - Mary Taub and Gordon Sato. University of California, San Diego, La Jolla,Calif. 92093. A hormone-supplemented, serum-free medium has been developed for a canine kidney epithelial cell line, MDCK. The supplements in the medium include insulin, transferrin, hydrocortisone, triiodothyronine (T_3) and PGE_1. MDCK cell growth occurred at equivalent rates in serum-free medium supplemented with either these five components or with fetal calf serum. This medium has been used to study: 1)the regulation of dome formation by hormones. (Domes are groups of cells slightly raised from the tissue culture dish surface, presumably as a result of transepithelial salt and water transport). Of the five components discussed above, PGE_1 was necessary in the medium for dome formation to occur. When medium supplemented with insulin, transferrin and PGE_1 was further supplemented with either hydrocortisone or T_3 the frequency of domes increased. Enhanced Na^+ transport by the Na^+ channel was observed in parallel with the increases in the frequency of domes observed above, which substantiates the hypothesis that dome formation depends upon the rate of salt transport across the monolayer. 2)The hormone-supplemented medium has also been utilized to grow primary cultures of kidney epithelial cells. Primary baby mouse kidney cultures grew at equivalent rates in medium supplemented either with hormones, or with fetal calf serum. Among the 5 added components, insulin, transferrin and PGE_1 caused significant growth stimulation when added individually to the medium. The dog kidney epithelial medium also maintained primary cultures derived from human embryonic and rabbit kidneys.

580 THE GROWTH OF SV3T3 TRANSFORMED CELLS IN A HORMONE-SUPPLEMENTED, SERUM-FREE MEDIUM, Delano V. Young, Michael Dean, Stewart Chipman, Fred W. Cox III, Eileen T. Nakano, and Standish C. Hartman, Dept. of Chemistry, Boston University, Boston, MA 02215

Simian Virus 40-transformed mouse fibroblasts (SV3T3) normally require serum for survival and growth in cell culture. Both the rate of cell proliferation and the final cell density are dependent on the level of serum in the culture medium (Dulbecco's Modified Eagle Medium, DME). Successful cultivation of SV3T3 cells in serum-free medium is possible if crystalline trypsin and trypsin inhibitor are used during the trypsinization process (Bush, H. and Shodell, M., J. Cell Physiol., 90 [1977] 573) and if DME is supplemented with biotin (DMEB) and transferrin or ferrous sulfate. Survival and moderate growth (20-30 hours population doubling time; 1 x 10^6 cells per 3.2 cm dish as a final cell density) are attainable in the serum-free, biotin-transferrin medium (DMEBT). The transferrin (or iron) growth effect is dependent on the presence of biotin in the medium.

Insulin added at high concentrations (200-500 ng/ml) to either DMEB or DMEBT causes an increase in the rate of cell proliferation as seen in a decrease in the population doubling time. If Epidermal Growth Factor (EGF, 50ng/ml) and/or Fibroblast Growth Factor (FGF, 10ng/ml are added to insulin-containing DMEBT growth nearly comparable to that found in saturating serum is achieved (a population doubling time of 14 hours and a final cell density of 1-2 x 10^6 cells per 3.2 cm dish in the hormone medium vs. 12 hours and 2-3 x 10^6 cells for 10% calf serum DME). The relationship of insulin, EGF, FGF, and transferrin to the unidentified SV3T3 growth factors present in serum is currently under investigation.

581 THE RELATIONSHIP OF EARLY MEMBRANE EFFECTS OF TETRADECANOYL PHORBOL ACETATE (TPA) AND PGF_2 TO CELL CYCLE KINETICS: AN APPROACH THROUGH G_1 MAPPING. L. D. Tomei and C. E. Wenner, Roswell Park Memorial Institute, Buffalo, New York 14263.

