001 SIALOGLYCOPROTEINS OF HUMAN ERYTHROCYTE MEMBRANES, Heinz Furthmayr and Vincent T. Marchesi, Department of Pathology, Yale University School of Medicine, New Haven, Connecticut 06511

Studies on the primary structure of the sialoglycoproteins from erythrocyte membranes have allowed to gain considerable insight into the organization of protein components of plasmamembranes. Two different polypeptide chains, glycophorin A and glycophorin B, can be separated by column chromatography in the presence of detergents (1). Both glycopeptides have similar molecular weights and carbohydrate compositions, but they can be distinguished on the basis of differences in their primary structure. On SDS-polyacrylamide gels complex patterns are observed. Glycophorin A shows two bands of apparent molecular weights of 83500 and 46000 daltons (1). This heterogeneity contrasts with the amino acid sequence data, which indicate a homogeneous protein of 31000 daltons including 131 amino acid residues and 16 oligosaccharide chains attached to amino acids in the amino-terminal portion of the molecule (2). The two bands represent, however, interconvertible forms of polypeptide subunits and subunit-complexes (3,4). The subunits interact within the hydrophobic segment (2) of the molecule, located between the N-terminal glycopeptide region and a C-terminal hydrophilic peptide portion. A tryptic-derived peptide from the hydrophobic region or modification of a single methionyl residue within this region can inhibit reassociation of the glycophorin A subunits. Glycophorin B forms similar oligomers, which are rather stable in detergents (1). The complex gel patterns that are observed under various conditions can now be explained simply in terms of two polypeptide chains, although the interpretation may be somewhat complicated by the effect of additional variation in the attachment of oligosaccharide chains (2).

Specific antibodies to an antigenic determinant located within 17 amino acid residues in the C-terminal region of glycophorin A react exclusively with glycophorin A complexes, subunits or small peptides, but not with glycophorin B or other proteins from the membrane. Structural data confirm the absence of an extended C-terminal hydrophilic peptide segment in glycophorin B. The results suggest that the sialoglycoproteins exist as multi-subunit complexes in the red cell membrane and that some of their properties are determined by the presence or absence of distinct regions in the molecule. The structural data will be reviewed and new information derived from the latter studies on the localisation of the antigenic determinant in intact cells will be presented.

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002 THE STRUCTURE AND EVOLUTION OF GENE PRODUCTS CODED BY THE MAJOR HISTOCOMPATIBILITY COMPLEX OF MAMMALS, L. Hood, M. Cecka, S. Ewald, M. Hunkapiller and M. McMillan, Division of Biology, California Institute of Technology, Pasadena, CA 91125

Structural analyses of the transplantation antigens and Ia antigens coded by the H-2 locus of the mouse will be presented (1,2,3). These data together with those obtained by other laboratories on the MHC gene products of man will be discussed with regard to their implications for biological functions, genetic organization and the evolutionary history of the major histocompatibility complex of mammals.

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003 ENVELOPE GLYCOPROTEINS OF SINDBIS VIRUS, Kenneth Keegstra and David J. Burke, Dept. of Microbiology, State University of New York, Stony Brook, New York, 11794.

Sindbis virus, an alphavirus, has two envelope glycoproteins (1), which are thought to be glycosylated by host glycosyl transferases (2,3). This suggests that Sindbis might be a useful probe to examine the glycosylation processes of the host cell. Since the viral oligosaccharides are synthesized by a subset of the glycosyl transferases which synthesize the host cell surface oligosaccharides, it follows that the viral oligosaccharides should also be similar in structure to a fraction of the cell surface oligosaccharides. Therefore in analyzing the structures of the viral glycoproteins, we also learn about the oligosaccharides of the host cell.

Each of the two Sindbis glycoproteins (called glycoprotein E1 and glycoprotein E2) has two different types of oligosaccharide attached to it (4). Each glycoprotein has one rather complex oligosaccharide which contains glucosamine, mannose, galactose, fucose, and variable amounts of sialic acid (4). In addition, each glycoprotein has a second oligosaccharide which contains only glucosamine and mannose (4). We have purified each of these oligosaccharide structures from virus grown either in baby hamster kidney (BHK) cells or in chicken embryo cells (5). We have examined the structures of these various oligosaccharides using methylation analysis and sequential enzymatic degradation (5). The results of these studies have shown that the oligosaccharide structures of the Sindbis glycoproteins are very similar, regardless of whether the virus is grown in chick cells or BHK cells. Preliminary evidence suggests that the oligosaccharide residues are attached to the same site on the viral polypeptide when virus is grown in either host. The details of our structural studies on the Sindbis glycoproteins will be presented. In addition, more recent comparisons between the Sindbis glycopeptides and the surface glycopeptides of uninfected host cells will be presented.

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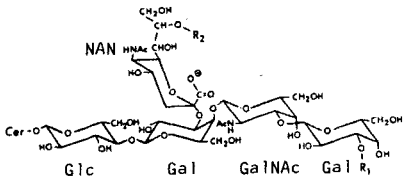
5) Burke, D. J. Ph.D. Thesis, 1976.

004 GANGLIOSIDE STRUCTURES OF NEURAL AND EXTRANEURAL TISSUES, Robert W. Ledeen, Departments of Neurology and Biochemistry, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

N-Acetylneuraminic acid (NAN) is the principal sialic acid in CNS gangliosides, while extraneural ones contain in addition N-glycolylneuraminic acid (NGN) and O-acetylated forms of both. The four major gangliosides of mammalian brain are built up from the G_{M1} backbone:



Structures of the four major gangliosides of mammalian brain. G_{M1}: $R_1=R_2=H$; G_{D1a}: R_1=NAN, $R_2=H$; G_{D1b}: $R_1=H$, R_2=NAN; G_{T1b}: $R_1=R_2$=NAN.

All glycosidic linkages have the β-configuration except those of sialic acid which are α-ketosidic bonds. A dozen or more minor gangliosides have been characterized in brain. The simplest is sialosyl-galactosylceramide ($G_7=G_{M4}$) which is unique in having a galactosylceramide unit (all others have glucosylceramide) and a pattern of hydroxy and normal long-chain fatty acids similar to that of myelin cerebrosides (most other brain gangliosides have normal acids only, with stearate the major component). The most complex ganglioside thus far is a pentasialo species with a G_{M1} backbone and 3 NAN's in a chain on the internal Gal. Aside from hematosides which lack hexosamine, the majority of brain gangliosides contain N-acetylgalactosamine (GalNAc) as the third sugar unit from ceramide. A minor species, present in peripheral nerve and extraneural tissues as well as brain, contains N-acetylglucosamine (GlcNAc) in a lacto-neotetraose (paragloboside) backbone: Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc-Cer. This structure forms the basic unit of a number of extraneural gangliosides, such as the major one of human erythrocytes which has NAN linked (2-3) to terminal galactose. Human kidney has this type with fucose linked to the C_3 hydroxyl of GlcNAc while human spleen has NAN(α2-3)Gal(β1-4)-GlcNAc(β1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc-Cer. A species with fucose joined (1-2) to the terminal galactose of G_{M1} was characyerized in boar testis while a closely related ganglioside with NGN in place of NAN was found in bovine liver. Most extraneural tissues have both GlcNAc- and GalNAc-containing gangliosides together with hematosides; the latter often predominate and occur in mono- or disialo forms. Brain gangliosides have both C_{18} and C_{20} sphingosine while extraneural species have C_{18} with little or none of the latter. (Supported by USPHS grants NS 03356, NS 04834 and NS 10931).

041 MOLECULAR PROPERTIES OF RHODOPSIN, Paul A. Hargrave, Southern Illinois University, Carbondale, Illinois, 62901.

The amino-terminal of bovine rhodopsin is blocked and has the sequence

X-Met-Asn(CHO)-Gly-Thr-Glu^{5}-Gly-Pro-Asn-Phe-Tyr^{10}-Val-Pro-Phe-Ser-Asn^{15}(CHO)-Lys-Thr-Gly-Val-Val^{20}-Arg-Ser-Pro-Phe-Glx^{25}-Ala-Pro-Glx-Tyr

where Asn^{15} contains a previously undetected second site for carbohydrate attachment in rhodopsin. The carboxyl-terminal sequence of this intrinsic membrane protein,

Val-Ser-Lys-Thr-Glu-Thr-Ser-Gln-Val-Ala-Pro-Ala

is also hydrophilic in composition and might be expected to be exposed to a membrane surface aqueous enviornment. Limited proteolysis of rod cell membranes by thermolysin [Saari, J. Cell Biol. 63, 480 (1974); Pober and Stryer, J. Mol. Biol. 95, 477 (1975)] produces two membrane-bound fragments F1 (~ 26,000 MW) and F2 (~ 10,000 MW) and some soluble peptides. F1 contains the amino-terminal glycopeptide region. F2 which contains the retinyl-binding site, originates from the carboxyl-terminal region of rhodopsin. The soluble peptides must originate from an internal region of the polypeptide chain which connects F1 and F2.

042 THE MOLECULAR STRUCTURE OF BOVINE SPERM SURFACE, Tae H. Ji, Inhae Ji, Bong Y. Yoo, and George Nelms. The University of Wyoming, Laramie, Wyoming 82071, and the University of New Brunswick, Fredericton, New Brunswick, Canada.

Once sperm were deposited in the female organ, the proper and continuous modification of the surface structure of sperm are essential for a successful fertilization in mammalian systems. The disruption in the process probably results in the infertility. Both fertility and sterility are obvious interest to human as means for the increase of cattle production and for the human population control. The critical elements determining the surface structure of a cell is now well known to be the surface molecules, some of which are the integral membrane components being exposed to the surface. Although sperm carry a variety of antigens and possibly other proteins on the surface, little is known of their chemical identity. Ejaculated bovine sperm were examined by surface labeling with the lactoperoxidase-^{125}I system and fluorescamine, along with the proteolytic digestion of the sperm by trypsin and chymotrypsin. Those molecules modified were identified on SDS-polyacrylamide gels by staining and autoradiograph, after solubilization and electrophoresis. More than seven species among over thirty were found to be susceptible to the labels and proteolysis. The results remained the same regardless of washed sperm, free of the seminal fluid, or sperm in the seminal fluid were labeled. Various controls for the labeling and proteolysis indicated that the modified, labeled or proteolyzed, molecules were accessible at the sperm surface to the surface labels and proteolytic enzymes. (Supported by grants from NSF BM 75-09230 and the National Research Council of Canada, A3651. Wyoming Experimentental Station SA-749).

043 POLYSACCHARIDES OF SPONGE AGGREGATION FACTOR, Susie Humphreys and Tom Humphreys, Pacific Biomedical Research Center, University of Hawaii, 41 Ahui St., Honolulu, Hawaii 96813.

The size and location of the polysaccharide chains of the sponge aggregation factor proteoglycan complex have been studied. Preparations of purified Microciona prolifera sunbursts treated with EDTA for several days at 0°C dissociate into three protein and polysaccharide containing components. These were fractionated on a 1,000 Å pore size micropore glass column. Fifteen percent of the material is excluded and appears in the electron microscope as the central circle of the sunburst. Digestion of the circles with 10^{-3} M DTT and 0.5 mg/ml Proteinase K for 72 hours at 37° C produces two polysaccharide chains of 65,000 and 5,000 daltons as fractionated and sized on a 240 Å pore size micropore glass column using Pharmacia dextrans as standards. The included fraction of the EDTA treated sunbursts is subunits of the arms which make up 70% of the preparation. A single polysaccharide of 5,000 daltons is released from these subunits by proteinase digestion. We calculate that there are approximately four 65,000 dalton chains and one hundred 5,000 dalton chains per circle and fifty 5,000 dalton chains per arm. The third component of the EDTA treated preparation is partially included on the column. It appears as linear fibrils in the electron microscope and contains polydisperse polysaccharides of several hundred thousand daltons. It may be an impurity since there is apparently less than one of the large polysaccharide chains per sunburst. Supported by a grant from the NSF.

044 GLYCOSYLATION OF VSV GLYCOPROTEIN IS SIMILAR IN CYSTIC FIBORSIS, HETEROZYGOUS CARRIER AND NORMAL HUMAN FIBROBLASTS, Lawrence A. Hunt & Donald F. Summers, Dept. Micro. University of Utah, S.L.C., Ut. 84132

The single envelope glycoprotein of vesicular stomatitis virus (VSV) can be used as a specific probe of glycosyl-transferase activities in tissue culture cells because:(1) the oligosaccharide side chains contain sugars normally found in plasma-like glycoprotein, and (2) cell- specific transferases are responsible for glycosylation of the virus-specified polypeptide.

VSV was grown in the presence of radioactive sugar presursors in fibroblasts from two cystic fibrosis patients, an obligate heterozygous carrier and a normal human. Gel filtration of pronase glycopeptides from both purified virions and cell-associated virus glycoprotein which had been labeled with (^{3}H) glucosamine did not reveal any significant differences in the glycosylation patterns between the different cell cultures. All four cell lines were apparently able to synthesize the mannose- and glucosamine-containing core structure and branch chains terminating in sialic acid which are characteristic of asparagine-linked carbohydrate side chains in cellular glycoproteins.

In addition, analysis of tryptic glycopeptides labeled with (^{3}H) glucosamine by ion-exchange chromatography indicated that the same two major sites on the virus polypeptides were recognized and glycosylated in all four virus-infected cell cultures. These studies suggest that the basic biochemical defect(s) in cystic fibrosis is not an absence or deficiency in enzymes responsible for the biosynthesis of complex carbohydrate side chains.

045 STUDIES ON THE CARBOHYDRATE MOIETIES OF THE H-2D GLYCOPROTEIN, W. A. Janeczek and R. D. Poretz, Rutgers University, New Brunswick, NJ 08903

The gene products of the D region of the H-2 complex specify serologically defined cell surface glycoproteins considered the major histocompatibility antigens of mouse. To explore the relationship of the structure of the carbohydrate moiety and serotype of the H-2D gene product we have examined the interaction of carbohydrate specific lectins with the intact cell bound antigen. Employing monospecific anti H-2.4 serum which we presume to be directed against the polypeptide portion of the antigen and various *N*-acetyl-D-galactosamine and D-galactose binding lectins from *S. japonica, M. pomifera,* and *W. floribunda* and the D-mannose binding lectin, concanavalin A, we have studied the competitive binding activities of these carbohydrate reagents and anti H-2.4 immunoglobulin in leucoagglutination, cytotoxicity and complement binding assays, and cellular binding of fluorescent and radiolabeled proteins. In addition we have examined the effect of these reagents on the induction of lymphocyte receptor mobility. Correlations from these techniques indicate that although 50% or more of the bound lectin reacts with structures distinct from the H-2 glycoproteins, the lymphocyte bound H-2D gene products in a single animal display microheterogeneity in regard to the structure of the carbohydrate moieties. This work was supported in part by grants from USPHS (CA 20889 and CA 17193).

046 ISOLATION AND PARTIAL CHARACTERIZATION OF THE INFECTIOUS MONONUCLEOSIS HETEROPHILE ANTIGEN FROM BOVINE ERYTHROCYTES, J.M. Merrick, R. Schifferle, K. Zadarlik and F. Milgrom, Dept. of Microbiology, Sch. of Medicine, SUNY at Buffalo, Buffalo, New York 14214

The infectious mononucleosis heterophile antigen (Paul-Bunnell antigen, PBA) was isolated by extraction of an aqueous suspension of bovine erythrocyte stromata with chloroform-methanol (C-M), 2:1 (v/v). The upper aqueous layer contained gangliosides, PBA and a high molecular weight glycoprotein (H-GP). PBA and gangliosides were separated from H-GP by extraction of lyophilized upper layer with C-M solvents. Separation of PBA from gangliosides was carried out by chromatography on DEAE-cellulose with C-M solvents. Purified PBA appeared to be a minor glycoprotein component of the erythrocyte membrane with unusual lipophilic properties, and was soluble in either organic or aqueous solvents. On SDS-polyacrylamide gel electrophoresis, it migrated as a single component that stained for protein with Coomassie blue, for carbohydrate with periodic acid Schiff reagent and for lipid with oil red O; it had an apparent molecular weight of 26,000. It was composed of 62% protein with major amino acids: glutamic acid, proline, glycine, isoleucine and leucine (152, 114, 95, 88, and 85 residues per 1000 residues respectively). Carbohydrate content was 9.5% with major sugar constituents: sialic acid, galactosamine and galactose. Serologic activity of PBA was destroyed by pronase but not by trypsin. (Supported by American Cancer Society Grant #IM24B).

047 COOPERATIVE BINDING PROPERTIES OF ^{125}I-WGA TO PARENTAL AND WGA-RESISTANT CHO CELLS, Pamela Stanley and Jeremy P. Carver, University of Toronto, Toronto, Canada M5S 1A8

Binding of ^{125}I-WGA (~ 20 μCi/μg) to CHO cells has been studied over a wide range of WGA concentrations (1 ng/ml to 1 mg/ml). Scatchard analysis gave rise to a curve with an initial region of positive cooperativity followed by a region which may correspond to WGA receptors possessing negative cooperativity or to the presence of numerous WGA-binding sites of decreasing affinity. The shape of this complex binding curve was not significantly altered by conditions which have been shown to affect agglutination and capping phenomena. Also, enriching the cell population to 50% mitotic cells had no effect on the binding curve. CHO cell lines resistant to the cytotoxicity of WGA were compared for their WGA-binding abilities under the above conditions. The three mutants ($Wga^{R}I$, $Wga^{R}II$ and $Wga^{R}III$) differ in their resistance to WGA (29-, 15-, 4.6-fold more resistant than parental CHO cells, respectively) and belong to different complementation groups. $Wga^{R}I$, previously shown to lack a specific glycosyltransferase activity which leads to the loss of a large proportion of surface carbohydrate, exhibited a marked decrease in WGA binding specific to the negatively cooperative region of the curve. $Wga^{R}II$ exhibited a decrease in the extent of positively cooperative binding of WGA as well as reduced binding in the negatively cooperative region of the curve. In contrast, $Wga^{R}III$ exhibited no significant alteration in WGA-binding ability. The binding characteristics of these mutants clearly demonstrate the heterogeneity in structure and function of WGA receptors on the CHO cell surface. Furthermore, they provide a means of correlating subclasses of receptor sites with specific biological phenomena.

048 MONOCLONAL ANTIBODIES DIRECTED AGAINST HUMAN CELL SURFACE ANTIGENS, Lois A. Lampson and Ronald Levy, Stanford University School of Medicine, Stanford, CA 94305

Antisera are widely used as reagents in characterizing and purifying cell surface determinants; this usefulness is ultimately limited by the heterogeneity of the antibody population in whole antiserum. Here we describe a method for obtaining monoclonal antibodies directed against human cell surface antigens. In an adaptation of the Klinman fragment culture system, "donor" mice are primed and boosted with human cells (HC); two weeks following the boost, donor spleen cells are injected, along with a stimulating dose of HC, into irradiated recipient mice; within one day, the recipient spleens are cut into mm^3 fragments, the fragments are cultured individually in vitro, and the supernatant fluids are screened for antibody activity against the immunizing HC. The dose of donor spleen cells is chosen so that each responding fragment contains the clonal progeny of at most one responding B cell. The greatly restricted heterogeneity of the antibody produced by each responding fragment is demonstrated by a restricted pattern of mobility in isoelectric focusing and a restricted pattern of cross-reactivity with a panel of HC related to the immunizing HC. In different experiments, we have obtained series of antibodies which (without further purification or absorption) discriminate between normal peripheral blood lymphocytes (PBL) from different individuals, between chronic lymphocytic leukemia (CLL) cells from different individuals, and between T and B cell lines from the same individual. (Supported in part by NIH Grant No. 1F32 AI 02530)

049 GLYCOSYLATION OF THE GLYCOPROTEIN OF A THERMOLABILE MUTANT OF VESICULAR STOMATITIS VIRUS
J. S. Robertson & D.F. Summers, Dept. Micro., U. of Utah, S.L.C., Ut. 84132

The single glycoprotein of Vesicular Stomatitis Virus contains two major oligosaccharide side chains, the structures of which are similar to those of serum glycoproteins. TL-17 is a thermolabile mutant of VSV which was selected for resistance to anti-VSV serum. The virus envelope glyprotein is the antigen which gives rise to and reacts with neutralizing antibody. To determine the effect of the mutation on glycosylation of the glycoprotein, both sugar and amino acid labeled tryptic digests of TL-17 and wt virus glycoprotein were analyzed.

By ion-exchange and gel filtration chromatography of ^{3}H-glucosamine labeled tryptic and pronase glycopeptides, we found that glycosylation of TL-17 and wt glycoprotein was virtually identical. The amino acid sequence of TL-17 was examined by ion-exchange chromatography of tryptic digests of ^{3}H-lysine or ^{3}H-arginine labeled glycoprotein. Comparison with the peptide map of wt glycoprotein indicated extensive differences in the amino acid sequence of the polypeptide portion of TL-17 and wt glycoprotein.

This alteration in the primary structure of TL-17 glycoprotein has undoubtedly caused drastic changes in the tertiary structure of the glycoprotein as evidenced by its altered antigenicity and thermal instability. Despite these changes the glycosylation of the polypeptide is identical to that of the wt glycoprotein indicating that the initial glycosylating enzyme must recognize a short amino acid sequence or small configurational feature of the polypeptide backbone. This result also indicates that the antigenicity of the VSV glycoprotein resides in the polypeptide moiety and not in the oligosaccharide side chains.

050 OLIGOSACCHARIDE STRUCTURE OF THE MEMBRANE GLYCOPROTEIN OF VESICULAR STOMATITIS VIRUS
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The oligosaccharide moieties of the membrane glycoprotein of vesicular stomatitis virus (VSV) have been analyzed by sequential chemical and enzymatic degradation of the peptidyloligosaccharides obtained after Pronase digestion of the purified glycoprotein. The data indicate that the two major oligosaccharide moieties of the glycoprotein have similar structures. These structures are acidic oligosaccharides, each having two or three branches terminating in the sequence sialic acid-galactose-N-acetylglucosamine at the non-reducing end of the oligosaccharide chain. These branch structures are attached to a tetramannosyl-di-N-acetylchitobiose core structure. Periodate oxidation studies indicate that the sialic acid is linked to galactose with α-2,3 bonds; the galactose to N-acetylglucosamine with β-1,3 or 4 bonds; and the N-acetylglucosamine to mannose with β-1,3 bonds.

The amino acid and carbohydrate constituents were analyzed by gas-liquid chromatography. The weight-average molecular weight of the peptidyloligosaccharides was estimated to be 3450 by molar composition of the amino acid and sugar residues. The number-average molecular weight was estimated to be 3150 by gel filtration analysis.

Growth of VSV in a mutant cell line lacking an N-acetylglucosaminyltransferase activity results in the production of virus with the membrane glycoprotein lacking the terminal branch structures in the oligosaccharide chains. The progeny virus was fully infectious and normal yields were obtained. This result indicates that the glycosylation steps involved in the synthesis of the oligosaccharide terminal branch structures are not obligatory steps for the maturation of membrane glycoproteins.

051 CHARACTERIZATION OF THE Fc RECEPTORS OF THE MURINE LEUKEMIA L1210,
Sheldon M. Cooper and Yugalkishore Sambray, University of Southern California School of Medicine, Los Angeles, California, 90033.

