BIOLOGICAL RECOGNITION AND ASSEMBLY
David S. Eisenberg, James A. Lake and Lubert Stryer, Organizers
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Plenary Sessions

Workshop and Poster Sessions

Recognition in Viral Assembly

194 BIOCHEMICAL AND GENETIC STUDIES ON BIOSYNTHESIS OF THE VESICULAR STOMATITIS VIRUS (VSV) GLYCOPROTEIN, Harvey F. Lodish, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

VSV Glycoprotein (G, mw 63,000) is synthesized as a transmembrane protein; thirty amino acids at the COOH terminus remain exposed to the cytoplasmic face of the endoplasmic reticulum, while the remainder of the chain, and the two asparagine-linked "core" carbohydrate chains (consisting of mannose and N-acetylglucosamine) face the lumenal surface. The two "core" carbohydrate chains are added, en bloc, presumably from a lipid donor, one-at-a-time to the growing nascent G chain at precise times (1). Recently, we found that completed, but incompletely glycosylated G molecules, cannot later receive the "core" oligosaccharides (2). Several <u>in vitro</u> and <u>in vivo</u> studies established that glycosylation is not required for normal transmembrane insertion (2).

Synthesis of G begins by a ribosome unattached to membranes, and endoplasmic reticulum membranes are required before the nascent chain is 80 amino acids long in order for proper membrane insertion and subsequent glycosylation to occur (1). A sequence of 16 predominantly hydrophobic amino acids is normally lost from the amino terminus of nascent G at some point before its synthesis is completed (3).

About 40 minutes after its synthesis G appears on the surface of the infected cell, from where it is incorporated into budding virus particles. Contrary to previous assumptions, it appears that a discrete subset of normal cell surface proteins are also incorporated into VSV particles. Mutants in the gene for VSV G can provide additional evidence concerning the synthesis and processing of this glycoprotein. Recently, we devised a scheme for selectively isolating ts VSV mutants specifically defective in G synthesis (4). This selection is based on the ability of unrelated oncornaviruses to rescue known VSV ts G mutants by providing an "infectious" surface glycoprotein. This results in a pseudotype consisting of a VSV nucleocapsid surrounded by a phospholipid membrane containing only the oncornavirus glycoprotein. In cells infected at the nonpermissive temperatures by ts G mutants, G is made normally, but remains in intracellar membranes, and is not found on the surface or in budding particles.

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195 ASSEMBLY OF SEMLIKI FOREST VIRUS, Kai Simons and Henrik Garoff, European Molecular Laboratory, Postfach 10.2209, D-6900 HEIDELBERG, F.R.G.

Semliki Forest virus contains four structural proteins. One is in the nucleocapsid, the C protein (M_r $30x10^3$), and three in the glycoprotein spikes in the lipid envelope, E1 (M_r $49x10^3$), E2 ($52x10^3$) and E3 ($10x10^3$). Each glycoprotein spike is a three-chain structure with one species of E1, E2 and E3. The spike is attached to the lipid bilayer by hydrophobic peptide tails deriving from both E1 and E2. The tail of E2 spans the membrane. The orientation of E1 and E2 in the membrane has been determined by introducing a gradient of radioactivity increasing from the amino- to the carboxyterminal end of the proteins (1). In this way the hydrophobic tails have been mapped to the carboxyterminal ends of both E1 and E2. Thus the polypeptides of the spike are oriented so that the aminoterminal ends of E1 and E2 are on the outside and the carboxyterminal on the inside of the viral membrane. All the structural SFV proteins are specified by one mRNA molecule (26S) with a single initiation site. An unusual feature of this mRNA is that it directs the synthesis of proteins destined for two different cellular compartments, the C protein for the cytoplasm and the E proteins for the endoplasmic reticulum. The proteins are synthesized sequentially (2). The C protein is read first followed by the p62 (the precursor to E3 and E2) and E1 protein. The C protein is cleaved from the nascent chain, and is rapidly bound to the 42S viral RNS to form the nucleocapsid in the cytoplasm of the infected cell. The membrane proteins become inserted into the rough endoplasmic reticulum from where they are transported to the plasma membrane. This segregation of the viral proteins between the cytoplasm and the endoplasmic reticulum can be reproduced in vitro using a cell free system programmed with 26S RNA in which the protein synthesizing machinery was derived from HeLa cells and the microsomes from dog pancreas (3). The results showed that the C protein is translated first by ribosomes in the free state. As soon as the C protein is completed it is released from the growing chain. The ribosome now becomes membrane-bound and continues synthesis of the p62 and E1 proteins which are inserted into microsomal membrane. If the microsomes were added after a period corresponding to the synthesis of about 100 amino acids of the p62 protein, segregation, glycosylation and cleavage between p62 and E1 failed to occur. Thus the region coding for the signal of membrane attachment is not present at 5'-region immediately following the initiation codon on the 26S mRNA as in the case in the mRNAs coding for VSV membrane glycoprotein (4) and for secretory proteins , but further along the chain right after the C region in the beginning of the p62 region.

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196 DYNAMICS OF VIRUS ASSEMBLIES, D.L.D. Caspar, Rosenstiel basic Medical Sciences Research Center, Brandeis University, Waltham, MA 02154.