An objective of the current research with mouse embryonic fibroblasts is to define the relationship of early membrane effects to activation of cell cycle. The earliest changes induced by the potent tumor promoter, TPA, are in membrane transport where TPA (10^{-8}M-10^{-6}M) stimulates the ouabain-sensitive uptake of the K^+ marker $^{86}Rb^+$ by 1.3-1.7 fold and the enhancement of $^{32}P_i$ incorporation within a few minutes of administration. Similar effects were obtained with prostaglandin $F_{2\alpha}$ at the same concentrations as TPA. Since $PGF_{2\alpha}$ and TPA exhibit similar activities, the question arises as to whether tumor promoting phorbol esters and $PGF_{2\alpha}$ have in common properties that influence cell growth regulation. Both TPA and $PGF_{2\alpha}$ have been associated with proliferative responses and cell cycle activation. Since both drugs exhibit early membrane effects, we approached the problem of relating cell cycle responses to stimulation of ion movements by attempting to map the g_1 phase of cell cycle. A working hypothesis has been developed and is based upon the rate at which a cell enters DNA synthesis and the manner by which a drug changes the cells' entry into DNA synthesis. This involves the proposal of a multi compartment model within g_1 defined by the responses to drugs in terms of 2 variables; (1) distance in time between drug addition and entry into S phase and (2) drug induced stimulation of ion uptake. TPA as well as $PGF_{2\alpha}$ stimulated ^{3}H-Tdr incorporation into DNA in postconfluent 3T3 cells. However, TPA, but not $PGF_{2\alpha}$, produced a further stimulation of ^{3}H-Tdr into DNA when fresh serum was introduced. The kinetic responses to TPA and $PGF_{2\alpha}$ are evaluated with respect to clonal variation as a test of the validity of the model.

582 EFFECTS OF THE TUMOR PROMOTER TPA ON CEREBELLAR NERVE FIBER OUTGROWTH IN VITRO, Alphonse Krystosek, Univ. Colorado Medical Center, Denver, Colorado 80262

The tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate (TPA) has been shown to inhibit cell differentiation in several in vitro systems. The effects of TPA on the outgrowth of nerve fibers in dissociated cell cultures of 7-9 day postnatal mouse cerebellum has now been examined. In contrast to the inhibition by TPA of the induced neurite outgrowth by clonal mouse neuroblastoma cells (Ishii et al.,Science 200:556, 1978; and my results with clone N18) and embryonic chick ganglia (Ishii, Cancer Res. 38:3886, 1978), the spontaneous outgrowth of nerve fibers in cerebellar cultures proceeded in the presence of TPA (1×10^{-10} - 5×10^{-6} M). Moreover, there was an enhancement of cell attachment and early fiber outgrowth seen by day 1 in the presence of TPA.This attachment effect was independent of cell proliferation as shown by treatment of cultures with 1×10^{-5} M cytosine arabinoside. TPA treated cultures did not show a stimulation over control cultures in the incorporation of ^{3}H-thymidine during day 1 (the time of increased cell attachment), but did show an approximately 2-fold increase in incorporation measured at 44-48 hr. The nature of the refractoriness of the cerebellar cultures to an inhibition of morphological differentiation is not understood at present, but several types of evidence suggested that it was not due to an inactivation of TPA. Supported by NIH grants F-32-CA-05949; NS-09818 (to N.W. Seeds); and a grant from the Milheim Foundation.

583 INITIATION OF DNA SYNTHESIS BY TPA AND GROWTH FACTORS IN NORMAL AND RSV-TRANSFORMED RAT RIBROBLASTS. B.E. Magun and G.T. Bowden, University of Arizona, Tucson, Az. 85724

Stimulation of DNA synthesis in response to different growth factors and the tumor promotor tetradecanoyl phorbol acetate (TPA) was examined in untransformed and RSV-transformed (B-77 strain) rat fibroblasts (Rat-1 cells). Following serum deprivation to achieve quiescence, semiconservative DNA replication was measured by incubation of cells in BrUdR and FUdR after stimulation, followed by CsCl density gradient analysis of newly replicated DNA. When added to quiescent cultures in serum-free medium, TPA induced a minimal increase in the amount of DNA made over a 30h period (<10% above controls) in both untransformed and transformed cells. However, if a short period of serum stimulation (4h) was followed by TPA for 1 h, a dramatic increase in DNA synthesis occurred in the untransformed, but not in the transformed, cells. The latter cells responded by a decrease in DNA synthesis under the same conditions. In all experiments, the RSV-transformed cells were refractory to, or inhibited by, TPA at all concentrations up to 1 μg/ml. We conclude that TPA acts as a comitogen on the untransformed, but not the transformed, cells. Because TPA has been found to interact with epidermal growth factor (EGF) receptors, we tested the mitogenic effect of EGF, fibroblast growth factor (FGF) and multiplication stimulating activity (MSA). Quiescent untransformed Rat-1 cells, but not the RSV-transformed cells, were stimulated by synthesize DNA by EGF, FGF and MSA. These data support the conclusion that the transforming function initiates a process which renders the normal hormonal control mechanisms ineffective in the regulation of growth.