A glycoprotein (GP) extract prepared from the plasma membranes of L1210 cells was passed over Sepharose 4B columns to which either aggregated intact IgG or $F(ab')_2$ fragments had been coupled. The IgG column bound 35.7% of the applied cpm, whereas the $F(ab')_2$ column bound 2.8%. Bound GP's were eluted with citrate buffer and analyzed by SDS-PAGE. Three components with molecular weights of 65,000, 45,000 and 28,000 daltons were purified by electroelution from polyacrylamide gels. Reduction with 2-mercaptoethanol did not change the molecular weights of the three components. Both the cells and the isolated GP's had the same affinity for mouse myeloma proteins of different subclasses, suggesting that these molecules are related to L1210's surface Fc receptors. Amino acid analyses of the two heavier GP's revealed marked similarity. The isolation of three Fc binding GP's from L1210 suggests that there may be molecular heterogeneity of Fc receptors from a single cell line. Since we have previously demonstrated that a 45,000 dalton GP is selectively released from L1210 during Fc receptor redistribution, another explanation is that the smaller components may be specific cleavage products of a larger molecule.

052 PROTEINS CONTAINING REDUCTIVELY AMINATED DISACCHARIDES. CHEMICAL AND IMMUNOCHEMICAL CHARACTERIZATION,
Gary R. Gray, Barbara A. Schwartz, and Barbara J. Kamicker, Department of Chemistry, University of Minnesota, Minneapolis, MN 55455

Synthetic glycoproteins can be prepared by reductive amination of protein and reducing disaccharide in the presence of sodium cyanoborohydride. The reaction proceeds readily in aqueous solution over a broad pH range to give high degrees of substitution. The degree of substitution can be determined by amino acid analysis, as the secondary amine linkage formed by reductive amination to the ε-amino groups of lysine is stable to acid-catalyzed protein hydrolysis conditions.

Bovine serum albumin conjugates containing reductively aminated maltose, cellobiose, and lactose are effective antigens in rabbits. In all three cases, antibodies cross-reacting with both hapten and carrier protein are formed. Hapten-specific antibodies were predominant in the immune sera of the lactose and cellobiose conjugates, but were not formed to a very great extent against the maltose conjugate. The specificities of antibodies formed to the cellobiose conjugate were established by examining the effect of various synthetic inhibitors on the agglutination reaction. These studies demonstrate that antibodies to the hapten recognize the terminal ß-glucopyranosyl residue, the *acyclic* reduced glucose residue, and the 2^{o} amine linkage. These studies demonstrate the importance of the reduced carbohydrate arm in the specificity of antibodies formed to disaccharide-BSA conjugates, in agreement with the results of other coupling methods. The cyanoborohydride procedure should, for larger oligosaccharides, however, provide a convenient method for obtaining hapten-specific antibodies useful for the detection of cell surface carbohydrate determinants.

053 CARBOHYDRATE STRUCTURE OF VESICULAR STOMATITIS VIRUS GLYCOPROTEIN. Christopher L. Reading, Edward E. Penhoet and Clinton E. Ballou, Department of Biochemistry, University of California, Berkeley, CA 94720.

A spinner culture of baby hamster kidney (BHK) cells has been derived from the BHK_{21} line that permits the large scale production of vesicular stomatitis virus (VSV). The viral membrane glycoprotein has been isolated in good yield with high purity. The monosaccharide composition has been determined by gas chromatography of trimethylsilyl ethers after methanolysis, and the structure of the oligosaccharides of the glycoprotein investigated using hydrazinolysis, nitrous acid deamination, serial periodate oxidation and methylation analysis. There are two oligosaccharide chains per glycoprotein molecule, each containing three residues each of sialic acid (NeuNAc), galactose (Gal), mannose (Man), one residue of fucose (Fuc), and five residues of N-acetylglucosamine (GNAc). The terminal sequence $NeuNAc\rightarrow^{3}Gal\rightarrow^{4}GNAc\rightarrow$ is identical with that of fetuin, which has been shown to inhibit adsorption of VSV to BHK cells [Schloemer and Wagner (1975) J. Virol. 15:882-893]. The core, to which the terminal trisaccharides are linked, contains a branched mannose structure linked to di-N-acetylchitobiose, similar to several of the serum type glycoproteins. In addition, it contains a fucose residue linked to N-acetylglucosamine as follows: $Man\rightarrow^{6}Man\rightarrow^{4}GNAc\rightarrow^{4}GNAc\rightarrow$.

$\uparrow^{3}$ Man $\uparrow^{6}$ Fuc

054 STRUCTURE OF SENDAI VIRUS FUSION FACTOR. Mary-Jane Gething and Michael D. Waterfield. Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, England.

The Fusion Factor (F) and Haemagglutinin-neuraminidase (HN) glycoproteins of the Sendai virus envelope can be separated using affinity chromatography on lentil-Sepharose utilizing the differential binding of the glycoproteins to lentil in the presence of the detergents Empigen BB and sodium deoxycholate. Final purification of F by molecular sieving chromatography yields pure protein for protein chemistry and reconstitution studies.

The active Fusion Factor (F) consists of two subunits F_1 and F_2 linked by disulphide bonds. These subunits arise from in vivo proteolytic cleavage of an inactive precursor F_0. F_1 and F_2 have been separated by chromatography on Sephadex G-100 after complete reduction and alkylation of the sulphydryl groups. Sequence studies suggest that the subunits are made biosynthetically as $NH_2\text{-}F_2\text{-}F_1\text{-}COOH$. The N-terminal sequence of F_1 is extremely hydrophobic and shows a high degree of similarity to that of the HA_2 subunit of Influenza virus. This similarity in sequence and hydrophobicity of the polypeptide chain following the cleavage activation sites in Sendai and Influenza suggests that this region of the protein may have a role in the interaction of the virus with the cell membrane.

055 KINETICS OF RELEASE OF GLYCOPROTEINS FROM THE TA3-Ha MURINE ADENOCARCINOMA, Douglas K. Miller & Amiel G. Cooper, Dept. Path., Tufts School Medicine, Boston, MA.

A high molecular weight glycoprotein, termed epiglycanin, found on the surface of the TA3-Ha murine adenocarcinoma has been implicated in the allogeneic transplantability of the cell by surface masking of H-2 histocompatibility antigens or, after shedding into the ascites fluid and serum, by blocking cytotoxic lymphocytes. We have studied in vitro the synthesis and release of glycoproteins by the TA3-Ha cell. Epiglycanin release can be measured by the presence in the medium of radioactive glucosamine labeled glycoproteins and of material inhibiting a lectin from Vicia graminea seeds, an inhibition in this system specific for epiglycanin. Aliquots of medium from cells labeled for 30 min with radioactive glucosamine are taken at various chase periods, treated with perchloric acid (PCA) to precipitate proteins, and dialyzed. The kinetics of release of the pulse-labeled glycoproteins show an overall biphasic pattern with half-lives of about 45 min and 70 hr with >90% being the slowly released component. When the aliquots of PCA-treated media are chromatographed over Sepharose 4B columns, 3 major peaks are found; the second, Peak B, contains most of the epiglycanin. The TA3-Ha subline releases epiglycanin at the rate of 8-10% of the amount found on the cell surface per day. The release rates for the 3 peaks vary; Peaks A & C have $t_{\frac{1}{2}}$ <1 hr, while Peak B has a $t_{\frac{1}{2}}$ of several days. When surface membrane glycoproteins are labeled externally by galactose oxidase and $^{3}H\text{-}NaBH_4$, the subsequent cell growth medium contains primarily Peak B. The rate of appearance on the cell surface of labeled epiglycanin, which is obtained by trypsin cleavage of intact cells has a $t_{\frac{1}{2}} \approx 45$ min. Most of the released epiglycanin probably comes from the cell surface, not directly from internal pools.

056 PLATELET PLASMA MEMBRANE DISULFIDES IN NORMAL AND GENETICALLY ABNORMAL PLATELETS. David R. Phillips, Dept. of Biochemistry, St. Jude Children's Research Hospital, Memphis, Tennessee 38101

The surface of the platelet plasma membrane is the site of a number of activities such as adhesion, stimulation, aggregation and clot promotion. One approach to determine what specialized structures on the membrane surface perform each of these activities is to compare the surface of normal platelets to genetically abnormal platelets where specific activities have been lost. In this study, we have used the lactoperoxidase-iodination technique to examine the platelets from patients with Glanzmann's thrombasthenia (absent aggregation) and Bernard-Soulier syndrome (absent adhesion) and have compared them to normal platelets. Labeled platelets were solubilized in SDS and electrophoresed on nonreduced-reduced two dimensional gels. Ten glycoproteins were identified on normal platelets. Three of these were shown to be attached to nonidentical subunits by disulfide linkages. Four other glycoproteins gave evidence for intramolecular disulfide bonds as well. Thus, contrary to the generalization that disulfide crosslinking is unimportant in membrane structure, many of the platelet membrane polypeptides contain disulfides as an integral part of their structure. Analysis of the glycoprotein composition of the platelets from the thrombasthenic individuals showed that this disease is characterized by an absence of two of the membrane glycoproteins, IIb and III. In contrast, Bernard-Soulier platelets had a decreased concentration of all glycoproteins, with the greatest decrease in glycoprotein Ib. The data demonstrate that both genetic abnormalities are caused by different defects in the platelet membrane glycoproteins. Supported by HL 15616 and HL 0080 (Career Development).

057 *A DISACCHARIDE ANTIGEN COMMON TO THE GROUP C STREPTOCOCCAL CARBOHYDRATE AND THE* FORSSMAN GLYCOLIPID. J.E. Coligan, B.A. Fraser and T.J. Kindt. The Rockefeller University, New York, N.Y. 10021.

A disaccharide hapten, isolated from the streptococcal Group C carbohydrate, was shown by chemical and immunochemical means to have identity with the immunodominant carbohydrate antigen of the Forssman glycolipid. This disaccharide was assigned the structure 3-O-α-N-acetylgalactosaminyl-N-acetylgalactosamine on the basis of chemical and physical data. The disaccharide was able to inhibit binding between the Group C carbohydrate and most antibodies directed against the Group C streptococci, indicating that this is an immunodominant element of the C carbohydrate. The ability of the disaccharide to inhibit these reactions was dependent upon the presence of the N-acetyl groups on the amino sugar units.

Group C carbohydrate consists of a linear rhamnose backbone with alternate 1,2 and 1,3 linkages. The amino sugar disaccharide is 1,3-linked to alternate rhamnose residues. Crossreactivity between the Group C carbohydrate and the Forssman antigen was predicted on the basis of this common di-N-acetylgalactosamine structural unit, and was demonstrated by the ability of the Forssman glycosphingolipid to inhibit Group C carbohydrate binding reactions with approximately the same efficiency as the disaccharide isolated from the Group C *carbohydrate. It was further demonstrated that antisera to the Group C carbohydrate would* agglutinate sheep red blood cells and this agglutination could be inhibited by the Forssman antigen.

058 **GLYCOSAMINOGLYCANS OF PLASMA MEMBRANES OF SEPARATED RENAL TUBULES AND LIVER OF THE GUINEA PIG.** **Dora Lis and Benito Monis. Instituto de Biología Celular. Universidad Nacional de Córdoba. Casilla Postal 362. 5000 Córdoba. Argentina.**

Cytochemical and cell electrophoretic data indicated that renal tubule and hepatoma cell surfaces contained glycosaminoglycans (Histochemistry 40:241,1974; Exp Cell Res 67:142, 1971). Plasma membranes were subjected to papain and RNAse digestions, lipid extraction, purification on Sephadex G-50 and separation on DEAE-Sephadex A-25 (Table). Fractions thus obtained were identified by digestion with leech hyaluronidase and chondroitinases AC and ABC, chromatography, and electrophoresis on cellulose acetate.

Plasma Membrane	Uronic acids	Hexosamines	Sulfates	DEAE-Sephadex fractions (% uronic acids)					
	(μg/100 mg D.W.)			0.1	0.5	1.0	1.5	2.0	2.5 M ClNa
Kidney	97.18	261.24	31.00	0	53.6	7.6	12.8	10.6	15.3
Liver	72.86	224.47	12.35	0	37.6	15.1	22.1	20.2	5.0

We conclude that plasma membranes of renal and liver cells of guinea pig contain hyaluronic acid, dermatan, chondroitin (4 and 6) and heparan sulfates. Sulfated glycosaminoglycans predominate in liver plasma membrane, whereas kidney plasma membrane contains similar amounts of both, sulfated and non-sulfated glycosaminoglycans.
This work was supported by CONICET (ARGENTINA).

059 Cell Surface Glycoproteins of Human Ovarian Tumor Cells, Edward A. Z. Johnson, M.S. Piver, J.J. Barlow, M. Gamarra, Howard J. Allen, Roswell Park Memorial Institute, Buffalo, New York 14263

Human ovarian tumor cells were isolated from patient effusions. The tumor cell preparations were incubated in vitro with [^{3}H] and [^{14}C] L-fucose and/or glucosamine. The culture media from the tumor cell incubates were subjected to gel filtration on columns of Sephadex G-200. Most of the radiolabel appeared in the excluded fraction. Using columns of Sepharose 6B, the latter, high molecular weight fractions were resolved into two fractions, an excluded fraction and a retarded fraction. The radioactivity profile of the retarded fraction, being somewhat displaced from its protein profile, indicated that the population of radioactive glycoproteins were different from the bulk proteins of the culture medium.

The excluded fractions from Sephadex gel filtration were subjected to affinity chromatography on Concanavalin A - Sepharose 2B. Quantitative differences in the radioactivity bound by the affinity columns, for both fucose and glucosamine radiolabeled glycoproteins, were observed for the various cell types.

When tumor cell surface glycoproteins were labeled by the galactose oxidase-borotritide method, the radiolabeled glycoproteins elaborated into the culture medium co-chromatographed with the metabolically radiolabeled glycoproteins.

Using the in vitro radiolabeled tumor cell surface glycoproteins as assay markers, the isolation and characterization of cell surface glycoproteins present in ovarian cancer patient effusions are in progress.

060 MECHANISM OF ACTIVATION OF PLASMA MEMBRANE Mg^{2+}-ATPase BY PLANT LECTINS John R. Riordan and Maria Slavik, Research Institute, Hospital for Sick Children, Toronto, Canada.

The Mg^{2+}-ATPase activity of liver plasma membranes decreases markedly with increasing temperature above 30°C. This negative temperature dependency is counteracted by the binding of wheat germ agglutinin, concanavalin A or *Ricinus communis* agglutinin (at concentrations $\geq$ 0.5 mg/ml) to membranes prior to assay of the enzyme. With one of these lectins bound, the enzyme has a single energy of activation between 20° and 45°C. The binding of dimeric succinyl concanavalin A, soybean agglutinin, fucose-binding lectin from *Lotus tetragonolobus*, or the leucoagglutinin from *Phaseolus vulgaris* does not alter the temperature dependency of the enzyme. The latter two lectins, however, do prevent the concanavalin A-induced activation of the enzyme at 37°. The enzyme is not inhibited by any of the lectins tested over a wide range of concentrations.

Cytochalasin B and colchicine separately or in combination do not influence the lectin-induced enhancement of enzyme activity. Chlorpromazine and vinblastine sulfate each partially prevent the activation and in combination do so completely. Treatment of the membranes with the detergent Lubrol-PX or phospholipase A prevents activation of the enzyme by concanavalin A. The results are consistent with a restriction by the lectin of an environment which is normally too fluid for maximal enzyme activity above 30°C.

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005 COMPARATIVE BIOCHEMISTRY OF NUCLEOTIDE-LINKED SUGARS

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Nucleotide-linked sugars have two general biochemical functions: they are (i) intermediates in the formation of monosaccharides found in complex carbohydrates and (ii) glycosyl donors of these monosaccharides (1, 2). Few sugars arise by reactions not involving nucleotide-linked intermediates. Of these few, glucose, mannose, and N-acetylglucosamine are important in that they are transformed after attachment to nucleotides into most other monosaccharides. Three nucleotides, GDP, UDP, and CMP,are utilized in the formation of the complex carbohydrates of animals, and a given sugar is associated with only one of them. For example, L-fucose is only carried by GDP, and sialic acid is only carried by CMP. Plants and bacteria, in contrast to animals, use ADP, CDP, and dTDP i addition to GDP, UDP, and CMP, and the same sugar can be carried by different nucleotides. Glucose, for instance, is linked to CP, GDP, ADP, CDP, and dTDP, and there are enzymes specific for each derivative. What factor governs the choice of a particular nucleotide carrier for a given reaction is not apparent, but the use of different nucleotides separates pathways of synthesis and offers a means for their independent control by creating reactions unique to the synthesis of certain products and therefore suitable for regulation. For example, ADP-glucose is the glycosyl donor for glycogen synthesis in bacteria, and the synthesis of glycogen is regulated by activation and inhibition of ADP-glucose pyrophosphorylase by various metabolites, implying not only that the pyrophosphorylase reaction is rate-limiting, but also that the ADP-glucose produced is used only for the synthesis of glycogen and is neither an intermediate in the synthesis of other sugars, nor a glycosyl donor for the synthesis of other complex carbohydrates (UDP-glucose is the glycosyl donor for glycogen synthesis in animals, and possibly because UDP-glucose has other functions, control of glycogen synthesis in animals is at the level of glycogen synthetase). Similarly, CDP-glucose formed by CDP-glucose pyrophosphorylase in bacteria is only used to produce deoxysugars, and the activity of the enzyme is regulated by cellular levels of CDP-deoxysugars. Carrying sugars on different nucleotides may also be advantageous by increasing the accuracy of synthesis of complex carbohydrates. For example, a transferase responsible for the transfer of fucose from GDP-fucose is less likely to transfer galactose from UDP-galactose by mistake than galactose from GDP-galactose. The role of nucleotides in the synthesis of complex carbohydrates thus appears related to the specificity of enzymes that catalyze the modification and transfer of nucleotide-linked sugars.

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006 INVOLVEMENT OF SACCHARIDE-LIPIDS IN GLYCOSYLATION OF MEMBRANE-BOUND AND SECRETORY GLYCOPROTEINS

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Initial studies on the participation of polyisoprenol-linked sugars in synthesis of hen oviduct membrane glycoproteins documented the synthesis of mannosylphosphoryldolichol (1), and its involvement in synthesis of an oligosaccharide-lipid (2) whose carbohydrate chain was shown to have the structure $(\alpha\text{-Man})_{4\text{-}6}\text{-}\beta\text{-Man-}\beta\text{-GlcNAc-GlcNAc}$ (3). Experiments with the isolated oligosaccharide-lipid demonstrated that the oligosaccharide chain could be transferred en bloc to endogenous membrane protein acceptors (2,4). None of the three endogenous glycosylated membrane proteins was identical to the major secretory glycoprotein of oviduct, ovalbumin. However, by utilizing oviduct tissue slices and tunicamycin, strong evidence has been obtained that polyprenol-linked saccharides are involved in glycosylation of ovalbumin (5).

Recently, we have examined in more detail the early steps involved in assembly of the oligosaccharide-lipid. The formation of GlcNAc-, GlcNAc-GlcNAc-, β-Man-β-GlcNAc-GlcNAc-lipids has been established (6). Moreover, it has been shown that both the disaccharide- and trisaccharide-lipid can either be elongated to form oligosaccharide-lipid, or can be directly transferred to endogenous membrane protein acceptors.

A major limitation of earlier studies was that the only polypeptide acceptors were endogenous membrane proteins. However, it is now known that several exogenous, soluble proteins can, after denaturation and disulfide bond cleavage, serve as acceptors (7). The minimal requirement for a protein to serve as an acceptor of an oligosaccharide chain from oligosaccharide-lipid is that it contain the tripeptide sequence -ASN-X-SER(THR)-. Current studies on the potential acceptor activity of proteolytic fragments of proteins containing this tripeptide sequence may cast further light on the specificity of glycosylation. All of these findings will be discussed in the context of the hypothesis that there is a common mechanism for glycosylation of membrane and secretory glycoproteins.

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007 INITIAL ATTACHMENT OF CARBOHYDRATE TO SINDBIS VIRUS GLYCOPROTEINS, Bart Sefton, Tumor Virology Laboratory, The Salk Institute, Post Office Box 1809, San Diego, California 92112

Studies have been carried out on the initial addition of carbohydrate to the two Sindbis virus glycoproteins during growth of the virus in chick embryo cells. It appears that both types of viral oligosaccharides (A-type and B-type) are assembled by way of the transfer of a large, 1,800 dalton, oligosaccharide from a lipid molecule to the nascent polypeptide.

The fact that glycosylation begins prior to release of the polypeptide from the polysome follows from two observations. First, the intervals between incorporation of radioactive mannose and radioactive amino acids into viral protein and the subsequent appearance of label in mature viral glycoproteins are indistinguishable. Second, it was not possible to identify unglycosylated viral glycoproteins in infected cells, even if cells labeled for only 30 sec with radioactive amino acids were examined.

The fact that both types of viral oligosaccharides are assembled on the polypeptide by transfer of an oligosaccharide from lipid was shown by pulse-labeling of infected cells with radioactive mannose. Infected cells, labeled for 50 sec with ^{3}H-mannose, contain large amounts of radioactive, incompletely assembled, lipid-linked oligosaccharides but no radioactive, incompletely assembled, protein-bound oligosaccharides.

008 SYNTHESIS AND SEGREGATION OF SECRETORY PROTEINS: THE SIGNAL HYPOTHESIS, Günter Blobel, Dept. of Cell Biology, The Rockefeller University, New York, N.Y. 10021.

Membranes emerged in evolution as a means to compartmentalize and therefore to function as diffusion barriers for large as well as for certain small molecules. A variety of mechanisms has evolved to modify this restriction and to permit transfer of molecules across membranes. Secretory proteins belong to a specific group of proteins which is assembled by cytoplasmic ribosomes and is transferred across the microsomal membrane. A possible mechanism to achieve this has recently been proposed in the so-called signal hypothesis (1). It was postulated that all mRNA's for secretory proteins code for a unique sequence--the signal sequence--which constitutes a metabolically short-lived amino terminal extension, present only in nascent, uncompleted chains.

The signal sequence, emerging from a putative tunnel within the large ribosomal subunit, is thought to cause the association of several ribosome receptor proteins in the membrane so as to form a proteinaceous tunnel in the membrane. Coordinated binding of the large ribosomal subunit of the translating ribosome to the associated ribosome receptor proteins would link the tunnel in the large ribosomal subunit with the newly formed tunnel in the membrane, with the ribosome acting as a crosslinker with respect to the ribosome receptor proteins. The signal sequence is removed before the chain is completed by a membrane associated proteolytic activity--referred to as signalase. Following release of the nascent chain from the ribosome the latter is detached from the membrane. This displacement is probably mediated by a recently isolated detachment factor (2). Ribosome detachment would permit the ribosome receptor proteins to dissociate and to diffuse in the plane of the membrane eliminating the tunnel. Evidence accumulated in this laboratory (1, 2, 3, 4) in support of the signal hypothesis will be presented.