584 TRANSFERRIN RECEPTORS ON NORMAL AND TRANSFORMED LYMPHOID CELLS, James W. Larrick and Peter Cresswell, Div. of Immunology, Duke Univ. Medical Center, Durham, NC 27710

Several investigators have reported that transferrin is among a small group of protein growth factors necessary for serum free growth of cell lines (e.g., Proc. Natl. Acad. Sci. 75:901, 1978). We have recently demonstrated the existence of receptors for iron-saturated transferrin on both normal human peripheral mononuclear cells and matched pairs of T and B lymphoblastoid cell lines. Binding of ^{125}I-labeled transferrin is rapid, saturable and reversible. It can be specifically inhibited by unlabeled transferrin but not by lactoferrin, ovalbumin, or bovine serum albumin. Receptor molecules have been solubilized in the non-ionic detergent octaethyleneglycoldodecylether. *Characterization of the receptor molecules solubilized from* lymphoblastoid cell lines and isolated by affinity chromatography will be presented. Approximately 60,000 binding sites per cell are found on T cell lines which is double the number found on B cell lines. The affinity of binding determined by scatchard analysis is high ($K_{aff} = 1 \times 10^{12}\ M^{-1}$) and approximately equal for the B and T cell lines. Mixed peripheral mononuclear cells have fewer receptor sites and less affinity for transferrin than the transformed cell lines. The number of binding sites varies with the growth rate of the cells. Rapidly proliferating (log phase) lymphoblastoid cells express more receptors than stationary cultures or untransformed cells. Implications of changes in transferrin receptor number and affinity during normal and transformed cell growth will be discussed.

585 THE GROWTH FACTOR ACTIVITY OF HUMAN AND BOVINE MILK. Michael Klagsbrun, David Tapper and Jacqueline Neumann. Children's Hospital and The Harvard Medical School, Boston, Mass. 02115.

Human and bovine milk contain growth factors capable of stimulating DNA synthesis and cell division in cells in culture. In both species, mitogenic activity is most pronounced in milk obtained shortly after parturition, that is, in the colostrum. Mitogenic activity is diminished by over 80% in lactating cows within 5 days after birth and in lactating women within 20 days after birth. The bovine and human milk mitogens are proteins whose molecular weights are between 5,000 and 20,000. When bovine and human milk are analyzed by isoelectric focusing between pH 3 and pH 11, only 1 peak of activity is found. The isoelectric points for bovine and human milk mitogen are 4.6-4.8 and 4.4-4.7 respectively. Since no activity is found in the high pH range, it is unlikely that milk contains platelet growth factor or fibroblast growth factor which have isoelectric points between 9.5 and 10. A partially purified preparation of bovine colostrum will support the growth of certain cells in culture in the absence of serum. A canine kidney epithelial cell line (MDCK) can be trypsinized, plated sparsely (2×10^3 cells/cm^2) and grown to confluence (1.5×10^5 cells/cm^2) in Dulbecco's modified Eagles medium supplemented with 1 mg/ml of partially purified bovine colostrum. There is no growth in unsupplemented medium. The MDCK cells can be subcultured at least twice more and grown to confluence with the colostrum. On the other hand, Balb/c cells when plated sparsely will not grow in medium in which colostrum has been substituted for serum. It is suggested that the mitogenic activity of colostrum has specificity for epithelial cells but not for fibroblasts.

586 SPECIFIC BINDING OF SERUM MITOGENS TO HYPERPLASTIC RAT INTESTINAL MUCOSA IN SITU, Peter Milthorp and James F. Perdue, Lady Davis Institute for Medical Research of the Jewish General Hospital, Montreal, Quebec, Canada H3T 1E2.

Hyperplasia of rat intestinal mucosa occurs after resection of 50% of the jejunum-ileum. Analysis of the mucosa at day 4 post operation showed a 46% increase in RNA and a 16% increase in DNA. Studies involving perfusion of the residual jejunum-ileum were initiated to determine whether compensatory hyperplasia is reflected in greater nutrient transport; protein, RNA and DNA synthesis, and if these changes can be correlated with greater specific binding of serum mitogens, i.e. insulin, IGF (insulin-like growth factors) and EGF (epidermal growth factors). The vascular system of the organ was perfused at 37°C and 110 mm Hg pressure with oxygenated media composed of a bicarbonate buffered salt solution, 5.5 mM glucose, 3% dextran and the appropriate radiolabelled ligands. The intestinal lumen was simultaneously perfused with 300 mOs mannitol. The movement of radiolabelled ligands from the vascular perfusate to the lumen and tissue was monitored over a 90 minute period. Preliminary data indicates that 2-deoxyglucose and uridine are specifically transported into mucosal cells. To date stimulation of this uptake by 200 mM insulin has not been demonstrated.

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587 QUANTITATIVE RECEPTOR OCCUPANCY STUDIES, Edward O'Keefe, University of North Carolina, Chapel Hill, North Carolina 27514

A transformed cell relatively deficient in ganglioside GM_1, the natural receptor for cholera toxin (choleragen), binds 2% of the choleragen bound by the parent line. Receptors blocked by the inactive derivative, choleragenoid, which binds with equal affinity to the receptor ($K_D=2x10^{-10}M$), can be reconstituted by incorporation of exogenous ganglioside GM_1 with restitution of adenylate cyclase activation, indicating that all receptors are biologically equivalent. Choleragenoid competes for binding in all cells, but inhibition of cyclase is seen readily only in fat cells, which lack receptors in excess of the number ($1-2x10^4$) required for full cyclase activation. Inhibition of cyclase activation requires 50-200-fold excess choleragenoid over choleragen in transformed cells, which have 10-fold excess receptors, and is poorly seen in the parent line, which has 100-fold excess receptors. Interpretation of competitor activity requires quantitative assessment of receptor occupancy in relation to a proximal, prompt biological effect in the membrane.

Inhibition in the parent line by choleragen of epidermal growth factor stimulated ^3H-thymidine incorporation, a distant effect of adenylate cyclase stimulation, is maximal at $10^{-13}M$ choleragen, but binding is less than 10% of that saturating adenylate cyclase activation. Thymidine incorporation overestimates "spare" receptors by at least 10-fold in comparison with adenylate cyclase; comparison of hormone receptor occupancy with distant biological effects is not valid.

588 A TOXIC, DISULFIDE-LINKED HYBRID MOLECULE CONTAINING FRAGMENT A OF DIPHTHERIA TOXIN AND CONCANAVALIN A. Gary Gilliland, R. J. Collier, Department of Microbiology, College of Letters and Science, UCLA, Los Angeles, CA 90024.

A disulfide-linked conjugate of Concanavalin A (Con A) and Fragment A from diphtheria toxin has been synthesized and is toxic for HeLa (human), CHO (hamster), and SV3T3 (murine) cells. The conjugate is constructed by first coupling cystamine to Con A with a carbodiimide reagent and then reacting the modified Con A with reduced Fragment A under conditions pormoting disulfide interchange. The desired conjugate, obtained in nearly 50% yield relative to input Fragment A, is purified by affinity chromatography on Sephacryl S-200 and NAD-Sepharose. The conjugate is about equally active in inhibiting protein synthesis in HeLa, CHO, or SV3T3 cells in culture, but is inactive relative to controls on a toxin resistant strain of CHO cells containing altered elongation factor 2, the target protein of Fragment A. On toxin-sensitive strains the conjugate was 100-1,000 fold more active than controls, including Fragment A, cystaminyl-Con A, and mixtures thereof, but was 50-500 fold less toxic than diphtheria toxin itself. The activity of the conjugate in tissue culture was inhibited by Con a or α-methyl mannoside, but not by galactose. This and similar conjugates should be useful in studying mechanisms of entry of biologically active proteins into cells.

589 MITOTIC FACTORS FROM MAMMALIAN CELLS INDUCE GERMINAL VESICLE BREAKDOWN AND CHROMOSOME CONDENSATION IN AMPHIBIAN OOCYTES, Prasad S. Sunkara[1], David A. Wright[2] and Potu N. Rao[1], Department of Developmental Therapeutics[1] and Biology[2], The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030.