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009 IN VITRO SYNTHESIS AND PROCESSING OF A PUTATIVE PRECURSOR OF THE SMALL SUBUNIT OF RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE

Bernhard Dobberstein, Günter Blobel, and Nam-Hai Chua, Rockefeller University, New York, N.Y. 10021

Ribulose-1,5-bisphosphate carboxylase (EC.4.1.1.39) is a predominant protein of the chloroplast stroma. In C. reinhardtii, the holoenzyme weighs 550 Kdaltons and is composed of several copies each of two non-identical subunits (1). It has been shown that the large subunit (55 Kdaltons) is synthesized inside the chloroplast (2) whereas the small subunit (S) (16.5 Kdaltons) is translated on cytoplasmic ribosomes (3) and therefore has to be transported into the organelle for assembly. To investigate the in vitro synthesis of S, polyadenylated mRNA from C. reinhardtii was translated in a cell-free wheat germ system. Among the polypeptides synthesized was a 20-Kdalton species which was immunoprecipitated specifically by antibodies raised against S. Since the immunoprecipitated polypeptide is larger than authentic S by ~ 3,500 daltons we identified the former as a putative precursor (pS) of the latter. In contrast to mRNA translation, completion of nascent chains ("readout") on free polysomes isolated from C. reinhardtii yielded S instead of pS. pS could be cleaved by a specific endoprotease into two products: one apparently identical in size to S and a smaller fragment (F) which presumably represents the remainder of pS.The endoprotease is present in an algal post-ribosomal supernatant but is also found in association with polysomes. The latter observation could account for the failure to detect pS accumulation among the products of polysome readout. The endoprotease was inhibited by sulfhydryl reagents but unaffected by metal ion chelators or serine protease inhibitors. We propose that pS is an extrachloroplastic form of S and that the F sequence plays a role in its specific transfer into the chloroplast by a mechanism distinctly different from that of the "signal" sequence-mediated transfer of nascent presecretory chains across the microsomal membrane (4).

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010 GLYCOPROTEIN AND PROTEIN PRECURSORS TO PLASMA MEMBRANES IN VIRUS INFECTED AND UNINFECTED HeLa CELLS

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Animal viruses maturing at the plasma membrane are known to contain as part of their own membrane glycoprotein (G protein) and also, often, a nonglycosylated species (M protein). In uninfected HeLa cells, glycoproteins and nonglycosylated proteins do not assemble into membrane simultaneously (1) and in vesicular stomatitis virus (VSV) infected HeLa cells G and M proteins do not associate with the HeLa plasma membrane at the same rate after their synthesis and probably do so by different routes (2). M protein associates with little delay and similar observations for M-type proteins have been made for fowl plaque virus (3), Rauscher leukemia virus (4). Moreover, it has been concluded that influenza virus (5) and sendai virus (6) components must assemble into virions by different pathways. VSV M mRNA is associated mainly with the free polysomes while G mRNA is associated with the membrane bound polysomes (7,8). Short term labeling studies with [^{35}S]methionine (1 min pulse, 1 min chase) confirm that G protein is found on the membrane bound polysomes while M protein is found almost entirely in the supernatant to the free polysomes. Newly synthesized M protein is not yet assembled into plasma membrane in 1' pulse, 1' chase and thus must pass through an internal pool, with a subsequent transit time to the plasma membrane of not more than 2 minutes. G protein, at the time fucose is added, is nearly complete in the oligosaccharide moeity, and is located internally on a membranous fraction of density 1.13 gm/ml in sucrose. Its transit time from this pool (which in uninfected cells is between 1-2% of the total cell fucosyl glycoprotein (9)) is 15 minutes. Preliminary indications are that M and G protein are not on the same internal membranous component. Association of M protein with the plasma membranes may thus occur from a soluble cytoplasmic pool by direct adsorption (and see 2).

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011 BIOSYNTHESIS AND MATURATION OF VIRUS MEMBRANE GLYCOPROTEINS, Flora Katz, Dyann Wirth, and Harvey Lodish, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

The glycoprotein (G) of vesicular stomatitis virus (VSV) is synthesized in association with the rough endoplasmic reticulum (er). The association of the VSV G mRNA with the er membrane is apparently mediated by the nascent glycoprotein chain since treatment with puromycin, even in a solution of low ionic strength, will remove polysomes containing G mRNA from the membrane.

Newly made glycoprotein is bound to the er membrane. Digestion with proteases of er vesicles containing newly made G protein removes only about 30 amino acid residues from each G polypeptide. Treatment of vesicles with deoxycholate before protease digestion results in complete degradation of G. These results mean that most of each G protein is either facing the lumin of the er or is imbedded in the lipid bilayer; about 30 amino acid residues are facing the cytoplasmic face of the er. Thus G appears to be a transmembrane protein after its biosynthesis.

Sindbis virus differs from VSV in that a single mRNA – 26S – encodes the three viral structural proteins, one internal protein, core, and two membrane glycoproteins, E_1 and E_2. This mRNA has one initiation site; core, E_1 and E_2 are derived by proteolytic cleavage. We showed that during infection, the 26S RNA is found mainly in membrane bound polysomes which synthesize all three virion structural proteins. These polysomes are released from the membrane upon treatment with puromycin and high salt. Newly synthesized core protein is localized on the cytoplasmic side of endoplasmic reticulum membranes, while newly synthesized envelope proteins are sequestered by the lipid bilayer. These results suggest that the nascent glycoproteins, presumably their amino termini, are of major importance in directing the binding of polysomes containing 26S mRNA to endoplasmic reticulum membranes and in the subsequent transfer of glycoproteins into the bilayer. At least one Sindbis envelope glycoprotein also appears to be a transmembrane protein after its biosynthesis.

061 CHANGES IN GLYCOPROTEINS ASSOCIATED WITH VIRAL TRANSFORMATION AND DIFFERENTIATION, Keith Burridge, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724.

The glycoproteins of whole cells have been analyzed by direct application of radioiodinated lectins to SDS gels. The technique, which analyzes glycoproteins according to their apparent molecular weight and oligosaccharide specificity, has been used (1) to study the glycoproteins of cultured vertebrate cells following viral transformation, and (2) to study glycoproteins during the development of Dictyostelium discoideum. With higher cells a surprising number of bands have been found reactive with some lectins (e.g. ConA), but the specificity of this interaction has been confirmed by sugar inhibition studies. Marked differences are seen when the glycoproteins from a number of viral transformants are compared with the glycoproteins from normal parental cells. Certain differences, however, are also seen when the glycoproteins are compared from two separately derived SV40 transformants of 3T3 cells, suggesting a degree of clonal variation between these lines that may not relate to transformation.

When gels of D. discoideum are reacted with labelled lectins extensive glycoprotein changes are seen as successive developmental stages are analyzed. Many more alterations are revealed using this method than have previously been detected by techniques such as lactoperoxidase iodination. For example, with the wheat germ lectin a series of high molecular weight bands begin to be detected after about eight hours of development and these increase in intensity and number as development continues. On the other hand, ConA reveals a progressive loss of several glycoproteins as well as the appearance of some new major bands.

062 THE "ACID" AND "NEUTRAL" α-GLUCOSIDASES OF HUMAN LIVER, Allen K. Murray and Ronald R. Townsend, Div. of Developmental Disabilities and Clinical Genetics, Dept. of Pediatrics, University of California,Irvine, Irvine, CA 92717

The relationship between the two α-glucosidases is of interest since the lysosomal and microsomal β-glucuronidases are products of the same structural gene(Owens,et.al.,Arch. Biochem. Biophys. 166:258, 1975) while the lysosomal, Golgi and cytosolic α-mannosidases appear to be genetically distinct(Tulsiani, et.al., Fed. Proc. 35:1727, 1976). The acid (lysosomal) α-glucosidase from human liver has been purified 2400-fold to apparent homogeneity and its carbohydrate composition has been determined. The binding of this mannose containing glycoprotein by Concanavalin A has been investigated. The Con A binding of the acid α-glucosidase is completely prevented by D-mannose or α-methyl-D-glucoside. At half saturation 12.5 moles of Con A are bound per mole of enzyme. The binding is demonstrable both with the purified enzyme and crude homogenates. The neutral (soluble) α-glucosidase has been assayed in the presence of D-turanose which specifically inhibits the acid glucosidase. In contrast to the acid glucosidase, Con A does not bind the neutral glucosidase which suggests that its carbohydrate moiety may differ from that of the acid glucosidase, if the neutral glucosidase is in fact a glycoprotein. Further, antiserum to the acid glucosidase inhibits its activity, but in contrast has no effect on the activity of the neutral α-glucosidase. We are isolating the neutral α-glucosidase to further investigate the relationship between the two α-glucosidases.(Supported in part by a grant from the California Research and Medical Education Fund.)

063 PURIFICATION OF A FACTOR REGULATING MACROPHAGE PRODUCTION AND GROWTH, E. Richard Stanley, The Ontario Cancer Institute, Toronto, Ontario, Canada M4X 1K9

A 5-step purification procedure involving concentration, chromatography on DEAE-cellulose, Sephadex G-200, Concanavalin A-Sepharose and gradient gel electrophoresis has been developed for a factor (colony stimulating factor, CSF) produced by cultured mouse L cells. CSF stimulates granulocyte-macrophage production from single hemopoietic progenitor cells and shares identity with macrophage growth factor, a cell surface component of mouse L cells. It was purified from a higher molecular weight, concanavalin A-adherent fraction that represented approximately 70% of the total colony stimulating activity of serum-free mouse L cell conditioned medium. The purified material had a specific biological activity of 1.62×10^8 units/mg protein and stimulated maximum colony formation in the standard assay system at a concentration of 10^{-11}M. On both standard and sodium dodecyl sulphate polyacrylamide gel disc electrophoresis it gave a single band of protein that co-electrophoresed with a single band of biological activity. It behaved heterogeneously on isoelectricfocusing in polyacrylamide gels yielding several bands of protein in the pH range 3.7-4.9, each of which was associated with biological activity. The protein and biological activity of the purified material were shown to combine with components in antiserum raised against the penultimate purification stage at equivalent antiserum concentrations. The purified CSF is a glycoprotein with a molecular weight of approximately 70,000 daltons, composed of two polypeptide chains of molecular weight approximately 35,000 daltons covalently bound through disulphide bonds.

064 BIOSYNTHESIS OF (Na^+, K^+)-ADENOSINE TRIPHOSPHATASE. Lynn Churchill and Lowell E. Hokin, Dept. of Pharmacology, Univ. of Wis. Med. Sch., Madison, WI 53706

Biosynthetic studies of (Na^+, K^+)-adenosine triphosphatase ((Na^+, K^+)-ATPase) are now feasible in vitro as well as in vivo in the electric organ of the electric eel, Electrophorus electricus. The enzyme was labeled with L-(3,4-(n)-^{3}H)- or DL-(l-^{14}C)-valine in vivo by direct injection into the Main electric organ or in vitro by incubation of dissected rows of single Sachs organ cells. Isolation procedures developed for the Main electric organ (Dixon and Hokin, Arch. Biochem. Biophys. 163 (1974) 749) were modified for the Sachs electric organ and for removal of Lubrol. With the Sachs electric organ tissue, hyaluronidase treatment of the homogenate was necessary in order to obtain solid pellets by centrifugation. The removal of Lubrol was achieved either with Bio Beads SM-2 or with a Servacel AE column. When the purity is analyzed by polyacrylamide gel electrophoresis, the ammonium sulfate fraction isolated by the Servacel AE column matches the fraction isolated by the zonal rotor. Amino acid analysis of the trichloroacetic acid supernatants from radioactive homogenates reveals that valine is not metabolized in vivo or in vitro. The relative rate of incorporation of labeled amino acids in the glycoprotein and catalytic subunit of (Na^+, K^+)-ATPase has been calculated by determining their specific radioactivities after SDS gel electrophoresis of the ammonium sulfate fraction. The relative rate of incorporation is similar in vivo and in vitro. The glycoprotein incorporates 2-3 times more radioactive label per valine molecule than does the catalytic subunit within 24 hours. This data suggests that the glycoprotein turns over faster than the catalytic subunit of (Na^+, K^+)-ATPase, indicating that the catalytic subunit and the glycoprotein of this integral plasma membrane protein are synthesized independently.

065 POLYPRENYL-PHOSPHATE MANNOSYL TRANSFERASES OF THE EMBRYONIC CHICK RETINA, Edward L. Kean, Case Western Reserve University, Cleveland, Ohio 44106.

The retina of the embryonic chick contains mannosyl transferases which catalyse the transfer of mannose from GDP-(^{14}C)-mannose to polyprenyl-phosphate-mannose (Lipid I), oligosaccharide-phosphate-lipid (Lipid II) and to glycoprotein. While guanosine nucleotides inhibited the transfer to all three products, ATP and ADP stimulated the transfer to Lipid II and to glycoprotein, but inhibited the transfer to Lipid I. The effect on these activities by a variety of other nucleotides was also examined. Although $MnCl_2$ (3.3 mM) is stimulatory to these reactions, high concentrations (greater than 20 mM) inhibited the transfer of label to Lipid II and to glycoprotein, while leaving the transfer to Lipid I relatively unaffected. The retina of the embryonic chick displayed a high degree of specificity for dolichyl phosphate (DP) as an acceptor among a variety of polyprenyl phosphates which were tested. The anomeric configuration of Lipid I and of DP-mannose was beta as shown by chemical and enzymatic procedures. The endogenous product, Lipid I, accumulated in situ during incubations performed in the presence of high concentrations of $MnCl_2$, as well as exogenously added purified DP-(^{14}C)-mannose, served as substrates for the transfer of radioactivity to Lipid II and to glycoprotein. Lipid II-(^{14}C) also served as a substrate for the transfer of label to glycoprotein. Lipid I displayed the same chemical and chromatographic properties as DP-mannose. The technique of catalytic hydrogenolysis, when carried out on Lipid I and DP-mannose, was encumbered with artifact and extreme variability, depending on the reaction conditions employed, making it a less than acceptable procedure for distinguishing between polyprenyl derivatives having saturated or allylic terminal groups. (USPHS Grant #EY 00393).

066 DIFFERENTIAL EFFECT OF TUNICAMYCIN ON THE VIABILITY AND CELL SURFACE PROPERTIES OF NORMAL AND TRANSFORMED 3T3 CELLS. Dan Duksin and Paul Bornstein. Departments of Biochemistry and Medicine, University of Washington, Seattle, WA 98195

Confluent normal and virally transformed 3T3 cells were treated with tunicamycin (TM), an inhibitor of protein glycosylation. TM (1 µg/ml) caused detachment and death of SV40-3T3 and Py-3T3 cells within 24 hrs; these effects were not seen with 3T3 cells or with embryonic chick tendon fibroblasts. However, the proliferation of 3T3 cells was inhibited by TM and, after several days, there occurred a drastic change from an epithelioid to an abnormally elongated shape. Both inhibition of growth and the morphological changes were reversible. One explanation for these observations is that impaired secretion or the synthesis of carbohydrate-poor glycoproteins in the presence of TM may affect a number of cell surface properties, including cell attachment and shape. In support of this hypothesis, a marked (approx. 100 fold) decrease in Con-A agglutinability was observed in virally transformed 3T3 cells grown in the presence of TM. Agglutination by wheat germ and soybean agglutinins was unaffected. The agglutinability of trypsinized 3T3 cells by all three lectins was unchanged by TM. These results suggest that TM interferes with the insertion or function of a specific Con-A binding protein on the surface of cells. The more marked effect of TM on transformed 3T3 cells may result from reduced synthesis, more rapid turnover or improper function of cell surface glycoproteins. (Supported by NIH Grants AM 11248 and DE 02600)

067 ROLE OF THE GOLGI APPARATUS IN THE SYNTHESIS OF GLYCOPROTEINS AND GLYCOLIPIDS. Becca Fleischer, Dept. of Mol. Biol., Vanderbilt Univ., Nashville, Tenn. 37235

Golgi is the main locus in mammalian cells for a number of enzymes involved in glycoprotein and glycolipid synthesis. Galactosyl and sialyltransferases which add terminal sugars to glycoproteins are localized in Golgi in both liver and kidney. Sulfo and galactosyltransferases involved in the terminal steps of sulfatide and lactosylceramide synthesis are present in kidney but not in liver and are localized in the Golgi apparatus of kidney. A sialyltransferase which forms sialyllactosylceramide from lactosylceramide is localized in Golgi of both liver and kidney. The bulk of the glycoproteins formed in the Golgi of liver and kidney are destined for secretion, but glycolipids synthesized in the Golgi are membrane components and are transferred to other membranes of the cell. Glycoprotein galactosyltransferase and glycolipid sulfotransferase of the Golgi apparatus are membrane bound and are released by treatment with Triton X-100. We have determined sedimentation constants (GT = 3.16 s, SuT = 3.61s) and partial specific volumes (GT = 0.82, SuT = 0.84) of these enzymes by sedimentation of the protein-Triton X-100 complexes in sucrose gradients made in water or D_2O containing Triton X-100. The large partial specific volumes imply that a large amount of detergent is bound to the enzymes. The weight fraction (g/g complex) of Triton X-100 bound is 0.52 (GT) and 0.58 (SuT). Stokes' radii of the complexes are 48 Å (GT) and 42 Å (SuT) as determined by chromatography on Sepharose 6B in the presence of Triton X-100. The molecular weights of the complexes are 97,000 (GT) and 104,000 (SuT), and of the enzymes are 46,600 (GT) and 43,500 (SuT). Both GT and SuT are highly lipophilic and appear to be intrinsic membrane proteins. [Supported in part by USPHS NIH Grant AM 17223.]

068 THE METABOLIC FATE AND THE POSSIBLE FUNCTION OF A HIGH MOLECULAR WEIGHT CELL SURFACE GLYCOPROTEIN, F.Doljanski and Y.Plesser,Dept.Exp.Med.&Cancer Research,The Hebrew University-Hadassah Medical School,Jerusalem,Israel. We have previously shown that surface membrane components are continually shed into the culture medium as a consequence of normal membrane turnover. To further test this conclusion,the rate of loss from the cell surface and the rate of accumulation in the medium of labeled surface components were studied.Monolayer cultures of chick embryo cells were labeled with ^{125}I by the lactoperoxidase catalysed reaction or by exposing cells to ^{3}H-glucosamine for 20h. At different times after labeling,cells and medium were analysed by SDS-PAGE. Characteristic and reproducible radioactivity profiles were obtained. In agreement with earlier reports, a high molecular weight (~220,000) component (complex I) was extensively labeled.Co-chromatography of iodinated cells with medium from glucosamine labeled cultures showed that the two radioactivity profiles were practically identical. This indicates that these surface constituents are shed as intact molecules.Mild trypsin treatment releases glucosamine labeled complex I almost without degradation.The rate of loss of complex I from the cells (determined as the amount of radioactive complex I in the trypsinisate) corresponds to the rate of it's accumulation in the medium.Within 24h most of complex I initially present in the surface membrane is found in the medium.The fate of iodinated complex I could not be followed since the iodinated molecules were found to be unstable. The possible role of complex I in cell-cell and cell-substrate contacts will be discussed.

069 THE TRANSFER OF MANNOSE FROM GDP-MANNOSE TO ENDOGENOUS ACCEPTORS IN LACTATING BOVINE MAMMARY GLAND. Inder K. Vijay and Steven R. Fram. University of Maryland, College Park, Md. 20742.

A particulate fraction from the lactating bovine mammary gland contains mannosyltransferase which catalyzes the transfer of (^{14}C)mannose from GDP-(^{14}C)mannose to endogenous polymeric acceptor(s) insoluble in $CHCl_3$-CH_3OH (2:1), C/M, $CHCl_3$-CH_3OH-H_2O (10:10:3), C/M/W, and TCA. The kinetics of incorporation of (^{14}C)mannose into C/M, C/M/W and TCA insoluble products are consistent with the following sequence of reactions:

GDP-(^{14}C)mannose + Lipid ⇄ (^{14}C)mannosyl-lipid → (^{14}C)mannosyl-oligosaccharide-lipid → (^{14}C)mannosyl-"Acceptor(s)"

Synthesis of (^{14}C)mannosyl-lipid is freely reversible; in the presence of GDP the transfer of mannosyl groups from endogenously labeled mannosyl-lipid to GDP-mannose is obtained. The (^{14}C)mannosyl-lipid has been partially purified by preparative TLC and DEAE cellulose acetate chromatography. This compound appears to be (^{14}C)mannosyl phosphoryl dolichol by a number of criteria including co-chromatography with chemically synthesized β-mannosyl-phosphoryl dolichol. The identity of the (^{14}C)mannosyl-oligosaccharide-lipid and the (^{14}C)-mannosyl "Acceptor(s)" is currently under way. (Supported by the Maryland Agricultural Experiment Station and Grant PRO-7042-11 from USPHS.)

070 MANNOSYLPHOSPHATE TRANSFERASE IN *SACCHAROMYCES CEREVISIAE* MANNOPROTEIN BIOSYNTHESIS, Evelyn M. Karson, Dorothy Lun Ballou, and Clinton E. Ballou, Dept. of Biochemistry, University of California, Berkeley, CA 94720.

An *in vitro* assay for a mannosylphosphate transferase, involved in biosynthesis of cell wall mannan in *Saccharomyces cerevisiae*, has been designed utilizing NaB^3H_4-reduced α1→2-linked mannotetraose as exogenous acceptor. Transferase activity is dependent on the sugar donor GDP-mannose and Mn^{++} or Co^{++}. The labeled product formed in the reaction is a reduced mannosylphosphate diester tetrasaccharide analogous in structure to the natural product. The enzyme is localized in a washed membrane fraction obtained by differential centrifugation of an extract of mechanically broken, freshly grown yeast cells. Triton X-155 was most effective of the 16 nonionic detergents tested in stimulating and solubilizing the activity, whereas detergent-urea treatment of the particulate preparation destroyed mannosylphosphate transferase activity. The solubilized enzyme has been partially purified by ammonium sulfate precipitation and by ion exchange, gel filtration, and affinity chromatographies. Many haploid yeast mutant strains with phosphate-deficient mannans have the mannosylphosphate transferase activity but lack the endogenous acceptor to which the mannosylphosphate group is attached. Others show less than 20% of the normal activity and several exhibit a dominant phenotype; that is, transferase activity is absent in the heterozygous diploid which possesses one normal gene copy for the enzyme. Experiments are underway to distinguish between alternative explanations of this property. *Supported by U.S.P.H.S. Grant AI 12522 and N.S.F. Grant PCM74-18893.*

071 BIOSYNTHESIS OF LIPID-LINKED OLIGOSACCHARIDES IN AORTA. A. D. Elbein and A. Heifetz. Department of Biochemistry, The University of Texas Health Science Center, San Antonio, Texas, 78284.

A particulate enzyme fraction from pig aorta catalyzes the transfer of Man from mannosyl-phosphoryl-polyprenol (MPP) into lipid-linked oligosaccharides (LLO). The reaction required detergent (Triton X-100 or NP-40) but did not require metal ion. The oligosaccharides, released by mild acid hydrolysis of LLO, were similar in mobility to those formed from GDP-Man and ranged in size from a hepta- to a decasaccharide. The larger [^{14}C]-oligosaccharides gave rise to [^{14}C]-mannobiose and [^{14}C]-mannotriose upon acetolysis indicating the presence of 1 → 6 branches. All of the radioactive Man was released from the smaller oligosaccharides (i.e., those having 5, 6 or 7 sugars) by α-mannosidase, but only 50 or 60% could be released from the larger oligosaccharides (8 or 9 sugars) by this treatment. In the presence of EDTA and detergent, no MPP is formed from GDP-[^{14}C]-Man, but radioactivity is still incorporated into LLO. Essentially all of this radioactivity is found in the heptasaccharide indicating that GDP-Man is the direct donor for this oligosaccharide. Treatment of the particulate fraction with Triton X-100 led to the solubilization of a number of Man and GlcNAc transferases. The enzymes forming MPP and (GlcNAc)2-P-P-polyprenol, were partially purified on DEAE-cellulose. Both enzymes required the addition of acceptor lipid for activity. A lipid fraction isolated from pig liver with properties of dolichyl-P, as well as commercial dolichyl-P, was active with both Man transferase and GlcNAc transferase. The (GlcNAc)2-lipid acted as an acceptor of Man for the formation of Man-β-GlcNAc-GlcNAc-lipid by this solubilized enzyme. The mannosyl donor for this trisaccharide-lipid was GDP-Man and no activity was detected with MPP. Other mannosyl transferases involved in the formation of the larger oligosaccharides were also solubilized by detergent. (Supported by grant HL-17783 from the National Institutes of Health.)

072 FUCOSYL-GLYCOPROTEIN KINETICS IN HELA CELLS. Peter D. Yurchenco and Paul H. Atkinson. Albert Einstein College of Medicine, Bronx, New York, 10461.

The pool sizes, label equilibration times, and specific radioactivity relationships of fucosyl-glycoproteins and precursors have been studied in exponentially growing HeLa S_3 cells using a quantitative radioisotopic approach. 10^7 cells contain about 0.52 nmoles of GDP-fucose and about 5.3 nmoles of glycoprotein-fucose of which 96-98% resides in or on the plasma membrane; 2% or less is in a small internal pool(s). Fucose flowing from the nucleotide pool to internal pool is in part precursor to the plasma membrane; the remainder is released as soluble glycoprotein directly into growth medium without random mixing with the plasma membrane glycoprotein. Furthermore, nearly twice as much cell surface glycoprotein-fucose destined to reach plasma membrane is synthesized per generation time as is required for cell doubling; the remainder represents plasma membrane turnover 75 to 80% of which appears in growth medium as free fucose. 2% of the total glycoprotein-fucose could be in a degradative pathway being recycled internally before release as free fucose. In a particular culture in which 10^7 cells contained 4.4 nmoles glycoprotein-fucose, a total of 11.1 nmoles glycoprotein-fucose was synthesized per generation (23 hours); of this, 2.5 nmoles per generation was released diretly into the growth medium and 8.6 nmoles per generation flowed into the plasma membrane, of which 4.2 nmoles, after mixing with plasma membrane glycoprotein, ultimately was released into growth medium, mostly as free fucose. Studies indicate that the internal pool(s) can be isolated from plasma membrane ghosts by sucrose density gradient centrifugation at a specific gravity of 1.13 compared with plasma membrane which bands at 1.16.

073 DISTRIBUTION OF GLYCOCONJUGATES IN MOUSE FIBROBLASTS WITH VARYING DEGREES OF TUMORIGENICITY, David J. Winterbourne and Peter T. Mora, National Institutes of Health, National Cancer Institute, Bethesda, Md. 20014

Radiolabelled glycoconjugates produced by cell cultures have been analyzed by anion exchange chromatography after papain digestion. Whole cultures were divided into three fractions: the medium, containing secreted macromolecules, material released from the cell surface by trypsinization and the viable trypsinized cells. These three fractions all contained peaks tentatively identified as glycopeptides, hyaluronic acid, heparan sulphate and chondroitin sulphate. The majority of the hyaluronic acid and chondroitin sulphate was secreted whereas the glycopeptides were mostly found associated with the trypsinized cells. Very little of the heparan sulphate was found in the trypsinized cells, with most about equally distributed between the medium and the trypsin released cell surface material. Two clones obtained by unbiased selection of single cells from a mass cell line, derived from whole embryos of the inbred AL/N strain of mice, were found to have lower tumorigenicity than the parent cell line (10^6 and 10^7 cells compared to $<10^5$ cells). In all three fractions, these clones produced less radioactively labelled hyaluronic acid than the more tumorigenic parent cell line, when growing at very similar rates and densities. Sister subclones with and without SV40 transformation were also compared.

074 DIFFERENCES BETWEEN THY-1 (+) AND (-) CLONES DERIVED FROM BALB/C LYMPHOMA CELLS. R.R. Szabadi, J. Buxbaum and R. Basch. Depts. of Pathology and Medicine, N.Y.U. Medical Center and Manhattan Veterans Administration Hospital, New York, N.Y.

Long term cultures of a Balb/c T-cell lymphoma, RLO^{-}1 express the surface antigens Thy 1.2, TL, $H_{2.31}$. We have used anti-Thy 1.2 antibody and complement to select stable Thy 1.2(-) clones (Som. Cell Gen. 1/77). The absence of Thy 1.2 and the presence of TL 1.2 and $H_{2.31}$ on the (-) clones was demonstrated by both cytotoxic and cytotoxic inhibition assays. In separate experiments, (+) and (-) clones were either incubated with ^{14}C amino acids or ^{3}H sugars, or surface iodinated with lactoperoxidase. Cell lysates were prepared by multiple freezing and thawings and were immunologically precipitated using mouse anti-Thy 1.2 and rabbit anti-mouse Ig. The immune precipitates were dissolved in SDS and electrophoresed on SDS acrylamide gels. The iodinated and sugar labelled preparations showed clear differences between the (+) and (-) clones. The slab gel electrophoresis showed the absence of the Thy 1 related T-25 glycoprotein (Trowbridge and Hyman, Cell, 6:279) in the negative clone. Similarly treated lysates obtained from amino acid labelled Thy 1.2+ and Thy 1.2- cells revealed no differences in electrophoretic patterns. A protein, corresponding in electrophoretic mobility to the T-25 protein was found in both lysates. The presence of the protein in both (+) and (-) clones coupled with the absence of expression of the Thy 1.2 antigen and the failure to demonstrate glycosylation of T-25 indicates that the defect in the prototype negative clone is an inability to incorporate sugars into the T-25 protein. Further, it suggests that glycosylation either accompanies, or is obligatory in, the insertion of T-25 protein into the plasma membrane of these cells. (Supported by NCI CA16247, ACS grant IM52 and V.A. Research Funds.)

075 PROPERTIES OF A PENICILLIUM GDP-MANNOSE:GLYCOPEPTIDE MANNOSYLTRANSFERASE SOLUBILIZED WITH TRITON X-100. J. E. Gander and Faye Fang, Department of Biochemistry, College of Biological Sciences, University of Minnesota, St. Paul, MN 55108.

Membranes from Penicillium charlesii were separated into six fractions by sucrose density gradient ultracentrifugation. The smallest density fraction (ρ=1.1 g/cc) contains GDP-mannose: glycopeptide mannosyltransferase(s) that transfers [^{14}C]mannose onto mannosyl-(seryl/threonyl)-polypeptide and phosphogalactomannan regions of peptidophosphogalactomannan. Approximately 90% of the [^{14}C]mannose incorporated was isolated as mannobiose following treatment of the glycopeptide with 0.5 N NaOH. The remainder was located in the phosphogalactomannan. About 10% of the mannosyltransferase activity was solubilized with 1% Triton X-100. The soluble mannosyltransferase was purified by DEAE-cellulose and gel permeation chromatography. Mannose incorporation was dependent on added acceptors. Up to 70% of the mannose from GDP-mannose was transferred to the glycopeptide. Treatment of the glycopeptide with 0.5 N NaOH released 90% of the [^{14}C]mannose as phosphogalactomannan and the remainder was released as mannobiose. Peptidophosphogalactomannan, phosphogalactomannan and phosphomannan all served as acceptors. In contrast, α-mannosidase treated peptidophosphogalactomannan did not serve as an acceptor of mannosyl residues. [^{14}C]Mannose-labeled phosphogalactomannan was subjected to acetolysis. Mannobiose was the major [^{14}C]-labeled product isolated. This shows that mannose was transferred to a (1→6)-linked mannopyranosyl residue. No evidence was obtained for the participation of a lipid-linked mannosyl-containing intermediate in this solubilized and partially purified system. This work was supported by Research Grant GM 19978 from the USPHS.

076 APPEARANCE OF EXTRACELLULAR SOLUBLE GLYCOPROTEINS OF VSV IS NOT DUE TO DEGRADED PROGENY VIRIONS, Sheila P. Little and Alice S. Huang, Department of Microbiology and Molecular Genetics, Harvard Medical School, 25 Shattuck St., Boston, Mass. 02115.

Vesicular stomatitis virus is a large, enveloped RNA-containing virus with 5 structural proteins, of which one is glycosylated and found on the surface of the virion. When progeny formation is inhibited, the release of extracellular soluble glycoproteins (Gs_1 and Gs_2) along with some internal proteins are not affected. Progeny formation is blocked by three methods: 1) co-infection of standard infectious particles with defective interfering (DI) particles; 2) non-permissive conditions for ts G41, a replication minus mutant; and 3) non-permissive conditions for ts 045, a mutant with a lesion in the glycoprotein. The soluble glycoproteins are sensitive to neuraminidase and incorporate glucosamine and fucose. A model for the appearance of soluble glycoproteins will be presented.

077 BIOGENESIS OF LIVER PLASMA MEMBRANE GLYCOPROTEINS. John Elovson, Dept. of Biology, University of California at San Diego, La Jolla, California 92093.

Two integral plasma membrane (PM) glycoproteins, NPPase and GP1, have been purified 10,000 and 2,000-fold, respectively, from rat liver, and their antibodies obtained. NPPase (nucleotide pyrophosphatase) is a sialoprotein with peptide MW 165,000. GP1, peptide MW 125,000, is the major PM receptor for wheat germ agglutinin, and accounts for 2 and 10% of PM protein and sialic acid; its antigenic determinants are exposed only on the outer surface of liver PM. Following 3H-leucine pulses in vivo the labeling kinetics of GP1 and NPPase in subcellular fractions have been analyzed by detergent lysis, immuneprecipitation and preparative SDS-gel electrophoresis. While PM GP1 and NPPase differ in turnover rates ($t_1/_2$ 5 and 1 day, respectively), their insertion into the PM, and its response to colchicine, show a similar time-course, which also parallels that for the release of secretory proteins: i.e. a 15 min lag, followed by a rise to plateau values at 1-2 hours. Such a lag is not seen for total PM proteins. GP1 activity in rough endoplasmic reticulum (RER) peaks at 15 min; that in the Golgi fraction continues to rise over 2 hrs. Labeled and unlabeled GP1 in RER differ in electrophoretic mobility and in their reactivity towards anti-GP1 when this is added to intact RER vesicles. These differences, and a simple computer modeling of the kinetic data, indicate 1) that the labeled GP1 in RER behaves as a precursor to the GP1 in PM, and 2) that this is not the case for the bulk of unlabeled GP1 which is found in RER and Golgi as prepared by established procedures. These results are discussed in terms of a modified membrane-flow model for PM biogenesis.

078 SITE AND SEQUENCE OF CELLULOSE MICROFIBRIL ASSEMBLY IN ACETOBACTER XYLINUM. R. Malcolm Brown, Jr. Department of Botany, University of North Carolina, Chapel Hill, N.C. 27514.

Studies of cellulose microfibril synthesis in eukaryotic plant cells indicate that terminal complexes are mobile within the plane of the plasma membrane and are responsible for the assembly of the microfibril (PNAS 73:143). Conversely, the site of cellulose synthesis in the gram negative prokaryote, Acetobacter is believed to be extracellular and at a distance from the cell (Science 189:1094). Analysis of in vivo microfibril synthesis in Acetobacter has been successful with darkfield light microscopy (PNAS,1976 December issue). Microfibrillar growth is extracellular ,but it is confined to the cell surface. The observed microfibrillar elongation rate corresponds to 4.7×10^{-10} umoles glucose/cell/hr assimilated into cellulose. Electron microscopy of the assembly process with negative staining, sectioning, and freeze etching indicates the presence of approximately 50 immobile microfibrillar synthesizing centers arranged in a row along the longitudinal axis of the bacterial cell. The glycosyltransferases appear to be in close association with the outer envelope. Another catalytic site,"assemblyase" may coordinate the crystallization of glucan chains into the microfibril. A combined biochemical and macromolecular model for cellulose microfibril synthesis in Acetobacter will be presented and compared with the process in certain algae and higher plant cells.

079 MEMBRANE SUGAR ANALOGS: BIOCHEMICAL CHARACTERISTICS, METABOLISM AND ANTI-TUMOR ACTIVITY OF SEVERAL ACETYLATED HEXOSAMINES, Ralph J. Bernacki, Moheswar Sharma, Nancy K. Porter, Mark Evans, Youcef Rustum and Walter Korytnyk, Roswell Park Memorial Institute, Buffalo, NY 14263.

We have synthesized several potential inhibitors and/or modifiers of the carbohydrate portion of plasma membrane glycoconjugate. These include fluorinated and acetylated analogs of D-glucosamine, D-galactosamine and D-mannosamine. These compounds have been tested to determine their effects on both [^{14}C]-glucosamine and [^{3}H]-leucine incorporation into glycoconjugate and on cell growth and viability using P-288 murine lymphoma cells maintained in tissue culture. Several specifically inhibit glucosamine incorporation. 2-Acetamido-2-deoxy-1,3,4,6-tetra-O-acetyl-β-D-glucopyranose or simply penta-acetylglucosamine has been shown to be toxic to cell growth in vitro having an ID_{50} of 5 x 10^{-6}M. In vitro metabolism studies with [^{14}C] and [^{3}H]-labeled penta-acetyl-glucosamine have indicated intracellular de-O-acetylation leading to the formation of UDP-N-acetyl-glucosamine, followed by the incorporation of this sugar into cellular glycoprotein. Concomitant with the formation of increased amounts of nucleotide-sugar intracellular UTP and CTP pools fell to one-third normal within three hours after the administration of 1 mM penta-acetylglucosamine. Combinations of hexosamine with pyrimidine antagonists are currently being evaluated for anti-tumor therapeutic synergism, in vitro, and these results will also be presented. (This work was supported by Public Health Service Grants CA-15757, CA-08793, CA-18420 and CA-13038 from the National Cancer Institute, National Institutes of Health, Department of Health, Education and Welfare.)

080 SIALIC ACID INCORPORATION INTO GLYCOPROTEINS AND GLYCOLIPIDS FOLLOWING ITS UPTAKE BY MAMMALIAN AND AVIAN CELLS, Carlos B. Hirschberg, Douglas Watson, Mary Yeh and David Carey, Dept. of Biochemistry, St. Louis Univ. Sch. Med., St. Louis, MO 63104.

We have recently reported (Hirschberg, C.B., Goodman, S.R. and Green, C., Biochemistry 15, 3591-3599, 1976) that the uptake of sialic acid (SA) by hamster and mouse fibroblasts (NIL,BHK and 3T3) grown on a monolayer, is linear up to 5mM, and that after a 1 hour incubation of NIL cells with ^{3}H-sialic acid approximately 25% of the radioactivity associated with the cells was insoluble in phosphotungstic acid (PTA). We have continued these studies and have found that when NIL, BHK and secondary chick embryo fibroblasts are grown for up to 24 hours in the presence of growth medium to which ^{3}H-SA has been added, approximately 75% of the radioactivity associated with the cells (0.1% of the radioactivity in the medium) is PTA precipitable. Extraction of this pellet with chloroform-methanol 2:1 removed 20% of the radioactivity. The remaining 80% was solubilized in phosphate buffer containing 1% sodium dodecyl sulfate (SDS) and had the following characteristics: (1) it eluted with the void volume on a Biogel P-10 column in 1% SDS (2) upon pronase treatment 80% of the radioactivity elutes on the former column with a retention volume larger than the void volume but shorter than free SA and (3) it is found associated with multiple bands on SDS acrylamide electrophoresis. Over 90% of the radioactivity in these cells remains as SA based on its behavior on Dowex-formate and paper chromatography. Similar results were found in kidney, liver and intestine of mice after a 24 hr injection of ^{3}H-SA, suggesting that this procedure may be used for specific in vivo labeling of sialoglycoproteins and sialoglycolipids. Supported by NCI Grant RO1-CA17015.

081 GENETICS OF CELL SURFACE GLYCOPROTEINS. Roger H. Kennett, University of Pennsylvania, Philadelphia, Pa. 19174

Cell surface glycoproteins isolated from solubilized membranes by affinity chromotography with Lens culinaris lectin were separated by two dimensional electrophoresis. Glycoproteins from mouse and human cells were compared and the synthesis of human glycoproteins in mouse-human hybrid cells examined. This approach makes it possible to identify the human genes involved in the synthesis of human cell surface glycoproteins.

082 PREPARATION OF DOLICHYL PHOSPHATE AND DOLICHYL PHOSPHATE GLYCOSES, Kenneth K. Carroll and C. Anthony Rupar, Dept. of Biochemistry, University of Western Ontario, London, Ontario, Canada. N6A 5C1.

Dolichyl phosphate has been synthesized in good yield by phosphorylating dolichol with 2-chloromethyl-4-nitrophenyl phosphorodichloridate (Rupar and Carroll, Chem. Phys. Lipids 17: 193-200, 1976). This reagent has also been used for preparation of mixed phosphodiesters (Mushika et al, Bull. Chem. Soc. Japan 44: 232-235, 1971). and experiments are being carried out to investigate the possibility of synthesizing dolichyl phosphate glycoses by this approach. (Supported by the Medical Research Council of Canada).

084 How a Single Sindbis Virus mRNA Directs the Synthesis of One Soluble Protein and Two Integral Membrane Glycoproteins. Dyann F. Wirth, Flora Katz, Harvey F. Lodish and P.W. Robbins. Department of Biology, M.I.T. Cambridge, Mass. 02139.

The synthesis of the Sindbis virus core protein and membrane glycoproteins in infected cells has been studied. Since, one soluble protein and two integral membrane proteins are synthesized on one mRNA with a single initiation site, this system can distinguish between the various models proposed to explain the specificity for the attachment of polysomes to membranes and the transfer of membrane proteins into the lipid bilayer, e.g., a special class of ribosomes or a membrane binding sequence in the mRNA. We have found that during infection, the Sindbis 26S RNA is found in membrane bound polysomes. As is the case with secretory tissues, these polysomes are released by treatment with puromycin and high salt. In vitro experiments which allow the completion of nascent protein chains show that all structural proteins can be synthesized by membrane bound polysomes. In extracts from infected pulse labeled cells,the core protein remains on the cytoplasmic side of the endoplasmic reticulum while the newly synthesized envelope proteins are sequestered by the lipid bilayer. These results suggest that the glycoproteins themselves, presumably their nascent amino termini are of major importance in directing the binding of the polysomes containing 26S RNA to the endoplasmic reticulum membranes and the subsequent transfer of glycoproteins into the bilayer. At least the specificity for the transfer of the membrane proteins into the bilayer cannot reside in the ribosomes or mRNA since they also direct the synthesis of the core protein which is not inserted into the bilayer, but remains on the cytoplasmic side of the endoplasmic reticulum.

083 PREPARATION OF DOLICHYL PHOSPHATE AND DOLICHYL PHOSPHATE GLYCOSES, Kenneth K. Carroll and C. Anthony Rupar, Dept. of Biochemistry, University of Western Ontario, London, Ontario, Canada. N6A 5C1.

Dolichyl phosphate has been synthesized in good yield by phosphorylating dolichol with 2-chloromethyl-4-nitrophenyl phosphorodichloridate (Rupar and Carroll, Chem. Phys. Lipids 17: 193-200, 1976). This reagent has also been used for preparation of mixed phosphodiesters (Mushika et al, Bull. Chem. Soc. Japan 44: 232-235, 1971) and experiments are being carried out to investigate the possibility of synthesizing dolichyl phosphate glycoses by this approach. (Supported by the Medical Research Council of Canada).

012 CONFORMATION AND INTERMOLECULAR INTERACTIONS OF CARBOHYDRATE CHAINS

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For consideration of their conformation and interactions, carbohydrate chains can conveniently be divided (1) into three classes on the basis of their covalent structure; namely (a) periodic, (b) interrupted periodic and (c) aperiodic types. In aqueous solution the hydrophilic carbohydrate chains often exist as highly disordered random coils. Under appropriate conditions, however, polysaccharides of types (a) and (b) can adopt (1,2) a variety of ordered conformations. We have used 'simple' interacting plant, bacterial and animal systems to characterize the sub-types of ordered conformations and intermolecular interactions and to develop ideas and techniques for application to more complex biological systems. Physical methods and in particular the chiroptical techniques of optical rotation and circular dichroism, and nuclear magnetic resonance, provide sensitive probes for the study of the mechanism and specificity of such interactions in aqueous solution.

Intermolecular interactions between polysaccharide chains arise from cooperative associations of long structurally regular regions of the molecules, in ordered conformations. For acidic polysaccharides cooperative associations may involve alignment of extended ribbons of regular sequences with cations sandwiched between them. In other systems the interactions involve the intertwining of chains into double helices which may then aggregate further. Geometric 'matching' of structurally regular regions of different polysaccharide chains can also occur. Ordered associated regions are terminated by structural interruptions or 'kinks' which prevent complete aggregation of the molecules.

The complex carbohydrate chains, covalently bound to both proteins and lipids, which occur at the periphery of animal cells have very different aperiodic /type (c)/ structures and their conformations are, as yet, poorly understood.

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013 DIMENSIONS AND SPECIFICITIES OF RECOGNITION SITES ON LECTINS AND ANTIBODIES

Elvin A. Kabat, Departments of Microbiology, Human Genetics and Development, and Neurology, Columbia University, Neurological Institute, Presbyterian Hospital, New York, N.Y. 10032

There are two major classes of substances in addition to specific enzymes which react with blood group A, B, H, Le^a, Le^b and precursor I glycoproteins and glycolipids - antibodies and lectins. Since the blood group antigenic determinants are predominantly sequential rather than conformational, it is possible to measure the relative capacities of oligosaccharides of varying sizes and structures to inhibit precipitation by blood group substances of the lectin or antibody of appropriate specificity. In this manner one can measure the size of the specific receptor site as being complementary to the largest oligosaccharide which contributes binding energy as indicated by reaching maximum inhibition per mole with oligosaccharides of increasing size. Thus in the dextran-antidextran system, antibody combining sites have been shown to vary in size, the lower limit being complementary to between a mono and a disaccharide, about 6Å, while the upper limit is complementary to a hexa- or heptasaccharide, about 34Å, arbitrarily measuring the dimensions in the most extended conformation. Moreover, studies on myeloma proteins with antidextran specificity established that combining sites may be either cavities in which the terminal nonreducing ends are held three dimensionally or grooves in which the site binds a linear sequence of up to six sugars not involving the nonreducing terminus. Animal and plant lectins with blood group specificity fall into the same range of sizes. The receptor sites of various lectins will be compared with antibody sites with respect to their similarities and differences. Lectin sites do not appear to be as specific as antibody sites. The lectin of Euonymus europeus shows both B and H specificity and is unable to distinguish between DGalα1→3[LFucα1→2]DGalβ1→3DGlcNAc and DGalα1→3[LFucα1→2]DGalβ1→4 DGlcNAc although the DGlcNAc interacts in the site. Two sponges, Axinella polypoides and Aaptos pappillata each yielded several lectins with different specific receptor sites. Axinella agglutinins I and II were specific for β1→6 linked terminal DGal and p-nitrophenyl βDGal, but differ in their behavior toward other oligosaccharides. Aaptos agglutinins I and II also differed in their combining sites, agglutinin II being specific for N, N', N'' tri-N acetylchitotriose, while agglutinin I was best inhibited by the tetramer N, N', N'', N''' tetraacetylchitotetraose (1, 2, 3).