The fusion between a mitotic and an interphase cell usually leads to the breakdown of the interphase nucleus and the condensation of chromatin into discrete chromosomes under the influence of the factors present in the mitotic cell. This phenomenon, which has been termed premature chromosome condensation (PCC) appears to be similar to the initiation of mitosis and meiotic maturation of amphibian oocytes. In amphibians, meiotic maturation of ovarian oocytes involves breakdown of the nuclear envelope (germinal vesicle), chromosome condensation and progression through first meiotic division. This maturation process can be induced either by incubating oocytes with progesterone in vitro or by injecting them with the cytoplasmic extracts from mature oocytes. Because of these similarities between PCC induction and meiotic maturation of amphibian oocytes, we decided to test whether mitotic factors from mammalian cells can induce maturation in amphibian oocytes. Cytoplasmic extracts of HeLa cells synchronized in various phases of the cell cycle were injected into fully grown Xenopus laevis oocytes to monitor the presence of factors that can induce meiotic maturation, i.e., germinal vesicle breakdown and chromosome condensation. Extracts from G1 and S phase cells had no activity. The maturation-inducing activity, which was found to be low during early and mid G2 phases, increased rapidly during late G2 and reached a peak in mitosis. The results of this study suggest that the factors that regulate the breakdown of nuclear membrane and chromosome condensation during mitosis, meiosis, and premature chromosome condensation appear to be very similar, if not identical, throughout the animal kingdom. (Supported in part by grants CA11520, CA14528, CA19856 and CA23878 from NCI, DHEW).

590 A MUTATION AFFECTING THE CYCLIC-AMP RECEPTOR OF DICTYOSTELIUM DISCOIDEUM, Ellen J. Henderson and Stephen L. Barclay*, Dept. of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139

Under conditions of starvation, the initially free-living amoebae of D. discoideum elaborate cell surface receptors for cAMP and aggregate into multicellular masses. Aggregation is a consequence of a chemotactic response of the amoebae to cAMP released by neighboring cells. The resulting cell mass undergoes a series of biochemical and morphological transformations resulting in formation of a fruiting body composed of stalk cells and spore cells.

We have isolated a mutant (D477) which aggregates without streams into small aggregation territories and forms small fruits. The ratio of spore mass diameter to stalk length in these fruits is tenfold higher than for the wild type parent. Scatchard analysis of cAMP binding to the mutant cells shows a single class of sites with an affinity for cAMP intermediate between the two affinity classes exhibited by wild type. The mutant has about half the total number of sites observed in the parent. As a consequence of these differences, the mutant binds more cAMP than the parent at physiological cAMP concentrations, yet in a light-scattering assay of responsiveness to cAMP, the mutant is less sensitive. The properties of this mutant confirm the indirect evidence of other workers suggesting a role for the cAMP chemosensory system in the selection of terminal differentiation pathways during fruiting body formation.

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591 REGULATION OF HORMONE-STIMULATED ADENYLATE CYCLASE BY MICROTUBULES IN INTACT S49 LYMPHOMA CELLS. Paul A. Insel & Michael Kennedy, U.of Cal.,San Diego and San Francisco.

Interaction of cytoskeletal elements with the plasma membrane has been shown in a number of experimental systems. In this regard, we have recently been studying the interaction between microtubule inhibitors(MI,e.g.colchicine,vinblastine)and cyclic AMP(cAMP)generation in murine S49 lymphoma cells. We have reported(Nature 273:471(1978))that MI enhance beta-adrenergic-stimulated cAMP levels in wild-type(WT) S49 cells by an action distal to beta-adrenergic receptors. In order to define further the mechanism of this enhancement, we have studied other stimulants of adenylate cyclase and have used S49 variants that have abnormalities in the pathway of cAMP generation and function. In intact WT S49 cells, MI also enhance PGE_1- and cholera toxin-stimulated cAMP levels, but the enhancement of PGE_1 and beta agonists is lost if cells treated with MI are disrupted or if MI are incubated with S49 plasma membranes. MI do not block desensitization to beta-agonists in intact WT S49 cells. In S49 variants(94.15,UNC) that lack coupling between PGE_1 and beta receptors and adenylate cyclase, MI alone do not facilitate PGE_1-or beta-stimulated cAMP, whereas S49 variants lacking cAMP-dependent protein kinase activity respond as do WT cells. Incubation of WT cells with cholera toxin(which inhibits GTPase in other cell types)also enhances PGE_1-and beta-stimulation of cAMP;this enhancement summates with that produced by MI. Moreover, in the UNC variant,cholera toxin and MI appear to potentiate each other so that beta-stimulated cAMP generation(normally absent) can be demonstrated. We conclude that MI increase cAMP probably by inhibiting microtubules in intact S49 cells, that microtubules may restrict adenylate cyclase activity in intact cells, and that this effect may occur by an alteration in the GTP coupling unit of adenylate cyclase.