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014 PLANTS INTERACT WITH MICROBIAL POLYSACCHARIDES, Peter Albersheim, Arthur R. Ayers, Jr., Barbara S. Valent, Jürgen Ebel, Jack Wolpert and Russell Carlson, Department of Chemistry, University of Colorado, Boulder, Colorado 80309.

Plants are resistant to almost all of the microorganisms with which they come in contact. In response to invasion by a fungus, bacterium or a virus, many plants produce low molecular weight compounds, phytoalexins, which inhibit the growth of microorganisms. Phytoalexins are produced whether or not the invading microorganism is a pathogen. The production of phytoalexins appears to be a widespread mechanism by which plants attempt to defend themselves against pests. Molecules of microbial origin which trigger phytoalexin accumulation in plants are called elicitors. Structural polysaccharides from the mycelial walls of several fungi elicit phytoalexin accumulation in plants. Approximately 10 ng of the polysaccharide elicits the accumulation in plants of more than sufficient amounts of phytoalexin to stop the growth of microorganisms in vitro. The best characterized elicitors have been demonstrated to be β-1,3-glucans with branches to the 6 position of some of the glucosyl residues. Oligosaccharides, produced by partial acid hydrolysis of the mycelial wall glucans, are exceptionally active elicitors. The smallest oligosaccharide which is still an effective elicitor is composed of about 8 sugar residues.

Bacteria also elicit phytoalexin accumulation in plants, but the Rhizobial symbionts of legumes presumably have a mechanism which allows them to avoid either eliciting phytoalexin accumulation or the effects of the phytoalexins if they are accumulated. The lectins of legumes bind to the lipopolysaccharides of their symbiont, but not of their non-symbiont, Rhizobia. It is not known whether the lectin-lipopolysaccharide interaction is involved with the establishment of symbiosis. However, evidence will be presented that suggests that lectins are, in fact, enzymes capable of modifying the structures of the lipopolysaccharides of their symbiont, but not of their non-symbiont, Rhizobia. It will also be shown that the lipopolysaccharides isolated from different Rhizobia species and from different strains of individual Rhizobia species have different sugar compositions. Thus, the different strains of a single Rhizobia species are as different from one another as the different species of Salmonella and other gram-negative bacteria. This conclusion is substantiated by experiments demonstrating that antibodies to the lipopolysaccharide from a single Rhizobial strain can differentiate that strain from other strains of the same species as well as from other Rhizobial species. The role in symbiosis of the strain-specific O-antigens is unknown.

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015 THERMODYNAMICS OF INTERACTIONS OF SACCHARIDES WITH PROTEINS, John A. Rupley, Department of Chemistry, University of Arizona, Tucson, AZ 85721

Two approaches will be taken in describing the interactions of small ligands, in particular saccharides, with enzymes and other proteins.

First, the crystallographic data available for various protein-ligand complexes can be used to evaluate the nature of protein-ligand interactions. The interactions at small molecule sites are exceptional, in that it is difficult to relate the thermodynamics of the interactions to either the extent of the protein surface affected or to the type of atom-atom contacts involved. Active sites may be more cleverly designed than the architecture of the protein itself.

Secondly, particular contacts within the lysozyme active site will be described. It is well known how the general features of the specificity of lysozyme reflect enzyme-saccharide interactions. It has been possible to examine the thermodynamics of several of these interactions. For example, the free energy and enthalpy of formation of the two hydrogen bonds between Asp-101 and the substrate are large and negative. In contrast, the hydrogen bond involving Trp-62 is of less importance as is the small movement associated with Trp-62 and its neighboring backbone that follows complex formation. Reaction of the glycosyl-enzyme with various acceptors shows inconsistencies that are difficult to explain using a simple model of the structure of the reactive enzyme-substrate complex. It is possible that the arrangement of the transition state depends on saccharide size.

016 THE MECHANISM OF BINDING OF MONO- AND OLIGOSACCHARIDES TO CONCANAVALIN A

Curtis F. Brewer and Rodney D. Brown, III, Albert Einstein College of Medicine, Bronx, N.Y. 10461 and IBM T.J. Watson Research Center, Yorktown Heights, N. Y. 10598

In previous studies of the interaction of solvent water molecules with the Mn^{++} ion in Mn-Con A by observation of the dispersion of the spin-lattice relaxation rate (T_1^{-1}) of the solvent water protons over a wide range of magnetic fields (Koenig et al., 1973), we have shown that this rate is dominated by the residence time of a single exchanging water ligand on the Mn^{++} ion. Additional measurements were made of Mn-Con A in the presence of saturating amounts of either α- or β-methyl-D-glucopyranoside and it was observed that the relaxation rate across the dispersion spectrum was reduced by approximately 15%. In the present study, we have measured the effects of binding of a series of mono- and oligosaccharides on the solvent water proton relaxation rate over a range of magnetic fields from 5 Oe to 12 KOe and show that the observed decrease in the relaxation rate is due to an increase in the residence time of the single exchanging water ligand. This effect is consistent with a conformational change in the protein upon binding of saccharides. We find that the binding of α- and β-methyl-D-glucopyranoside, α-methyl-D-mannopyranoside and β-(o-iodophenyl)-D-glucopyranoside produce the same increase in residence time and therefore the same conformational change in the protein, whereas galactose and β-(o-iodophenyl)-D-galactopyranoside show no effects. The same reduction in relaxation rate as that caused by the above monosaccharides was observed with the following oligosaccharides: D-maltose, D-maltotriose, D-maltotetraose, o-α-D-mannopyranosyl-(1→2)-D-mannose, o-α-D-mannopyranosyl-(1→2)-o-α-D-mannopyranosyl-(1→2)-D-mannose, o-α-D-mannopyranosyl-(1→2)-o-α-D-mannopyranosyl-(1→2)-o-α-D-mannopyranosyl-(1→2)-D-mannose and melezitose. As observed by Goldstein and coworkers, the first three oligosaccharides have nearly the same affinity as monosaccharides, whereas the α-(1,2) linked mannans show increasing affinity constants with increasing chain lenght. Melezitose also shows enhanced binding by a factor of four relative to α-methyl-D-glucopyranoside. The water relaxation data suggest that the above mono- and oligosaccharides bind to Con A by a similar mechanism involving only a single saccharide residue combined with the protein at one time. The greater affinity of melezitose and the α-(1→2)-mannose oligosaccharides appears to be due to a statistical increase in probability of binding because of the presence of more than one binding residue in the chain and not to an extended binding site on the protein. The results have important implications regarding the molecular properties of so-called "Con A receptors" on the surface of cells.

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017 SPECIFICITY OF THE INTERACTION BETWEEN HYALURONIC ACID AND CARTILAGE PROTEOGLYCANS

Vincent C. Hascall, NIDR, NIH, Bethesda, MD 20014

Most proteoglycans are present in hyaline cartilage matrices as aggregates with as many as 100 molecules, each with MW of about $2x10^6$, bound through specific, non-covalent interactions to individual strands of hyaluronic acid, HA. The interactions with HA are mediated by the HA-binding region of the core protein, which is located at one end of each of the interactive proteoglycans. A fragment of the core protein, MW of about $6x10^4$, which contains the HA-binding site, can be isolated in an active form from trypsin digests of proteoglycan aggregates (1). The "active" HA-binding site in this preparation interacts strongly with HA-10 but weakly with HA-8, [oligomers of HA derived from partial digests of HA with testicular hyaluronidase]; HA-9, derived from β-glucuronidase digestion of HA-10 also interacts strongly (2). No polysaccharide other than HA has been found to interact. Christner et al. (3) modified the carboxyls on glucuronic acid groups in a mixture of HA-10 to HA-30, and they found that the interaction with proteoglycan no longer occurred if about 60% of the total carboxyls were (a) methyl esterified, (b) reduced to glucose, or (c) substituted with glycine in amide linkage. Saponification of the methyl esters restored activity. Dansylation of lysine residues in the HA-binding region preparation abolished binding activity (4). However, when the dansylation reaction was done in the presence of HA, the HA-binding activity was protected. Acetylation of the same residues did not abolish binding activity but did prevent subsequent inactivation by dansylation. Hardingham et al. (5) studied the effect of various amino acid modifiers on the interaction of intact proteoglycans with HA and showed that reaction of arginine residues with low concentrations of butane-2,3-dione was particularly effective in destroying binding. In sum, the data above suggests that the HA-binding region (a) contains accessible arginine residues necessary for activity, (b) contains nearby lysine residues which, when substituted with bulky groups such as dansyl, but not acetyl, sterically block interaction, and (c) requires a length of HA with at least 4.5 repeat disaccharides containing three, and possibly four, un-modified glucuronic acid carboxyls for interaction.

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085 Bacterial carbohydrate-specific antibodies distinguish between mouse cerebellar cells of different developmental stages, Ekkhart Trenkner* and Siddhartha Sarkar**, *Dept. Neuropath., Harvard Med. Sch., Boston, Ma. 02115, **Div. Med. Genetics, Dept. Med. M-013, Sch. Med. UCSD, La Jolla, Ca. 92093. High titered anti-carbohydrate antibodies were used to identify cell surface carbohydrates during different stages in histogenesis of mouse cerebellum in a micro tissue culture system, which mimics selected features of in vivo cerebellum development. Blockage of fiber formation within the first few days in vitro and inhibition of cell migrations by carbohydrate specific antibodies served as an assay system for possible contributions of surface carbohydrates into the behavior of developing cerebellar cells.

Microbial strains were selected on the basis of carbohydrate structures of their cell wall antigens, and anticarbohydrate antibodies were raised against treated whole bacteria in rabbits. We found that antibodies to mannan were active at all stages of development tested (embryonic day 13, E13; the day of birth, P0; and postnatal day 7, P7). Antibodies to sialic acids prepared against strains B and C of Neisseria meningitidis distinguish different subterminal structures; anti-B reacted with E13 and P0 cerebellar cells, and anti-C mostly with cells older than P1. Anti-fetuin antibody recognized E13 and P0 but not P7 cell populations. Pneumococcus C strain R36A specific antibodies were effective only after coating cells to C type carbohydrate before application of the antibody.

The results demonstrate that anti-microbial carbohydrate antibodies crossreact with mammalian cell surface carbohydrate structures and therefore can be used as a powerful tool in tissue culture to analyze those structures which might control cell behaviors pertinent to cerebellar development.

086 INHIBITION OF ANTIBODY-COMPLEMENT KILLING OF TUMOR CELLS BY CONCANAVALIN A (CON A) M. D. P. Boyle, J. J. Langone, S. H. Ohanian and T. Borsos, NIH, Bethesda, MD 20014

The site of action of antibody and complement is known to be the cell membrane. It has also been shown that Con A can bind specifically to α-D-glucosyl and sterically related sugars on the cell membrane. For this reason we tested the effect of Con A on the killing of line-1 guinea pig hepatoma cells (T) by anti-Forssman antibody (A) and complement to determine if any topographical relationship existed between the binding sites on the cell membrane for Con A and any of the complement components. It was found that Con A (at a concentration lower than that required to agglutinate the cells) inhibited the killing of line-1 cells sensitized with anti-Forssman antibody (TA) by guinea pig complement (GPC), but not by human complement (HuC). The inhibitory effect of Con A was observed when the GPC was mixed with Con A or when the antibody sensitized cells were pretreated with Con A prior to the addition of GPC. The inhibitory effect of Con A could be reversed by treatment with the sugar specific for Con A binding. Measurement of the ability of TA and TA-Con A to fix the first component of complement demonstrated inhibition of binding of GPC1 by > 90% while HuC1 binding was only inhibited to around 50%. There was no difference in the number of GPC1 molecules fixed to anti-Forssman sensitized sheep erythrocytes (EA) or these cells treated with Con A. It would consequently appear that the inhibitory effect of Con A on the binding of GPC1 to TA is not solely attributable to the interaction of Con A with the antibody. These observations suggest that Con A may prove useful for studying the location of antibody and complement binding sites on mammalian cell membranes.

087 A Mg^{++}- or Ca^{++}-Activated Cell Surface ATPase and Its Perturbation by Concanavalin A. Coralie A. Carothers Carraway, Douglas D. Fogle and Kermit L. Carraway, Department of Biochemistry, Oklahoma State University, Stillwater, Oklahoma 74074.

Cell surface glycoproteins have been implicated in a number of surface-mediated phenomena, such as the immune response, cell-cell interactions and adhesion to substratum. Lectins modify specifically the activities of a number of enzymes, including galactosyl transferase, 5'-nucleotidase and both Na^+, K^+- and Mg^{++}-ATPases, suggesting that these are glycoproteins or that they are closely associated with glycoproteins. Concanavalin A (Con A) activates ATPases from several cell and tissue types. A cell surface ATPase whose activity is dependent upon either Mg^{++} or Ca^{++} has been found in 13762 MAT-A rat mammary ascites tumor cells and its isolated plasma membranes. The activity is unaffected by oligomycin, NaN_3 or ouabain in either whole cells or membranes, indicating that the enzyme is not similar to either the mitochondrial or the Na^+, K^+-ATPase. ADPase and GTPase activities in cells and membranes are relatively lower and show more complex kinetic plots than the ATPase. These results indicate that these activities are not entirely due to the enzyme which expresses the ATPase activity. ATPase from isolated membranes shows an apparent activation by Con A, but kinetic analysis indicates that the activation is actually a release from substrate inhibition. Neither substrate inhibition nor Con A activation is found with intact cells. A model will be presented to explain the behavior of the enzyme and its regulation by external effectors such as Con A.

088 THE SELF-RECOGNITION CONCEPT: AN ACTIVE FUNCTION FOR THE MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) BASED ON PROTEIN CARBOHYDRATE COMPLEMENTARITY, Barry E. Rothenberg, Scripp Clinic and Research Foundation, La Jolla, CA 92037.

This concept answers a basic question-how does a phagocytic cell of any multicellular organism distinguish self from non-self? The answer which follows is based on molecular, genetic and evolutionary arguments which coalesce present facts and theories of cellular recognition and the evolution of the immune system into a unifying concept-a concept which serves to describe the function of the MHC and its relationship with cellular recognition mechanisms. The following are the major points of the hypothesis: 1) There exists on the membrane of all multicellular organisms containing a distinct population of phagocytic cells a unique chemical entity for self-recognition; 2) the process of self-recognition is mediated by the complementary interaction of protein and carbohydrate; 3) the biological counterparts of these molecules are the products of the MHC; 4) the protein and carbohydrate molecules coded by this complex are proposed to be encoded for by a series of separate but linked constant and variable genes similar both in structure and organization to immunoglobulin genes; 5) foreign recognition and immune systems are direct descendants of the self-recognition genes; 6) the MHA have an active as opposed to a passive function; 7) this active function would necessitate the ubiquitous distribution of the MHA; 8) one germ-line determines the molecular specificity of three distinct macromolecules (protein and carbohydrate). The postulate provides a unique and ingenious way to overcome the problems associated with parallel mutations; 9) Polymorphism is generated and selected externally by disease association and internally by mechanisms insuring the non-interaction of codominantly-expressed self-recognition molecules.

089 SIALIC ACID: A SPECIFIC ROLE IN SPLEEN COLONY FORMATION, Quentin J. Tonelli and Russel H. Meints, School of Life Sciences, University of Nebraska, Lincoln Nebraska 68588

We have recently reported a 50% reduction in hematopoietic spleen colonies following Vibrio cholerae neuraminidase (VCN) treatment of donor bone marrow (J. Cell Biol. 70 (2 part 2):7a and Science, in press), thereby implicating sialic acid in hematopoietic stem cell (CFU-S) site recognition and implantation. Currently, we are attempting to assess the function of sialic acid in spleen colony formation. Innoculum viability, as measured by in vitro incorporation of tritiated precursors of protein, nucleic acids, and oligosaccharides, is unaffected after VCN treatment, as is the ability of marrow in culture to respond to the hormone erythropoietin, as measured by the incorporation of 59Fe into cyclohexanone-extractable heme. A cytotoxic effect of the VCN preparation therefore seems unlikely. In addition, mild Malapradian oxidation of donor cells with sodium periodate also reduced CFU-S approximately 50%, while subsequent potassium borohydride reduction restored CFU-S to 75% of control levels. Incubation of VCN-treated marrow with either β-galactosidase or trypsin had no effect on the VCN-induced reduction in CFU-S. These results are consistent with the idea that membrane sialic acid plays a direct and specific role in the implantation and development of CFU-S.

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090 ROLE OF MEMBRANE GANGLIOSIDES IN THE ANTIVIRAL EFFECT OF INTERFERON, Paula M. Pitha and Morley D. Hollenberg, The Johns Hopkins University, Baltimore, MD 21205

The antiviral activity of human and mouse interferon can be neutralized by preincubation with gangliosides before application to cells. No particular specificity was observed for neutralization of human interferon. A ganglioside mixture was as effective as pure gangliosides. Ganglioside-deficient transformed mouse cells were much less sensitive to interferon action than the non-deficient parental cell lines. Treatment of such cells with gangliosides led to an increase in the ganglioside content and to an increase in cell sensitivity to interferon. Results on the correlation between the ganglioside content of the cells and their sensitivity to interferon will be discussed with respect to the possible role of membrane gangliosides in the antiviral effect of interferon.

091 CHEMICAL MODIFICATION OF THE HYALURONATE BINDING SITE OF CARTILAGE PROTEOGLYCANS. Tim Hardingham, Roger Ewins, and Helen Muir, Kennedy Institute, London W.6., U.K.

Proteoglycans are present in cartilage matrices at high concentration primarily as multimolecular aggregates in which many proteoglycans bind to single chains of hyaluronate. The binding is mediated by a gobular region at one end of the proteoglycan core protein through a specific site with a high affinity for a decasaccharide unit of hyaluronate.

The binding region is stable to exposure to 4M guanidinium chloride, 2M, KSCN, 2M $CaCl_2$, 3M $MgCl_2$, 6M urea, 33% acetic acid or absolute ethanol or acetone. It also resists thermal denaturation with little loss of binding activity when heated in solution at neutral pH at 60°C and with a half life of 14.2 min at 100°C. The binding activity was further studied after modification of various amino acid residues in the proteoglycan core protein. Purified disaggregated protoeglycan from pig laryngeal cartilage was reacted with acetic anhydride, 2-methylmaleic anhydride, butane-2,3-dione, N-bromosuccinimide, 2-nitrophenyl sulphenyl chloride and dithiothreitol. The results confirmed that the binding was dependent upon intact disulphide bridges and that the modification of lysine, arginine, and tryptophan residues all abolished activity. Lysine and arginine residues may be involved in direct sub-site interactions with hyaluronate carboxyl groups, but examination of the tryptophan fluorescence of the proteoglycan showed no change on binding, suggesting that tryptophan residues were not directly involved. It was possible to regenerate binding activity by reoxidation of reduced disulphide bonds or by the removal or 2-methyl maleyl groups from lysine residues at pH 3.0. This provided further evidence that the native conformation of the unmodified proteoglycan was thermodynamically preferred and of high inherent stability.

092 GLYCOPEPTIDE BINDING TO CONCANAVALIN A, J.P. Carver and B.J. Fuhr. Depts. of Medical Genetics and Medical Biophysics, University of Toronto, Toronto, Ontario, Canada.

Lectins interact with cell surfaces through the complex carbohydrate moieties of cell surface glycoproteins and glycolipids. As part of an ongoing study of the mechanism of lectin induced cellular events, we are attempting to determine the molecular structure of Concanavalin A (ConA) complexes with cell surface receptor analogues. To this end we have examined the binding of the monosialylated, Asn-linked, glycopeptide of human IgG with ConA:Co^{2+}:Ca^{2+} by the following the resultant changes in the 220 MHz high resolution proton magnetic resonance spectrum of ConA:Co^{2+}:Ca^{2+}. Previous studies in this laboratory have led to the assignment of separate histidine C2-proton resonances of ConA:Co^{2+}:Ca^{2+} complexes to specific residues in the primary sequence. Using these assignments we find that the intact glycopeptide causes a significant perturbation in the position of the side chain of histidine 205 but in no other histidine side chain. This perturbation can be reversed by α-methyl-D-mannoside. Partially degraded IgG glycopeptide in which the non-reducing terminal sialic acid,galactose and N-acetyl glucosamine residues have been removed does not perturb His-205 nor any other histidine side chain. However, it does bind and can be displaced by α-methyl-D-mannoside. On the basis of these and other studies a detailed model for the binding to ConA of these cell surface receptor analogues has been constructed.

093 ESTIMATION OF MOLECULAR WEIGHTS OF PEPTIDES AND GLYCOPEPTIDES BY THIN-LAYER GEL FILTRATION, Billy G. Hudson, Chung-Ho Hung, and Dudley K. Strickland, University of Kansas Medical Center, Kansas City, KS 66103.

The technique of thin-layer gel filtration using Sephadex G-50 in 6 M guanidine hydrochloride was evaluated for estimating the molecular weights of peptides and glycopeptides that have molecular weights below 10,000. A linear relationship between log molecular weight and relative distance of migration was obtained for peptides and glycopeptides ranging in molecular weight from 1,700 to 10,500, and 1,500 to 3,300, respectively. The migration behavior of glycopeptides in the range of 1,900 to 3,300 daltons did not differ significantly from that of peptides, indicating that peptides can be used as calibration standards for the estimation of the molecular weight of glycopeptides in this molecular weight range and possibly beyond 3,300. The procedure is rapid and simple, requires only small amounts of sample (30-100 μg), and molecular weights can be estimated with an average error of <10%.

094 A NEW APPROACH TO THE STRUCTURAL DETERMINATION OF GLYCOPROTEINS AND POLYSACCHARIDES: ANHYDROUS HF SOLVOLYSIS. Andrew J. Mort, MSU/ERDA Plant Research Laboratory, Michigan State University, E. Lansing, MI 48824.

Structural studies of glycoprotein polypeptides are often hampered by the oligosaccharide sidechains. These sidechains sometimes affect the rate of proteolysis, cause heterogeneity and therefore difficulty in purification, and interfere with normal sequencing methods. Therefore a general method for the removal of sugars from glycoproteins is needed.

From experiments with glycoproteins containing the glycopeptide linkages, arabinose-O-hydroxyproline and galactose-O-serine (plant cell wall glycopeptides), N-acetylgalactosamine -O-serine/threonine (pig submaxillary mucin), and N-acetylglucosamine-N-asparagine (fetuin), it is apparent that anhydrous liquid HF, a reagent commonly used by synthetic peptide chemists for the complete removal of protecting groups from synthetic peptides, cleaves the O-glycosidic linkages of neutral sugars in 1 hour at 0°, and the O-glycosidic linkages of amino sugars in 3 hours at 23°. The N-glycosidic linkage of N-acetylglucosamine to asparagine is not cleaved under any conditions that have been tested. SDS gel electrophoresis of bovine serum albumin treated in HF does not show any degradation of peptide bonds. Some relatively stable enzymes (lysozyme and RNAse) have been shown by others to retain most of their enzymic activity after short treatment (1 hour at 0°) in HF.

With the specificity of HF at 0° for neutral sugars it should be possibletto generate di- or trisaccharide in high yield from polysaccharides containing both neutral and amino sugars with neutral sugars as the reducing termini.

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095 GLYCOPROTEIN SYNTHESIS AS A FUNCTION OF EPITHELIAL CELL ARRANGEMENT, Zoltán A. Tökés and Gerald B. Dermer, Cell Membrane Lab, LAC/USC Cancer Center, Depts. Biochem. and Path., USC School of Medicine, Los Angeles, CA 90033.

Human breast and prostate epithelial cells are arranged in vivo in three-dimensional, glandular structures. These cells exhibit tight adhesion and unidirectionally secrete into the lumen. Since glycoproteins (GP) are responsible for organ-specific cell aggregation and recognition, conditions must be established for studying GP synthesis and turnover where the 3-D arrangement of cells is maintained. A comparison then becomes possible with cells growing in 2-D monolayers. We have established conditions where GP synthesis, as measured by ^{3}H-glucosamine incorporation, is maintained in organ cultures where 3-D tissue integrity is unaltered. Surgical specimens of both prostate and breast maintained their GP synthesizing capacity for 4 days as determined by autoradiography and there was a migration of label from the Golgi apparatus to the cell surface and into lumens. The released products were analyzed by SDS-PAGE. A remarkable simplicity of labeled GP pattern was observed. In organ cultures of both tissues, a predominant peak, mol. wt. 48,000, was recovered from the medium. Preliminary data indicate a progressively more complicated pattern with less differentiated cell types. Breast epithelial cells in 2-D monolayers synthesize GP and at least three additional components are detectable. Although a number of interpretations are available, an intriguing possibility exists that adhesion and contact restrict turnover, synthesis and release of a variety of GP, which might be responsible for cementing cells into 3-D structures. Therefore cells escaping from such a topographic restriction could express additional families of GP. Our preliminary data are in agreement with this supposition.

096 CARBON-13 AS A TOOL FOR THE STUDY OF CARBOHYDRATE STRUCTURES, CONFORMATIONS AND INTERACTIONS, Robert Barker, Hernan Nunez and Thomas C. Walker, Michigan State University, East Lansing, MI 48824

Natural abundance ^{13}C nmr provides a useful means for identifying carbohydrates and assigning structures to certain types of complex carbohydrates. This application is often limited by the low natural abundance of ^{13}C. Enrichment of carbohydrates with ^{13}C in specific carbons greatly increases sensitivity and provides opportunities to examine carbohydrate conformations and the interactions of carbohydrates with enzymes and lectins in solution. As examples: specific enrichment permits the unequivocal assignment of chemical shifts for individual carbons of the simple sugars. The coupling between carbons and between carbon and hydrogen in specific locations can also be observed in enriched carbohydrates and can be related to their conformations. Changes in coupling can be related to changes in conformation that occur on binding of complexing agents or to proteins. Coupling between carbon and phosphorus can also be used to assign conformational preferences in glycosyl phosphates and their derivatives. In disaccharides, coupling through the glycosidic bond can be interpreted in terms of the conformation about that bond. The interactions of carbohydrates with other substances can also be interpreted on the basis of changes in the relaxation times of carbons that can be expected to accompany binding. Evaluation of these relaxation times is greatly facilitated by ^{13}C-enrichment at selected centers.

Examples of each of the above applications will be presented involving ^{13}C-enriched glucose, galactose, mannose, fucose, N-acetyl glucosamine, their glycosyl phosphates, nucleoside diphosphate derivatives and derived disaccharides.

097 DISTRIBUTION OF FIBRONECTIN IN CULTURES OF ADHERENT CELLS. D.F. Mosher, O. Saksela, J. Keski-Oja, and A. Vaheri, U. Helsinki, Finland and U. Wisconsin, Madison, WI 53706

Fibronectin is a major glycoprotein of cultured adherent cells and is missing from surfaces of malignantly transformed cells. We have studied the distribution of fibronectin in early passage cultures of adherent cells grown from human embryonic skin, heart, lung, body wall, liver and kidney. Fibronectin was quantitated by radioimmunoassay and visualized by immunofluorescence, using antiserum directed against homologous plasma fibronectin. Subunit size was estimated by polyacrylamide gel electrophoresis of iodinated cell-surface fibronectin and of metabolically-labeled fibronectin purified from cell extracts or medium by double antibody immunoprecipitation.

Twenty-four hr after passage, cultures of all adherent cell strains contained large amounts (50-150 ug/mg cell protein) of fibronectin. Approximately 95% was in the medium, 2-3% was associated with cell surfaces, and 2-3% was intracellular. No differences in subunit size were detected among cell-surface fibronectins of different cell strains or between fibronectin in the medium and fibronectin associated with the cell layer. However, the arrangements of cell-surface fibronectin did vary among different cell strains: body wall, skin, liver--discrete fibrillar; kidney--discrete punctate; lung--dense fibrillar; and heart--discrete fusiform. To explain the polymorphism of cell-surface fibronectin, there must be either subtle structural differences among fibronectins synthesized by the various cell strains or factors in the cell layer which influence fibronectin binding and aggregation.

098 STUDIES ON THE MECHANISMS OF NEURAL CELL ADHESION

Bernard PESSAC, Françoise ALLIOT and Arlette GIRARD, Institut d'Immuno-Biologie Hôpital Broussais, 96, rue Didot, 75674 Paris Cedex 14 - France.

We have previously shown with a "modified collecting aggregate" and a "stationary monolayer" assay that adhesion of cells from various tissues of 8-9 day chick embryos is not tissue specific (1). In particular, cells from neuroretina, cerebrum and optic tectum, when matched in mutual combinations show no preferential adhesion for like tissues (2).
In the course of these studies, we have noticed a very important r.p.m. dependance of the number of cells adhering on aggregates in the M.C.A. assay. The results indicate that for neuroretina and optic tectum, there is a different rotation speed at which maximal cell collection is obtained. The data may be interpreted in terms of mechanisms for cell cell adhesion, such as a difference in the affinity of adhesive sites for different tissues. An other interpretation of these data is a change in the number and (or) the affinity of adhesive sites during the development of retina and tectum. If the maximal speed of cell collection is the same at different stages of development of a given tissue, one may anticipate that the sites of adhesion and their affinity are identical, although their number may be different as reflected by the percentage of adhering cells. These data will be discussed in context with the current views on the molecular basis of intercellular adhesion.

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099 BEHAVIOUR OF GLYCOLIPIDS IN LIPID BILAYERS, Chris W. M. Grant and Frances J. Sharom, The University of Western Ontario, London, Ontario, Canada N6A 5C1

Glycolipids are now recognized as playing important roles at the cell surface. As part of a program to investigate the behaviour and interactions of glycolipids, a spin labeled neutral glycolipid, galactosyl ceramide (GC), has been synthesized with the label at three different positions on the fatty acid chain. The same approach should work equally well for a variety of more complex glycolipids. The behaviour of spin labeled GC has been studied in bilayers of different lipids and lipid mixtures, and compared to that of corresponding phospholipid spin labels. Qualitatively, the physical behaviour of glycolipids in membranes is similar to that of phospholipids. For example, spin labeled GC has a low flip-flop rate and tends to be excluded from phosphatidylserine-enriched domains formed by Ca^{2+}-headgroup crosslinking in both binary and ternary lipid mixtures. The three GC labels probe different depths of lipid bilayer membranes, and the measurement of order parameters has shown the existence of a fluidity gradient similar to that seen for phospholipids. However, order parameters measured with GC labels are higher than those found for the corresponding phospholipid labels, and this has been interpreted in terms of a general capacity for glycosphingolipids to increase packing density in fluid lipid bilayers.

100 TYROSYLATION OF MEMBRANE BOUND TUBULIN, Jayasree Nath and Martin Flavin, Section on organelle Biochemistry, NHLBI, NIH, Bethesda, Maryland 20014

Colchicine effects on the mobility of cell surface receptors, and on secretion, have suggested that cytoplasmic microtubules might have a role in these processes.However in brain a substantial portion of tubulin is also in a non-tubule, membrane bound compartment (Bhattacharyya and Wolff, JBC 250, 7639).. We have been studying a post-translational modification of tubulin catalyzed by an enzyme (tubulin tyrosine ligase) which reversibly adds a tyrosine residue to the C-terminus of the α chain (Raybin and Flavin, BBRC 65, 1088); one possible function is to influence the partition of tubulin among cell compartments. Preliminary in vivo experiments with neuroblastoma cells suggest that, in the presence of cycloheximide, 3/4 of the tyrosine fixed is in a non-cytoplasmic fraction of tubulin (Raybin and Flavin, in press). Current studies with detergent-solubilized tubulin from a crude rat brain membrane fraction indicate that, like cytoplasmic tubulin, it can be tyrosylated by partially purified tubulin tyrosine ligase. Preliminary results suggest it can be tyrosylated to a greater extent than assembly-competent cytoplasmic tubulin, suggesting a smaller proportion of the α chains have C-terminal tyrosine already present. The membrane fraction also has appreciable ligase activity. The latter is not detectable in invertebrate ganglia or other tissues, and has a specific activity (nmole/min x mg) as follows in extracts of mammalian nerve cells: adult rat brain, 0.05; neuroblastoma NG108-15 0.05 (undifferentiated),or 0.06 (differentiated); SV-40 transformed glial cells, 0.13; sympathetic ganglia from 7 day old rats cultured 2 days with or without nerve growth factor, 0.44.

101 THE USE OF FLUORESCENCE RECOVERY AFTER PHOTOBLEACHING (FRAP) TO MEASURE LATERAL MOBILITY OF LECTIN-RECEPTOR COMPLEXES ON THE CELL SURFACE, Kenneth Jacobson and Yu Hou, Roswell Park Memorial Institute, Buffalo, N.Y. 14263

The use of fluorescence recovery after photobleaching (FRAP) to measure lateral transport of cell surface components is critically examined using the example of wheat germ agglutinin (WGA) bound to human fibroblast cells. The question of photodamage produced during the photobleaching pulse is approached by comparing the results with three different fluorophores conjugated to WGA. Internalization of the lectin receptor complexes by endocytotic pathways at the time of the measurement is checked by light microscope autoradiography of cell sections. Limitations on the interpretation of the FRAP data imposed by the contribution of top and bottom cell surfaces, by the presence of microvilli and by receptor heterogeneity will be discussed. Our present results show that the fraction of immobilized WGA receptor complexes (~15%) is appreciably smaller than that measured for the Con A receptor complexes (~60%) on the same cell type. However, the average lateral diffusion coefficient for both complexes are similar ($1x10^{-11} \leq D \leq 5x10^{-11} cm^2/sec$). In addition, the fraction of immobilized WGA-receptor complexes is small over most of the cell's surface with the exception of regions slightly back from the leading ruffled membrane where it increases to 50 to 70%.

This investigation was supported by Grant No. CA 16743, awarded by the National Cancer Institute, DHEW.

102 DISTRIBUTION OF GLYCOPROTEINS TO THOSE AREAS OF THE PLASMA MEMBRANE MOST LIKELY TO BE INVOLVED IN CELL-CELL AND CELL-SUBSTRATUM RECOGNITION, Joseph A. McClure and Kenneth D. Noonan, Department of Biochemistry, University of Florida, Gainesville, Florida.

We have recently improved a membrane isolation technique in which the "cap" of plasma membrane covering the cell nucleus of substratum-attached cells can be isolated free of the substratum-apposed plasma membrane as well as that part of the plasma membrane which extends out into the ruffled area of the cell surface. If glycoproteins are involved in recognition phenomena, one might expect them to be localized to the cell margins (where cell-cell contact occurs) or to the surface of the plasma membrane responsible for cell-substratum adhesion. In such a case many glycoproteins might be excluded from the "nuclear cap" (NC). Our data clearly show that plasma membrane glycoproteins are preferentially distributed to areas of the plasma membrane other than the NC. Using glucosamine as a precursor of glycoprotein biosynthesis, we have demonstrated a 20-fold reduction in the specific activity of glycopeptides in the NC region of the plasma membrane as compared to whole membrane ghosts. Using fucose as a precursor of glycoprotein biosynthesis, we have identified a 7-8 fold reduction in the specific activity of the glycopeptides in the NC as compared to whole membrane ghosts. Fluorographs of the glucosamine-labeled components of the various plasma membrane compartments have allowed us to localize glycoproteins to specific regions of the membrane. Taken together, our evidence suggests that, in vitro, glycoproteins are preferentially distributed to those areas of the plasma membrane where cell-cell or cell-substratum contact occurs. Such findings, by implication, strengthen the argument that cell-surface localized glycoproteins may be involved in recognition phenomena.

103 CARBOHYDRATE COMPOSITION AT HIGH SENSITIVITY USING CH_4-CHEMICAL IONIZATION GLC-MASS SPECTROMETRY OF THE ALDITOL ACETATES, Roger A. Laine, Linda C. Hodges and Allen M. Cary, Dept. of Biochemistry, University of Kentucky, Lexington, KY 40506

Analysis of chemical structure in somatic cell glycoproteins and glycolipids has been hampered both by low quantity of purified components available and by limits of detection methods. Free sugars released by hydrolysis from glycolipids and glycoproteins have been conveniently analysed in the past by either preparation of, a.) the methyl glycosides and trimethylsilylation, or, b.) by $NaBH_4$ reduction and acetylation, followed by gas-liquid chromatography using flame-ionization detection. The minimum level of detection is usually 100 nanograms per peak; however, plasticisers and other contaminants often produce ambiguous chromatograms.

We have significantly increased the sensitivity of detection for alditol acetates by chemical ionization mass spectrometry (CH_4) and computerized (signal-to-noise ratio enhancement) methods. Specific single ion monitoring of the M-59 (loss of O-acetyl) ion for per-acetyl: pentoses(m/e 303), fucose(m/e 317), deaminated products of aminosugars(m/e 273), hexoses(m/e 375), and inositol(m/e 373), allows detection of 1-10 nanograms injected on column as determined by standard mixtures. By analogous methods, linkage studies can be performed at much higher sensitivity using the conventional Lindberg technique, monitoring the M-59 ions and retention time indices of resulting peaks.

In theory, given ideal hydrolytic conditions, and using this method, it should be possible to perform sugar composition studies and preliminary linkage investigations on single bands from analytical slab gels of glycoproteins or thin layer plates of glycolipids.

104 ^{3}H-THYMIDINE IS INCORPORATED INTO MACROMOLECULAR MATERIAL WHICH IS NOT DNA, Phyllis R. Strauss and Elisabeth Daub, Dept. Biology, Northeastern University, Boston, MA 02115

Recently we demonstrated a large discrepancy between the amount of accumulated ^{3}H-thymidine and that apparently incorporated into nucleic acid by lymphocytes (1,2). The latter was 200 to 2000 percent of the former in nonadherent spleen cells from normal or conA stimulated mice. We report here that some of the discrepancy can be accounted for by labelled macromolecular material which is stripped off the cell surface when the cells are separated from medium by passage through a column of silicone oil (1). Once freed from cells the macromolecular material is poorly precipitated by 5% TCA and is incompletely precipitated by 5% TCA containing 0.5% phosphotungstic acid. The label is hydrolyzed from the macromolecular material by exposure to 0.01 N HCl at 90°C for 15 min and is recovered as thymidine, thymidine monophosphate, and thymidine diphosphate. After hydrolysis in 1N HCl at 100°C for 4 hrs, all the label is recovered as thymine. Exposure to DNase does not release low molecular weight radiolabel. We conclude that some ^{3}H-thymidine in these lymphocytes is incorporated into a macromolecular material which is not DNA and may be glycoprotein. (Supported by ACS BC-171 and NSF PCM 75-20323)

1. J. Exp. Med. 140: 1009 (1976)
2. J. Immunol., Manuscript submitted

018 THE ROLE OF MEMBRANE GLYCOPROTEINS IN RECOGNITION PHENOMENA, Gilbert Ashwell, Laboratory of Biochemistry and Metabolism, NIAMDD, NIH, Bethesda, MD 20014

Earlier studies, in vivo, revealed a protective role for the terminal sialic acid residues of serum glycoproteins in regulating their survival time in the circulation. The receptor responsible for the recognition and removal of those proteins from which part, or all, of the sialic acid had been removed was identified originally as an intrinsic constituent of the hepatic plasma membranes of mammalian species. Subsequently, the mammalian receptor was isolated from rabbit liver and characterized as an aqueous soluble glycoprotein composed of two subunits with molecular weights of 48,000 and 40,000, respectively. Pronase digestion of the intact protein resulted in quantitative recovery of the carbohydrate moiety which was shown to consist of two glycopeptides of varying composition. The carbohydrate sequence of both was determined and the relative distribution between the subunits was indicated. The availability of the purified binding protein permitted the development of an affinity column capable of detecting microquantities of circulating asialoglycoproteins. The low levels found in normal human serum were doubled or tripled in sera obtained from patients with a clinical diagnosis of cirrhosis or hepatitis. Subsequent examination of avian and reptilian species revealed an absence of the above galactose specific binding protein in these species--a finding which correlated with excessively high levels of circulating asialoglycoproteins. In this case, an alternate regulatory mechanism was found to be operative in that a new binding protein, specific for N-acetylglucosamine-terminated glycoproteins, was isolated and purified from avian liver.

The recent observations that the mammalian binding protein possesses the lectin-like ability to agglutinate erythrocytes and to stimulate mitogenesis in desialylated T-cells suggests a broader role in recognition phenomena than had been recognized heretofore. The implications of the latter finding will be discussed.

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019 COMPLEMENTARITY BETWEEN THE GANGLIOSIDE GM_2 AND THE ENZYME GM_1 SYNTHETASE IS A POSSIBLE RECOGNITION MECHANISM IN THE CHICK RETINO-TECTAL PROJECTION, Richard B. Marchase, Michael Pierce and Stephen Roth, Department of Biology, The Johns Hopkins University, Baltimore, MD 21218

Chick embryo retinal cells adhere to dissected and bisected tectal halves in a fashion that mimics the ultimate projection of the retinal ganglion cells onto the tecta. That is, dorsal retina cells preferentially adhere to ventral tectal halves and vice versa.

Using this in vitro manifestation of cell recognition as a biochemical assay, various agents can be tested for specific perturbations. We have focused on purified enzymes as biochemical probes since their effects are more precisely understood than are those of lectins and metabolic poisons, for example. In terms of retinal cell adhesion to tectal halves, proteases severely affect the ventral-most areas of the retina and tectum but leave the dorsal-most areas relatively unaffected. Of all the glycosidases tested, only beta-N-acetyl galactosaminidase perturbs retino-tectal adhesion negatively. In contrast to the proteases, this glycosidase affects only the dorsal-most areas of both the retina and the tectum. These data are consistent with the hypothesis that recognition results from complementary molecules distributed identically over the surfaces of the tectum and retina and that the dorsal-most molecules are protease resistant and terminate in N-acetylgalactosamine while the ventral-most components are protein in nature.

A search for an asymmetric distribution of glycoproteins gave negative results with great consistency. The ganglioside GM_2 appears to be morphogenetically active in this system, however. This ganglioside terminates in a beta-linked N-acetylgalactosaminide and, when incorporated into ^3H-labeled dipalmitoyl lecithin vesicles, causes the vesicles to adhere preferentially to ventral tectal halves. Lecithin vesicles alone, or vesicles with the gangliosides GM_1 or GM_3 all adhere poorly to tectal halves and none of these show any preference for either tectal half.

Finally, retinal homogenates assayed for GM_1 synthetase activity show more activity in ventral retina than in dorsal retina. Furthermore, this activity difference is only detectable after embryonic day six, precisely the time at which ventral retina acquires its ability to differentiate dorsal from ventral tectal halves. No other enzyme assayed showed this difference.

Since the gangliosides are often located on plasma membranes and since GM_1 synthetase is within a class of enzymes (the galactosyltransferases) that have also been partially localized to the plasma membrane, it is possible that the enzyme-substrate complementarity that exists between the two plays a role in the recognition underlying the retino-tectal map.

020 A ROLE FOR GLYCOSYL TRANSFERASES IN THE TURNOVER OF TISSUE-TYPE SPECIFIC CELL SURFACE LIGANDS, Jack Lilien, Joseph Hermolin and James McDonough, Department of Zoology, The University of Wisconsin, Madison, WI 53706.

Embryonic chick neural retina and cerebral lobe cells have at their cell surfaces tissue-type specific ligands which both prevent lectin induced "capping" (1) and participate in the formation of intercellular adhesions (2). The mechanism whereby these ligands are released from the cell surface into the environment has been examined. The release process is inhibited by hydroxyurea, cytosine arabinoside, EDTA, and certain nucleotide diphosphates, and is potentiated by Mn^{++}. These properties are characteristic of cell surface glycosyl transferases (3,4). Inhibition by nucleotides is highly specific: UDP is the most effective inhibitor of release of the neural retina ligand while GDP is the most effective inhibitor of release of the cerebral lobe ligand. The sugar derivatives of these nucleotides are the usual donors for glycosyl transferases catalyzing addition of the terminal sugars for the two ligands (5): N-acetyl galactosamine for neural retina and a mannosamine-like residue for cerebral lobe.

Activity is lost upon enzymatic removal of the terminal sugar from the ligand and can be restored by incubation of the deglycosylated ligand with intact cells. Furthermore, the reactivated ligand has the original tissue specificity and can be inactivated by the same glycosidase used initially to remove the terminal sugar.

These data are consistent with the hypothesis that release of ligand from the cell surface is mediated by enzymatic addition of the terminal sugar.

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021 ENDOGENOUS, DEVELOPMENTALLY REGULATED LECTINS IN CELLULAR SLIME MOLDS AND EMBRYONIC MUSCLE, Samuel H. Barondes, Department of Psychiatry, University of California, San Diego, La Jolla, CA 92093

The cellular slime molds, Dictyostelium discoideum and Polysphondylium pallidum, contain polyvalent carbohydrate-binding proteins, referred to as lectins, and assayed as agglutinins of appropriate erythrocytes. The lectins appear as the cells differentiate from a vegetative, non-cohesive form to a cohesive form. The lectins from the two species have been purified and shown to differ both in their carbohydrate-binding specificity and in a number of other properties. The cell surface location of these lectins was demonstrated using antibodies to the purified proteins as well as by a number of other techniques. Cell surface high affinity receptors on cohesive P. pallidum and D. discoideum cells have also been identified. They bind the homotypic lectins relatively specifically.

The role of pallidin, the lectin from P. pallidum in cell cohesion, has been studied in some detail. Cohesiveness of P. pallidum cells can be inhibited by: 1) specific simple sugars that interact with pallidin; 2) univalent antibody fragments prepared from antisera raised against purified pallidin; 3) asialo-fetuin, a potent inhibitor of pallidin as measured by hemagglutination assays. The asialo-fetuin is far more potent than native fetuin or asialo-fetuin modified by ß-galactosidase or galactose oxidase; both as an inhibitor of pallidin hemagglutination activity and as an inhibitor of P. pallidum cohesiveness. This series of findings suggests that the cell surface endogenous lectins in cellular slime molds play a role in specific cell cohesion (1).

A lectin from embryonic chick muscle, like that originally identified by Teichberg, et al. (2) has been shown to increase strikingly in activity with pectoral muscle differentiation (3). This lectin has been purified by affinity chromatography on a Sepharose column containing coupled lactoside. The possible role of this lectin in cell surface interactions of developing muscle will be considered.