592 MICROTUBULE/MICROFILAMENT ORGANIZATION AND MITOGENIC HORMONE RECEPTOR EXPRESSION. Mohan K. Raizada and Robert E. Fellows, University of Iowa, Iowa City, IA 52242.

Our earlier studies, using ^{125}I-insulin as a model mitogen, demonstrated that cytochalasin B (CB) treatment of uninfected cultured chick embryo fibroblasts (CEF) results in an increase in the number of mitogen receptors and suggested a role of microfilaments in this process. In contrast, RSV-transformed CEF, with 2-4 fold more mitogen receptors, do not respond to treatment with CB. Recently we have investigated the relationship between mitogen receptor expression, the organizational pattern of microfilament bundles and changes in cell surface morphology. Treatment of uninfected CEF with local anesthetics, including dibucaine, xylocaine, tetracaine, and mepivicaine, results in disruption of microfilaments (MF) and microtubules (MT) and an increase in the number of mitogen receptors. This effect is time and dose-dependent and removal of the agent results in a time-dependent decrease in these receptors. Mild trypsin treatment of uninfected CEF, whichdisrupts organization of MT/MF, also results in an increase in mitogen receptors. However, trypsin treatment is not synergistic with anti-MT/MF reagents. The increase in mitogen receptors is associated with rounding up of cells, the appearance of ruffles, blebs and microvilli on the cell surface, and a decrease in the number of microfilament bundles. Treatment of RSV transformed CEF, which have round morphology, decreased microfilament bundles and increased mitogen receptors, with anti-MT/MF agents does not result in a further increase in receptors. These results indicate that the expression of mitogen receptors in cultured CEF is related to alterations in cell morphology and the organization of cytoplasmic MT/MF. Supported by NIH grants AM 21018 and AM 19901.

593 RECONSTRUCTION OF MAMMALIAN CELLS BY PEG-INDUCED FUSION OF CELL FRAGMENTS,
Jerry W. Shay and Mike A. Clark, The University of Texas Health Science Center at Dallas, Department of Cell Biology, Dallas, TX 75235
Enucleation techniques permit cells to be divided into nuclear (karyoplast) and cytoplasmic (cytoplast) cell fragments. Even though these nuclear and cytoplasmic fragments are metabolically stable for short periods of time ultimately they degenerate. It is possible, however, to reconstruct viable cells by PEG-induced fusion of karyoplasts to cytoplasts and such reconstructed cells may provide a clearer understanding of the interactions between the nucleus and the cytoplasm. Utilizing the Y-1 cell line which was originally derived from a murine adrenal tumor and which retains the ability to secrete steroids in response to adrenocorticotropic hormone (ACTH), we have reconstructed cells containing the Y-1 karyoplast and a cytoplast from a non-responsive cell (AMT). In addition we have reconstructed cells containing the Y-1 cytoplast and AMT karyoplast. The Y-1 cytoplasts when treated with ACTH results in steroid secretion and morphological rounding up which indicated that the nucleus was not necessary for this response. The results of our experiments indicated that the reconstructed cells containing the Y-1 karyoplast initially did not respond to ACTH but after a delay of approximately 10-20 generations became responsive. The reconstructed cells containing the Y-1 cytoplast initially did respond to ACTH but after a short delay became unresponsive. These experiments suggest that the cytoplasm initially controls the phenotypic expression of this differentiated cell function but that ultimately the nucleus dominates.