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022 USE OF COMMON PLANT LECTINS FOR ISOLATION AND CHARACTERIZATION OF CONSTITUTIVE AND DEVELOPMENTALLY REGULATED CELL SURFACE ASSOCIATED GLYCOPROTEINS OF DICTYOSTELIUM DISCOIDEUM, Richard A. Lerner, Jasodhara Ray, and John E. Geltosky, Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California 92037

Because only minor changes in the protein composition of the plasma membrane of Dictyostelium discoideum accompany the onset of mutual cellular cohesiveness (1), it is necessary to use specific probes to select out potentially interesting molecules. One class of interesting molecules is cell surface glycoproteins, which can be isolated by use of lectin affinity chromatography.

We have previously reported that there are at least 15 Con A binding proteins associated with the cell surface, and that the appearance of at least one of the molecules (MW=150,000) is under developmental regulation (2).

Using plant lectins with different sugar specificities, we have been able to define other cell surface associated glycoproteins. The analytical approach is as previously reported: cell surfaces are first selectively radioiodinated (I^{125}) with lactoperoxidase, the cells solubilized with NP40, and the supernatant subjected to lectin column chromatography. (Lectins are coupled to Sepharose 4B activated with CNBr). The material eluted with the specific sugar is analyzed by SDS-PAGE, followed by autoradiography.

Wheat germ agglutinin, which has a sugar specificity for N-acetyl-D glucosamine recognizes the same cell surface molecules as does Con A, which is specific for α -methyl-D-mannoside. Elution of either column with the other non-specific sugar is not effective in releasing material from the column. Therefore, these 15 or so molecules contain both of these sugars in their carbohydrate side chains.

The lectin isolated from the seeds of Abrus precatorius which is specific for galactose, recognizes some of the same molecules as do the other 2 lectins; however, a number of unique molecules are also recognized by this lectin. In total, the Abrus precatorius lectin recognizes a minimum of 12 proteins, with mw's ranging from 18,000 to 130,000.

Of all the surface glycoproteins the 150K Con A binding protein is the most interesting because it is developmentally regulated and appears at the time cells become cohesive. Currently we are purifying this protein, using DEAE, hydroxyapetite and gel chromatography. Preliminary results indicate that the Abrus precatorius lectin does not bind gp150, but it does interact with a number of other Con A binding proteins, thereby providing another possible purification procedure.

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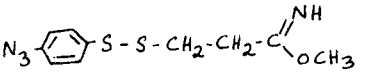
023 IDENTIFICATION OF A CELL SURFACE RECEPTOR FOR EPIDERMAL GROWTH FACTOR, Das, M., Miyakawa, T. and Fox, C.F. Department Bacteriology and The Molecular Biology Institute, University of California, Los Angeles, CA 90024.

The membrane receptor for epidermal growth factor (EGF) on 3T3 fibroblast cells has been identified and specifically labeled radiochemically using a novel heterobifunctional cleavable crosslinking reagent (I):



The reagent was covalently attached to Iodine-125 labeled EGF via the site specific imidate, to produce photoactivable radioiodinated EGF. The latter was incubated with 3T3 cells and then photolyzed 'in situ' to generate a nitrene. Analysis of the system by sodium dodecyl sulfate/polyacrylamide gel electrophoresis revealed, besides the band of EGF, only one other radioactive band of molecular weight approximately 200,000. This band was absent when a non-responsive and non-binding variant of 3T3 was used. A direct proportionality between binding activity and crosslinked complex formation was demonstrated using a vareity of binding conditions. 'Down regulated' cells, whose EGF binding activity was greatly reduced by prolonged incubation of the cells with high concentration of EGF, also showed a proportionate decrease in covalent complex formation. These observations indicate that the hormone-receptor complex can be specifically crosslinked, and that there may be only one receptor protein involved in specific recognition and binding of EGF to 3T3 cells. Preliminary experiments indicate that this procedure can also be used to identify the insulin receptor.

024 MODULATION OF CELL SURFACE CARBOHYDRATE RECEPTORS FOR LECTINS AND EFFECTS ON CELL ADHESION

R.C. Hughes, National Institute for Medical Research, Mill Hill, London NW7, 1AA, U.K.

1) Ricin resistant BHK cell lines have been classified into a small group (3 lines) showing no detectable surface change and in which resistance may be mediated intracellularly (e.g. Ric^R22), and a larger group (about 20 lines) in which some change in surface properties can be demonstrated. Many of the cell lines in the second group (e.g. Ric^R21) show a lower content of surface galactose containing glycopeptides and greatly reduced binding activity for ricin and other galactose binding lectins. 2) The cell lines showing altered surface properties adhere in general poorly to a substratum and also aggregate poorly compared to parental cells or the cell lines such as Ric^R22 in short term incubations of single cell suspensions. 3) One such cell line (Ric^R14) is deficient in a specific N-acetylglucosaminyl transferase shown to be lacking in other lectin resistant cells by Kornfield and Stanley and, alone among the 20 cell lines showing a surface change, expresses normally at its cell surface the high molecular weight 250K glycoprotein labelled by lactoperoxidase catalysed iodination. Since this cell line (Ric^R14) behaves abnormally in cell-cell adhesion and cell-substratum attachment, a role for the 250K glycoprotein in normal cell adhesion is questioned, unless some structural change not detected by our methods has occurred in the 250K glycoprotein to affect its function but not its localization at the cell surface. 4) Treatment of receptor-deficient cells including Ric^R14 and Ric^R21 with *neuraminidase* to release surface bound sialic acid renders the cells very sensitive to ricin toxicity without affecting appreciably their adhesive properties, suggesting structural heterogeneity of surface receptors for ricin and diverse roles in adhesion and in mediating ricin toxicity.

025 RELATIONSHIPS IN THE STRUCTURE AND FUNCTION OF CELL SURFACE RECEPTORS FOR GLYCOPROTEIN HORMONES, BACTERIAL TOXIN, AND INTERFERON

Leonard D. Kohn, National Institute of Arthritis, Metabolism, and Digestive Diseases, NIH, Bethesda, Maryland 20014

Gangliosides inhibit ^{125}I-thyrotropin (TSH) binding to the thyrotropin receptor. This inhibition, which appears to be hormonally specific, is critically altered by the number and location of the sialic acid residues within the ganglioside structure. The inhibition results from the interaction of gangliosides with TSH, rather than with the membrane receptor. The ganglioside-TSH interaction is associated with a distinct conformational change of the TSH molecule. The possibility that a ganglioside or ganglioside-like structure is a component of the thyrotropin receptor is suggested by the finding that gangliosides more complex than G_{M3} are present in bovine thyroid membranes in much higher quantities than have been previously found in extraneural tissue and are absent in a thyroid tumor with no TSH receptor activity. The finding that cholera toxin, which also interacts with gangliosides, has peptide sequences in common with TSH, suggests that TSH and cholera toxin may be analogous in their mode of action. It is suggested, therefore, that a ganglioside or ganglioside-like structure is a basic component of the thyrotopin receptor and that cholera toxin and TSH have a common mechanism by which their message is transmitted to the cell machinery (1-4).

Data will be presented which relate these results to the structure and function of cell surface receptors for other glycoprotein hormones (luteinizing hormone, human chorionic gonadotropin, and follicle-stimulating hormone) (5); for interferon (6, 7); and for other bacterial toxins such as tetanus toxin. The date indicate that each of these agents is similar in its mechanism of cell surface interaction and in its mechanism of modulating the cell machinery.

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026 RECEPTOR-MEDIATED UPTAKE AND CLEARANCE OF LYSOSOMAL ENZYMES,

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Neufeld and co-workers discovered in vitro corrective factors for enzyme deficient fibroblasts that indicated specific pinocytosis of lysosomal hydrolases by fibroblasts. They also found indirect evidence that carbohydrate components of the enzyme were recognized in the uptake process, and that I-cell fibroblasts produce enzymes defective in uptake capacity for fibroblasts. (1)

We have used β-glucuronidase deficient fibroblasts to study this process. Charge heterogeneity was found for human β-glucuronidase and the "high uptake" forms of the enzyme were identified in acidic fractions of the enzyme. "High uptake" enzyme was converted to "low uptake" enzyme following pinocytosis. The kinetics of pinocytosis of "high uptake" enzyme from platelets were characterized, and competitive inhibition of the uptake process demonstrated for certain hexoses, hexose phosphates, and yeast mannans which contain phosphate. These inhibitor studies, plus the observation that alkaline phosphatase destroys the high uptake capacity of human platelet β-glucuronidase, suggest a novel receptor on fibroblasts that may recognize hexose phosphate on glycoproteins.

Another type of carbohydrate recognition mediates clearance of some lysosomal enzymes in vivo. Human placental β-glucuronidase is predominantly a "low uptake" form of enzyme, i.e., not specifically pinocytosed by human or rat fibroblasts. Yet infused enzyme was rapidly cleared from rat plasma, was localized in liver, and was demonstrated in Kupffer cells by indirect immunofluorescence. Clearance of infused enzyme was inhibited by mannose-terminal glycoproteins and by free mannose. Although asialoglycoproteins did not inhibit clearance, agalacto-orosomucoid did inhibit clearance. Unexpectedly, when the cross-specificity of clearance of yeast mannan and agalacto-orosomucoid was examined, each was found to inhibit clearance of the other.

Thus clearance of human placental β-glucuronidase appears to be mediated by a system which recognizes mannose. This suggests a new receptor system, or mannose recognition by the system previously described for clearance of N-acetylglucosamine-terminal glycoproteins.

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027 BIOSYNTHESIS, SECRETION AND ATTACHMENT OF LETS GLYCOPROTEIN IN NORMAL AND TRANSFORMED CELLS,

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LETS glycoprotein is a cell surface glycoprotein of normal fibroblasts and myoblasts. It is reduced in level or absent on the surfaces of transformed derivatives (1). LETS protein is present in fibrous networks around the normal cells (2,3). Addition of purified LETS protein to transformed cells causes them to acquire more normal morphology and adhesive properties (4,5). This added LETS protein is found in a network similar to that on normal cells (5).

Metabolic labeling studies of synthesis of LETS protein show that there is a lag of about 1 hour between the completion of the polypeptide chain and its appearance on the cell surface. During this period, sugar residues are added to the protein.

In addition to the LETS protein on the cell surface, there is a large pool of LETS protein secreted into the culture medium. Prelabeled LETS protein on the cell surface is released into the medium and the molecules in the medium can attach to normal or transformed cells under a variety of conditions.

There is evidence from a variety of experiments for intermolecular attachments between surface polypeptides, both covalent and non-covalent. These appear to be involved in the attachment of LETS protein to the cell surface. Evidence for transformation related alterations in these various steps in processing of LETS protein will be presented.

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105 INDUCTION OF CELL ADHESION AND CELL SPREADING BY VARIOUS CELL SURFACE LIGANDS. Frederick Grinnell, University of Texas Health Science Center, Dallas, Texas, 75235.

Under routine conditions of cell culturing, baby hamster kidney (BHK) cells attach to the substratum by virtue of a unique serum glycoprotein (spreading factor) adsorbed to the substratum surface. Adsorption of other serum proteins to the substratum (e.g., albumin) prevents cell adhesion and spreading. Spreading factor can be substituted for by adsorption onto the substratum of various types of ligands which specifically bind to cell surface receptors. Cell spreading occurred onto substrata pretreated with concanavalin A (Con A), polycationic ferritin (PCF), or immunoglobulin directed at the BHK plasma membrane (Anti-BHK). The shape of cells spread onto Con A or Anti-BHK substrata was triangular (similar to the shape of cells spread onto spreading factor); however, the shape of cells spread onto PCF substrata was circular. Divalent cations, which are required for spreading of cells onto spreading factor, were not necessary for spreading of cells onto Con A, PCF or Anti-BHK substrata. On the other hand, energy metabolism inhibitions, sulfhydryl binding reagents, low temperature, and prefixation of cells inhibited cell spreading onto all the substrata tested. Specific blocking reagents of the various ligands also inhibited their induction of cell spreading; i.e., α-methylmanoside for substrata coated with Con A, heparin for substrata coated with PCF, and anti-immunoglobulin for substrata coated with Anti-BHK. The data suggest that cell behavior can be readily modulated by cell surface ligands which are part of the extracellular matrix. (Supported by grants from the N.I.H., CA-14609 and GM-34552.)

106 CELL SURFACE GLYCOSYLTRANSFERASES: DO THEY EXIST? Wolfgang Deppert, Max-Planck-Institut für biophysikalische Chemie, 34 Goettingen, FRG, and Gernot Walter, The Salk Institute, San Diego, Ca. 92112.

The presence of glycosyl transferases on surfaces of mammalian cells has been reported by many investigators and a biological role for these enzymes in intercellular adhesion and cell recognition has been postulated. Critical analysis, however, showed two major complications regarding the assay for cell surface glycosyl transferases: (1) hydrolysis of the nucleotide sugar by cell surface enzymes and subsequent intracellular use of the free sugar (2) loss of cell integrety, if trypsinized or EDTA treated cells were used in suspension assays. Therefore, many of the experiments reported may have been performed with dead or dying cells. We have assayed intact, viable cells in monolayer for cell surface glycosyl transferases using conditions, under which intracellular utilization of free sugars generated by hydrolysis of the nucleotide sugar was prevented. No evidence could be detected for the presence of glycosyl transferases on a variety of cells, including established (normal and virally transformed) as well as non established cells.

107 ADHESION CHARACTERISTICS OF CHINESE HAMSTER CELL MEMBRANE MUTANTS WITH ALTERED SURFACE GLYCOPROTEINS, Rudy Juliano, Research Institute, Hospital for Sick Children, Toronto, Ontario.

I have attempted to investigate the role of surface carbohydrate moeities in cell adhesion by examining the adhesive characteristics of several CHO cell mutants which possess altered membrane glycoproteins. The lines employed include wild type (WT) CHO cells, C2P1 a phytohaemagglutinin resistant mutant, and C4S4 and C5S3 which are drug permeability mutants. It has previously been deomonstrated that lectin resistant variants such as C2P1 have radically altered cell surface glycoproteins in that the oligosaccharide side chains are truncated and lack penultimate galactose residues and probably sialic acid residues as well. The drug resistant variants C4S4 and C5S3, on the other hand, possess an extra cell surface glycoprotein of MW 170,000 which is absent in WT cells. I have studied the rate of adhesion of WT and mutant cells to protein coated glass and plastic surfaces, and have also examined the detachment of cells from such surfaces by trypsin treatment. The adhesion kinetics of the various lines are all very similar; thus, at 30°C, 52% of WT cells, 57% of C2P1 cells, and 58% of C4S4 cells adhere during a 20 minute interval. By contrast the wild type and mutant lines manifest distinct differences in the ease with which they are detached by trypsinization. Thus treatment with 100 μg/ml crystalline trypsin at 25°C for a 20 min interval detached 25%, 7%, and 64% of WT, C2P1 and C4S4 respectively. The fact that gross modification of the cell surface glycoproteins does not substantially alter the adhesive rates of CHO cells suggest that much of the surface carbohydrate is not directly involved in the initial events of cell to substratum adhesion.

108 INTERCELLULAR INTERACTION: A NOVEL MODEL INVOLVING COVALENT INTERPEPTIDE LINKAGES. B.T.Walther & B.Barlam, Dept. of Anat. Sciences, HSC, SUNY at Stony Brook, NY11794,& Dept. of Biology, The Johns Hopkins University, Baltimore, MD. 21218.

Cell:cell recognition and adhesion proceeds by unknown molecular codes. A specific molecular model is proposed, and tested for its relevance to cellular interactions. -Isopeptide-bonds (between γ-carboxyl groups of glutamine, and ε-amino groups of lysine) have wide biological distribution, and the responsible transglutaminase-enzymes exhibit a high degree of specificity for the polypeptides being crosslinked. Such linkages have also been reported in eukaryote cell membranes. -We propose that the formation of isopeptide-bonds between surface proteins of 2 different cells may account for adhesion or recognition between such cells (in a manner somewhat resemblant of bacterial cell wall construction). -Some support for this model has been obtained in experiments using purified pigeon liver transglutaminase, and Balb/c 3T3 and 3T12 cells forming adhesive bonds in the cell layer assay. Thus, these cells contained a substrate (about 220 000 D) for exogeneous transglutaminase (detected by ^{3}H-putrescine incorporation into TCA-ppt. material).Furthermore, Monodansylcadaverine (at 10^{-4}M) was 6 and 12fold more effective than resp. monodansylethanediamine and monodansyl-ε-lysine, in blocking cell adhesion. However, only background levels of endogeneous transglutaminase activity was detected (^{3}H-putrescine incorporation with or without added α-casein), and exogeneous transglutaminase, with or without added putrescine, did not affect rates of adhesion of these fibroblasts, when added at 0.1mg/ml. (Supported by a grant from the A.Jahres Foundation.)

108A GLYCOPROTEINS AND GLYCOLIPIDS IN INTERCELLULAR ADHESION OF MOUSE FIBROBLASTS. Bernt Walther, Dept. of Anat. Sciences, H.S.C., SUNY at Stony Brook, NY 11794.

The cell layer assay was used to probe the involvement of cell surface carbohydrates during intercellular adhesion of Balb/c 3T3, 3T12 and SV40 3T3 cells. Competitive and non-competitive carbohydrate-analogs were screened for possible effects on Type I,II & III adhesive reactions (Walther et al.(1976)J.Cell Biol. 70,70a). The data fail to substantiate any participation of the carbohydrate-moieties of glyco-proteins and -lipids in such early adhesive reactions, in accordance with previous studies (Walther, B.T. (1975) Fed.Proc.34,498a). -Thus, p-nitrophenyl-D-glycosides (α-&β-) of glucose, galactose, mannose, xylose, N-acetyl-glucosamine and -galactosamine, mannosamine, and L-fucose, all failed to affect the rates of Type I,II &III adhesion, at levels of 10mM. Thiodigalactoside and γ-D-galactolactone (3mM)also were ineffective, as were 3mM of n-hexane-6-amino-1-thio-β-glucoside,-galactoside and -N-acetylglucosamine.-A series of affinity-labels for carbohydrate-reactants of the glycocalyx were synthesized,purified,crystallized, and tested for effects on cell adhesion. At 5mM levels of allylglycosides of (α&β) glucose,galactose,N-acetylglucosamine, and (β-)lactose and mannose, rates of adhesion (I&II) were normal. In addition, n-3,4-butenyl- and n-4,5-pentenyl-β-galactoside did not affect rates of adhesion, nor did 2,3-epoxypropyl-(α&β)-galactoside (5mM). Since these compounds have been shown to irreversibly inactivate both glycosidases and glycosyltransferases, the negative evidence is considered significant.-Finally, two mixtures of glycosidases (0.1mg/ml) from T.Cornutus and C.Lampus (MILES) did not affect rates of Type I,II&III adhesion. (Supported by Leukemia Society of America Special Fellowship)

109 CELL SURFACE CARBOHYDRATES OF PREIMPLANTATION EMBRYOS, Anna G. Brownell. Develop. Biology Lab., School of Dentistry, Univ. of Southern Calif., Los Angeles, Ca. 90007.

Implantation of the mammalian embryo into the uterus occurs at the blastocyst stage and can be divided into two discrete steps: (1) attachment of the embryo to the endometrium, and (2) invasion of the maternal tissue. The attachment step represents initial contact between embryonic cells and the maternal endometrium. Knowledge of the types of macromolecules on embryonic and maternal cell surfaces should aid in understanding heterotypic cell interactions leading to implantation. To this end, fluorescent lectin labeling of isolated mouse blastocysts was used to study the carbohydrate components. FITC-Concanavalin A (Con A) was observed to bind blastocyst cell surfaces as well as the zona pellucida, an acellular mucoprotein covering of the embryo. Lateral mobility of membrane receptors for Con A was demonstrated by incubation of blastocysts at 4° and 37°. Capping of embryonic cell surfaces occurred at 37°. FITC-Wheat germ agglutinin also bound to the blastocyst surface. In this case capping did not occur. FITC-Fucose-binding-protein did not bind to the blastocyst. Specificity of lectin binding was demonstrated by two methods: a) competitive inhibition with monosaccharides known to bind the lectin, and b) binding of FITC-bovine serum albumin to the blastocyst as an indication of non-specific protein binding capacity. From these studies, it was determined that α-D-glucose and/or α-D-mannose, as well as N-acetylglucosamine, were present on blastocyst cell surfaces presumably as portions of glycoproteins and/or glycolipids. L-fucose was not detected. Invasiveness, the second stage of the implantation process, was studied using in vitro implantation of blastocysts on collagen substrates analyzed by scanning electron microscopy. Supported by NIDR fellowship #5 F22 DE01631-02.

110 TANDEM EVENTS IN MYOBLAST FUSION, Karen A. Knudsen, Alan F. Horwitz, University of Pennsylvania, Philadelphia, Pa. 19174

Myoblasts, derived from primary chick pectoral muscle explants and grown on collagen coated culture dishes in a low calcium medium, are harvested with EDTA and gently agitated in suspension. In the presence of calcium the cells rapidly form easily dissociable aggregates which exclude fibroblasts. The apparent strength of adhesion increases with time until, under appropriate conditions, the myoblasts fuse in suspension to form multinuclear cells. The calcium dependent, dissociable aggregation shows optima for pH, temperature, calcium concentration, and culture age that closely parallel those observed for myotube formation measured with cells attached to tissue culture plates. We conclude from this marked correlation between the effects of these variables on myoblast aggregation and myotube formation that cell-cell adhesion is an integral part of myoblast fusion. Furthermore, we suggest that the formation of multinuclear cells is the result of a sequence of steps beginning with cell-cell recognition and adhesion, progressing to membrane union and ultimately ending after subsequent morphologic changes producing the morphologies characteristic of multinuclear cells both in suspension and on tissue culture plates.

111 HETEROGENEITY IN THE CELL ADHESION CARBOHYDRATE BINDING PROTEINS OF THE CELLULAR SLIME MOLDS,William A. Frazier and Beverly Pardos, Washington Univ.School of Medicine,St. Louis,Mo. 63110

The basis of species-specific cell adhesion in the cellular slime molds is believed to be the interaction of membrane-associated carbohydrate binding proteins with complementary oligosaccharide receptors. Both components appear during differentiation to an aggregation competent,multicellular state and are absent in vegetative cells. These carbohydrate binding proteins have been identified in all species of slime mold as intracellular,soluble agglutinins of red blood cells (lectins),each having a unique sugar binding specificity. In Dictyostelium discoideum two tetrameric lectins called discoidin I and II are made, having subunit molecular weights of 26,000 and 24,500 and isoelectric points of 6.0 and 7.0 respectively. We now report that both proteins can exist in several different states regarding pI,quaternary structure and consequently agglutination activity. Nonagglutinating forms of both have been found as the only constituents of the intracellular pool. They have pI's(app) from 8.0 to 9.5 and are monomeric as judged by sedimentation and cross linking experiments. Their amino acid compositions,however,are very similar to the tetramers of low pI. In spite of these gross differences in the soluble proteins,the cells aggregate and sporulate normally. These and other data suggest that the functional form of the carbohydrate binding protein on the cell surface may be quite different from the form which accumulates inside the differentiating cells.

112 A GRADIENT OF CELL-CELL ADHESIVE SPECIFICITY IN THE DEVELOPING CENTRAL NERVOUS SYSTEM - David I. Gottlieb and Charlotte Arington, Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri 63110 - The telencephalon is the most rostral of the primitive vesicles of the developing brain and is followed caudally by the diencephalon and mesencephalon. Cell-cell binding studies using a monolayer adhesion technique show that single telencephalon cells bind to mesencephalon cell monolayers more rapidly than to telencephalon cell monolayers. Mesencephalon cells bind more rapidly to telencephalon than to mesencephalon monolayers. Diencephalon cells bind equally well to telencephalon and mesencephalon monolayers. Finally, rostral mesencephalon cells bind preferentially to caudal mesencephalon cells and vice-versa. These results are best explained by assuming that two complementary gradients of adhesive determinants run along the rostro-caudal axis of the developing brain. Further results show that these gradients are functionally related to the gradients of adhesive specificity found in the developing retina[1]. The brain adhesive determinants are highly trypsin sensitive and will regenerate if trypsinized cells are allowed to recover. Plasma membrane vesicles from the telencephalon bind preferentially to tectal cells.

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Supported by PHS Grant 1R01-NS12867-01.

113 Cell Interaction and Migration in Primary Cultures of Embryonic Mouse Cerebellum: Synthetic Carbohydrate-derivatized Surfaces, Mary E. Hatten and Richard L. Sidman, Dept. Neuropath., Harvard Med. Sch., Boston, Ma. 02115 and Dept. Neurosci., Children's Hosp. Med. Ctr., Boston, Ma. 02115 When plated at high cell density in a microwell culture system, freshly dissociated embryonic mouse cerebellar cells assemble into reproducible three-dimensional patterns. Other cell populations such as embryonic midbrain, medulla or cerebral cortex assemble into different patterns, suggesting distinct sets of cell-cell interactions for different cell populations of the developing brain.

Agglutination studies with lectins of dissociated cell populations harvested from different regions of the embryonic brain reveal that different lectins agglutinate cell populations from different embryonic brain regions. Cells from E.13 cerebellum are agglutinated with ConA, WGA, RCA-60, RCA-120, and lens culinaris, but not by SBA or a fucose-binding protein. Cells from the midbrain are agglutinated only with ConA, RCA-60 and RCA-120, those from the cerebral cortex are agglutinated only with lens culinaris, and those from the medulla are agglutinated only with RCA-60 and RCA-120. In addition, agglutination of cerebellar cells with ConA, WGA and RCA is diminished over the course of development from embryonic day 13 to postnatal day 7. These studies suggest regional differences in the cell surfaces of the developing brain that are further modulated during the differentiation of the tissue.

On Poly-D-lysine treated substrata in microwell cultures cell migration is unique to the cerebellum. Surfaces treated with carbohydrate-derivatized Poly-D-lysine are currently being tested for their efficacy as substrates for differential cell migration.

114 AVIAN CELL SURFACE AND SERUM GLYCOPROTEINS WHICH CROSS REACT WITH THE VIRAL SLYOCPROTEIN (GP85), John E. Smart and Valerie Bosch, Imperial Cancer Research Fund, Lincoln's Inn Fields, London

^{3}H-glucosamine labeled cell extracts were precipitated with mono-specific antiserum against avian sarcoma Gp 85. Cross reacting molecules were found on all chicken cells (infected, uninfected, checked helper factor positive (Chf^{+}) and negative (Chf^{-})). SDS polyacrilamide gel electrophoresis of lectin affinity fractionated cross reacting antigens revealed not only the viral Gp85 (infected cells), but also a glyco-protein of slightly higher molecular weight (infected and uninfected cells), plus a third glycoprotein of c.a. 130,000 daltons (infected and uninfected cells).
We have also observed that the serum of leukosis free chickens strongly competes in a radio immunoprecipitation assay of ^{125} I-labeled viral Gp85 and monospecific aniti-Gp85 antiserum. The competing activity is a glycoprotein which reacts with the anti-Gp85, is non-dialysable, and is resistant to denaturation at 100°C. in SDS and reducing agents. Preliminary results indicate that the activity resides in a serum glycoprotein of c.a. 85,000 daltons.

115 SEA URCHIN SPERM-EGG BINDING AS AN in vitro SYSTEM FOR CELL SURFACE RECOGNITION: ISOLATION AND CHARACTERIZATION OF THE VITELLINE LAYER OF THE EGG, Charles G. Glabe University of California, Davis. Davis, Ca. 95616

An early event in fertilization of Echinoderms is the binding of sperm by the material contained in the acrosomal vesicle to the vitelline layer (VL) covering the plasma membrane of the egg. Sperm do not bind to eggs of another species and this binding is a recognition step to ensure species specific fertilization. The vitelline layer of the egg has been isolated as an intact structure with its sperm binding abilities preserved. Gel electrophoresis in SDS reveals that the isolated VL is composed of many polypeptide bands, two of which stain for carbohydrate with the PAS stain. Lactoperoxidase catalysed ^{125}I iodination of the egg surface before the VL is isolated labels a subset of the polypeptide bands and labels one of the PAS positive bands heavily. If the VL is labelled after isolation, essentially all of the polypeptide bands are accessible to labelling. When sperm are added to iso-VLs, they adhere only to the outer surface, suggesting that the inner and outer surfaces are of different composition. It is possible that one of the bands most accessible to lactoperoxidase iodination on the egg surface may be involved in sperm binding. Isolation of the VL could provide an advantageous in vitro system for determining the molecular mechanism of sperm-egg recognition. Work supported by NIH Grant No. HD 08645.

116 CELL SURFACE CARBOHYDRATE RECOGNITION AND THE VIABILITY OF ERYTHROCYTES IN CIRCULATION, David Aminoff, William C. Bell and William F. VorderBruegge, Dept. of Internal Medicine (Simpson Memorial Institute), University of Michigan, Ann Arbor, MI 48109.

Sialidase from V. cholera removes 95+% of the total sialic acid present on the erythrocytes tested; dog, goat, rabbit and chicken. The effect of this removal on the viability of RBC in circulation was monitored by the $Na_2{}^{51}CrO_4$ tagging procedure. Autologous transfusions of asialo-erythrocytes result in their rapid removal from circulation in the dog, goat and rabbit, but not in the chicken. When the sialidase treatment is followed by galactose oxidase, chicken erythrocytes are also removed as are those of dog and rabbit. These results are compatible with the interpretation that the chicken nucleated erythrocytes, in contrast to those of mammalian erythrocytes, has the capability to resialate the asialo sites. We have, moreover, shown that there is a preferential recognition of sialidase-treated, as contrasted to -untreated erythrocytes, by either mononuclear spleen or hepatic Kupffer cells. The decreased sialic acid content found in older erythrocytes, taken together with the above observations in autologous systems, suggests that sialic acid plays an important role in the viability of erythrocytes in circulation under normal physiological conditions. This system is analogous but not identical to the system of Ashwell, et al. for the clearance of asialoglycoproteins from circulation.
(Supported in part by USPHS HL AM 17881 and in part by ONR N-00014-76-C-0269.)

117 SPECIFIC ROTATION-MEDIATED AGGREGATION TO PURIFY ENDOCRINE CELLS FROM ISLETS OF LANGERHANS. Ronald C. Merrell, Dept. of Surgery, Washington University, St. Louis.

In vitro studies of normal (i.e. non-dividing) endocrine cells have been hampered by the difficulty in maintaining the cells in optimal culture conditions without the proliferation of fibroblasts. Islets of Langerhans from male Lewis rats were isolated on a ficoll gradient after collagenase digestion of the minced pancreas. Further digestions of the islets after a period of recovery in monolayer culture with crude trypsin gave a single cell suspension. Rotation-mediated aggregation in complete medium resulted in α,β cell coaggregates containing approximately 90% β cells. Fibroblasts and fibrous elements did not adhere to these aggregates and could be completely excluded by successive decantation. Returning the endocrine aggregates to monolayer culture failed to reveal fibroblast appearance even over periods of 3-4 weeks. The β cell enriched coaggregate responded appropriately to a glucose challenge with a biphasic insulin secretion curve attaining a maximal secretory rate of 650μ U/10^4 cells/hr. Insulin secreted into the medium correlated with β cell degranulation observed in microscopic sections stained with aldehyde fuchsin. Insulin was measured by double antibody radioimmunoassay. The endocrine cells would adhere to Chang liver cells in rotating culture to form random coaggregates which in 24 hours sorted out with endocrine domains within a Chang cell matrix. The functional properties of these coaggregates showed only a slightly blunted insulin secretion curve.

118 MYOBLAST SPECIFIC CELL SURFACE ANTIGENS. Martin Friedlander and Donald A. Fischman. Department of Biology, University of Chicago, Chicago IL 60637

Antisera raised in rabbits against pre-fusion myoblasts (Anti-M-24) has been characterized and will distinguish this cell type from mature skeletal muscle fibers and cells from other embryonic chick tissues (Friedlander and Fischman, 1976, J. Cell Biol. 67:124a). Complement dependent ^{51}Cr release cytotoxicity experiments indicate that: 1) freshly trypsinized (20,000 U/ml of minced tissue X 60 min/37°C) myoblasts more effectively inhibit the cytotoxicity of Anti-M-24 tested on myoblast monolayers than myoblasts allowed to recover from trypsin during 3.5 hr of preaggregation; 2) high concentrations of trypsin (30,000 U/0.1 ml packed, freshly dissociated cells X 10 min/37°C) and neuraminidase (3.0 IU) remove the antigen from the cell surface of myoblasts; 3) the ability of non-muscle cells to lower cytotoxicity of Anti-M-24, by removing species-related antibody specificites for myoblast target cells, is unaffected by trypsin; and 4) absorption of Anti-M-24 with increasing numbers of trypsin- or neuraminidase-treated fibroblasts from skeletal muscle rapidly lowers the cytotoxicity of this serum for fibroblast target cells but not for myoblasts. Immunofluorescence reveals: 1) more surface antigens are exposed on freshly dissociated than preaggregated muscle cells; 2) Anti-M-24 stains only myoblasts after absorption with embryonic heart, liver, brain, myotubes and RBC's or adult liver and skeletal muscle; and 3) distribution of myoblast-specific antigenic sites differ if crude or absorbed Anti-M-24 is used for staining. We conclude that myoblasts possess trypsin- and neuraminidase-sensitive surface antigens that distinguish myoblasts from other cell types of the chick embryo and adult muscle. (M.F. is an M.D.A. post-doctoral fellow; supported by grants ACS 1N-41-P, PHS 1-P01-CA1926501 and NHLI 13505-05.)

119 EVIDENCE FOR POST-TRANSCRIPTIONAL MODIFICATION OF MEMBRANE PROTEINS EXPOSED ON THE OUTER SURFACE OF VIRUS-TRANSFORMED FIBROBLASTS.
P.M.Comoglio and G.Tarone,Dept.Human Anatomy, Univ.of Torino (Italy)
Plasma membrane proteins exposed on the outer surface of control and RSV or Polyoma Virus transformed BHK-fibroblasts were selectively labelled with the probe TNBS (Trinitrobenzene sulphonate). Labelled membrane proteins were solubilized, purified by affinity chromatography on insoluble anti-probe antibodies ,separated on hydroxylapatite column and analyzed in SDS-PAGE. It has been shown that control and transformed fibroblasts expose at their surface ten major protein components with molecular weights ranging between 25 K and 200 K. Transformation by both viruses caused the appearance of a non-virion extra protein band of apparent mol.w. of 80 K. In BHK fibroblasts transformed by the FU-19 temperature sensitive mutant of RSV, this band was observed in patters obtained with cells grown at the permissive (37°C) but not at the restrictive (41°C) temperature. The extra band disappeared from the gel pattern of transformed cells after digestion of the cell surface with vibrio cholerae neuraminidase. These findings suggest a possible alteration of glycoprotein biosynthesis in transformed cells that leads to abnormal sialylation of one membrane component.

120 WHAT ARE THE ALTERNATIVES AFTER CELL-CELL RECOGNITION? A THEORETICAL APPRAISAL OF POSSIBLE ROLES FOR CELL SURFACE-ASSOCIATED PROTEOLYTIC ACTIVITY, Zoltán A. Tökés, Cell Membrane Lab, LAC/USC Cancer Center, Dept. Biochem., USC School of Med., Los Angeles, CA 90033

The first step in cell surface interactions leads to a choice among alternative events which will determine the consequence of recognition. In numerous systems recognition occurs through membrane glycoproteins (GP) which may result in either temporary restriction of their mobility, or in their relocation into patches and subsequently into a cap. This often leads to sudden shedding of membrane components. The availability of neutral proteolytic activity at the vicinity or on the surface of viable cells is supported by experimental observations. These proteases are active in modifying the surface of a "recognized entity". Their activity can be enhanced by membrane modifications, such as partial oxidation by periodate or by the disruption of membrane integrity. The enzyme active site is approachable from outside by inhibitors like ^{3}H-DFP. Intact membrane topography restricts the enzyme from randomly digesting the cell's own components. Since recognition results in the adhesion of two cells, it is conceivable that transmission of a signal via surface enzymes occurs either unidirectionally or mutually. Restriction of membrane GP mobility may trigger the proteolytic activity. Some GP acts as shields or as inhibitors for such enzymes. The absence of these GP would leave the recognized cell defenseless and thus cytotoxicity could be the consequence of recognition. A partial surface modification may result in promoting cellular differentiation or in allowing the cell to escape from junctions under early stages of differentiation at the time of cellular migration. Examples will be reviewed for each of these circumstances. (Supported by NIH Grant No. CA-14089.)

121 PREPARATION AND CHARACTERISTICS OF CELLS MADE PERMEABLE WITH TOLUENE, Jack R. Robinson, Jr., and Paul A. Srere, Veterans Administration Hospital and the University of Texas Health Science Center of Dallas 75235.

The cell and mitochondrial membranes of baby hamster kidney strain 13b and HTC cells have been made permeable to small molecules with mild toluene treatment. Intact cells (20 mg/ml cellular protein) are treated with 0.1% toluene at 37° in $CaCl_2$-free Hank's balanced salt solution containing 10% polyethylene glycol and 0.1% bovine serum albumin. After 5 minutes of constant shaking, the cells are centrifuged, and resuspended in the same volume of the solution above, and enzyme activities associated with the pellet and supernatant solution are determined spectrophotometrically. In both cell lines, release of citrate synthase from cells is ∿3%, yet 70-80% of the enzyme present (measured after sonic oscillation of the cells) can be measured. The toluene treated cells are stable, in that 24 hours later no further citrate synthase (or malate dehydrogenase) was released into the supernatant solution.

Comparative electron micrographs of treated and non-treated cells have been examined and the differences in gross membrane structure will be presented. Studies have been carried out to determine the size of molecules which can now enter the cells.

Using this method, the activities of citrate synthase and malate dehydrogenase have been examined, both in separate assays and as a coupled enzyme system of both enzymes with the malate dehydrogenase supplying the oxalacetate for the citrate synthase reaction. The treated cells show an enhanced rate for the coupled system at low malate concentration, when compared to cells disrupted by sonic oscillation.

122 CHEMICAL AND IMMUNOLOGICAL STUDIES OF CELL SURFACES FROM NORMAL AND TRANSFORMED CELLS, William J. Grimes, Gary A. Van Nest, Arthur R. Kamm, Univ. of Arizona, Tucson, AZ 85724.

Immunological and chemical studies of cell surfaces from normal and transformed BALB/c fibroblasts have shown alterations associated with transformation. The cells studied include normal lines which do not cause tumors when injected into BALB/c mice, viral transformants, and spontaneous transformants which cause tumors that either regress or grow progressively, killing the host. The spontaneously transformed progressors include cell lines which are immunogenic and non-immunogenic as determined by the ability of tumor excision to protect an animal from subsequent rechallenge by tumor cells. Tumor-bearing mice produce lymphocytes which are non-specifically cytotoxic for all the normal and transformed lines. Some of the cell lines induce specific antibody formation in BALB/c hosts. Antisera has been prepared in rabbits which is specific for the transformed cell lines. These antisera are being used to determine specific surface changes on the transformed cells. Chemical studies have shown glycolipid alterations between the normal cells and some, but not all, of the transformants. Carbohydrate compositions of the various lines have been determined by gas liquid chromatography. Glycoproteins labeled by ^{125}I-lactoperoxidase or [^{3}H]-glucosamine were compared by SDS gel electrophoresis. Results from these studies do not show changes associated with malignancy. Individual glycoprotein regions from gels were treated with pronase, and the glycopeptides compared by G-50 Sephadex chromatography. Alterations in glycopeptides from several cellular glycoproteins are the only changes which predict malignancy. Results will be correlated between the chemical studies and the immunoreactivity of cell lines in tumor-bearing mice. (Supported by grants CA 12753 from NCI and BC 131 from ACS).

123 INCREASED CELL-CELL "STICKINESS" IS ASSOCIATED WITH GROWTH INHIBITION Raphael J. Mannino*, Kurt Ballmer and Max M. Burger, Dept. of Biochemistry, Biocenter, Univ. of Basel, CH4056 Basel, Switzerland.

Succinylated concanavalin A (succinyl-conA) a non-toxic, non-agglutinating derivative of the jack bean lectin concanavalin A (conA), induces a cell-cell "stickiness" in both 3T3 and SV40 transformed 3T3 cells. This increased cell-cell "stickiness" is prevented by and reversed by α-methyl mannoside, a ConA specific hapten. Succinyl-conA also inhibits the growth of both 3T3 and SV40 3T3 cells. Growth inhibition is dependent upon both succinyl-conA concentration and cell density. Succinyl-conA inhibited 3T3 and SV40 3T3 cells accumulate in the G_1 phase of the cell cycle and exhibit a rate of ^{3}H-thymidine incorporation into acid precipitable counts similar to that of a 3T3 contact inhibited monolayer. Growth inhibition is reversed by and prevented by α-methyl mannoside. 3T3 and SV40 3T3 cells released from succinyl-conA growth inhibition proceed synchronously through the cell cycle. Morphologically, 3T3 and SV40 3T3 cells in the presence of succinyl-conA form patches leaving large areas of the substratum void of cells. Time lapse movies visually demonstrate the succinyl-conA induced cell-cell "stickiness" and the reversible growth inhibition of both 3T3 and SV40 transformed 3T3 cells. These data suggest that among the various possible mechanisms of growth control, some type of intercellular surface interaction may be involved.

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124 CELL SURFACE CHANGES ASSOCIATED WITH MYOBLAST DIFFERENTIATION, L.T. Furcht, G.W. Crabb, P. Woodbridge, Dept. Pathology, Univ. of Minnesota, Minneapolis, MN 55455

L6 myoblasts are mononucleated and with differentiation fuse to form multinucleated myotubes. Spontaneous differentiation may be modulated by various hormones. Insulin (15 μg/ml) initially promotes proliferation which is followed by enhanced differentiation. Dexamethasone (dex) has a biphasic effect; 10^{-5} M stimulates proliferation and blocks differentiation while 10^{-4} M has the opposite effect. We have been interested in cell surface changes that accompany differentiation and have examined the dynamic state of cell membrane receptors for concanavalin A (Con A). In these studies we have treated unfixed or prefixed cells with 30-50 μg/ml Con A for times ranging from 5-20 minutes at 4° and 37° and then localized the Con A at the ultrasturctural level by the peroxidase reaction. Con A is uniformly distributed over the surface in undifferentiated myoblasts regardless of whether cells are prefixed or not prior to incubation with Con A. Differentiated cells which are prefixed, and then reacted with Con A also exhibit a uniform distribution of lectin. However, when differentiated cells are exposed to Con A at 37° prior to fixation, there is a significant lectin redistribution. In differentiated cells, the prevalent pattern is a global redistribution or capping of the lectin with pronounced shedding of Con A and cell surface components although clustering and patching are also seen. The redistribution and shedding suggests that there is less restriction of Con A receptor mobility in differentiated cells. These studies suggest the myoblast differentiation is associated with changes in cell surface organization (or perhaps composition) and that differentiation may be modulated by polypeptide or steroidal hormones.

125 EVIDENCE FOR PHOSPHOHEXOSE RECOGNITION BY THE HUMAN FIBROBLAST RECEPTORS WHICH MEDIATE PINOCYTOSIS OF LYSOSOMAL ENZYMES, Arnold Kaplan, Daniel T. Achord and William S. Sly, Dept. of Microbiol., St. Louis University, St. Louis, MO 63104 and Dept. of Ped., Washington University, St. Louis, MO 63130

"High uptake" forms of human β-glucuronidase which are specifically pinocytosed by fibroblasts are more acidic than poorly pinocytosed forms. Two lines of evidence suggest that a portion of the recognition component on "high uptake" forms of the human platelet enzyme is a phosphoryl-group on, or in proximity to, a hexosyl-moiety. First, the following compounds, listed in decreasing order of potency, were found to be inhibitors of specific pinocytosis: S. cerevisiae mannan from mutant X2180-mnn1, mannan from wild type S. cerevisiae (Sigma Co.), α-D-mannose-6-phosphate, α-D-fructose-1-phosphate, α-D-mannose-1-phosphate, 2-amino α-D-mannose, Pi, a group of 2-axial-hydroxyl-4-equitorial-hydroxyl-pyranoses (including α-D-mannose and α-L-fucose), and α-D-fructose. In addition, platelet concanavalin-A binding proteins were found to be highly potent inhibitors. By contrast, 2- and 4-epimers of the pyranoses, other carbohydrates frequently present in glycoproteins, 2-deoxy-α-D-glucose-6-phosphate, and mutant mannans which lack phosphate were not comparably potent inhibitors. Competitive type inhibition of enzyme pinocytosis was demonstrated for the following compounds: α-D-mannose, α-L-fucose, α-D-mannose-6-phosphate, S. cerevisiae mannan, and mannan (X2180-mnn1). Second, alkaline phosphatase treatment abolished the "high uptake" capacity of human platelet β-glucuronidase.

126 EFFECT OF A HIGH FAT DIET ON INSULIN BINDING TO RAT LIVER PLASMA MEMBRANES, Jane V. Sun, Helen M. Tepperman and Jay Tepperman, Department of Pharmacology. State Univ. of New York, Upstate Medical Center, Syracuse New York 13210

The interaction of ^{125}I-insulin with purified liver plasma membrane (LPM) from rats fed a high glucose (G) diet or a high fat (L) diet was studied with respect to specific binding, insulin degradation, binding site degradation and rate of hormone association and dissociation. Scatchard analysis suggested the presence of high and low affinity binding sites for membranes of both G and L diet-adapted rats. However, LPM from G diet rats bound 50% more insulin or binding site degradation. The results suggested that an apparently reduced number of insulin binding sites (G=10.2 ± 2.45 x 10^{-12} moles/mg membrane protein , L= 4.5 ± 1.73 x 10^{-12} moles/mg membrane protein) associated with fat feeding was responsible for the reduced insulin binding by L diet membrane. The effects of Concanavalin A (Con A) on insulin binding to G and L diet membranes were also investigated. Con A enhanced the specific binding of insulin to both G and L diet LPM at concentrations lower than 50 ug/ml, whereas at concentrations higher than 50 ug/ml con A inhibited insulin binding to these membranes. The stimulatory effect of Con A on insulin binding at low concentrations was greater and inhibition of of binding at high concentration was less in the case of LPM prepared from L diet-adapted animals.