Switching mechanisms in the assembly and action of biological structures can be controlled by the interactions among the component molecules. Studies on the assembly of viruses show that the growth from smaller to larger aggregates is self-controlled. Structural proteins can act as their own allosteric effectors to trigger the conformational changes required for their self-assembly. Determination of the packing arrangement of TMV protein in the disk and virus helix has made it possible to formulate questions about the switching between these structures that can be related to observations on the kinetics and equilibria of stages in assembly. Switching from disk to helix can be triggered by binding protons to groups with abnormally high pK in the helix structure. The linkage between proton binding and TMV protein association is like the ligand linked switching described by the allosteric model. A thermodynamic model for the proton linked association of TMV protein can be formulated which takes into account the different structural states that have been experimentally identified.

Quasi-equivalent bonding in icosahedral virus particles requires different conformational states of identical subunits. The tomato bushy stunt virus (TBSV) subunit consists of two rigid parts connected by a hinge which occurs in two conformations in three quasi-equivalent environments of the capsid. TBSV, southern bean mosaic virus (SBMV) and other small icosahedral plant viruses swell reversibly when divalent cations are removed and then added back. Diffraction patterns from solutions and microcrystals of SBMV show that the swollen form is more disordered than the compact form. Swelling from a rigid compact form to an expanded, more flexible form can be represented by a simple mechanical model; the subunit in this model consists of two rigid domains connected by a flexible hinge corresponding to the structure of the virus subunit. In the compact form, the two conformations of the quasi-equivalently related units are determined by the interactions of the units in the surface. In the expanded form, there is considerable internal flexibility that arises from movement at the hinge. Flexibility and disorder of virus particles and cellular assemblies are related to the forces that move the subunits during assembly and action.

The switching process in bacteriophage tail sheath contraction is a paradigm for switching mechanisms in active protein assemblies. A mechanical model has been constructed that illustrates how the contraction process is controlled by the interactions among the parts. The mechanism operates utilizing energy stored in the extended structure by switching of variable linkages that are constrained by conserved connections. Any controlled process must operate by a similar purposeful balance of conserved and variable elements. Building mechanical models provides a direct way to explore the anticipation built into the action and reaction of biological machinery.

197 PROTEIN - RNA INTERACTIONS DURING TMV ASSEMBLY, K.C.Holmes, Max-Planck-Institut f.med. Forschung, Jahnstr. 29, 6900 Heidelberg, Germany

The rich polymorphism of TMV protein has been known for more than 20 years. In 1971 Butler and Klug established that the disc is the most important precursor of the virus. With the determination of the structure of the disc at 5.0 Å and then 2.8 Å (Champness et al, 1976; Bloomer et al, 1978) and of the intact virus to 4.0 Å (Stubbs et al, 1977) one begins to understand the role of the disc initiating assembly by binding to a specific RNA sequence. The RNA binding site has a hairpin structure with the repetitive structure AGU (Zimmern and Butler, 1977). The conformational change involved in passing from the disc to the helical virus, which involves the folding of a flexible segment about 30 residues long, can in a general way be followed. The flexible segment includes part of the RNA binding site so that the RNA binding is apparently an induced fit phenomenon. The initial step involves the clustering of 3 bases around an alpha-helix which forces the phosphates to group so that they bind 3 arginine residues. This in turn stabilises the flexible segment. The detailed interactions responsible for the specificity are not yet apparent.

Recognition in Protein-Nucleic Acid Interactions

198 RIBOSOME STRUCTURE AND TRANSFER RNA BINDING SITES, James A. Lake, Molecular Biology Institute and Department of Biology, University of California, Los Angeles, CA 90024.

Protein locations have been mapped on the ribosomal surface and relative orientations of the large and small subunits within the monomeric ribosome have been determined by immune electron microscopy using antibodies directed against specific ribosomal proteins. Functional regions such as the codon-anticodon interactions site, the tRNA binding sites and the peptidyl transferase are suggested by the locations of functionally important ribosomal proteins.

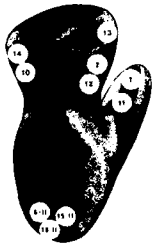


Figure 1

The locations of some of the ribosomal proteins mapped in collaboration with L. Kahan, W. Strycharz and M. Nomura are shown in Figures 1 and 2. The small ribosomal subunit (Figure 1) is divided into a one-third and a two-thirds region. A platform, located on the lower two-thirds, forms a cleft between it and the upper one-third of the subunit. The cleft, the upper one-third and the platform are thought to comprise the binding sites for mRNA, for initiation factors IF1, IF2 and IF3 and for initiator tRNA. Protein locations suggest that the codon-anticodon site is located on the platform.

Based on current knowledge of the elongation cycle, codon recognition first occurs with the aminoacyl-tRNA bound in a recognition "R" tRNA binding site that is functionally and structurally distinct from the A site. The recognition site is thought to be located on the external surface of the smaller ribosomal subunit distal from the interface between subunits where the aminoacyl(A) and peptidyl(P)tRNA binding sites are located. This location is shown in Figure 2.

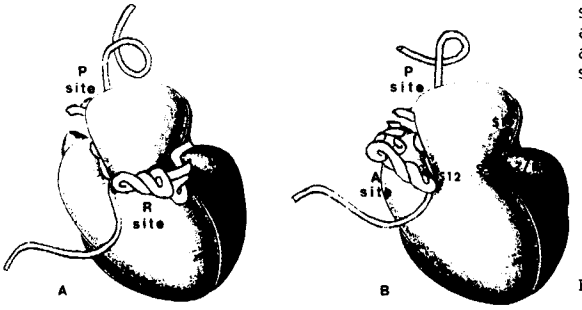
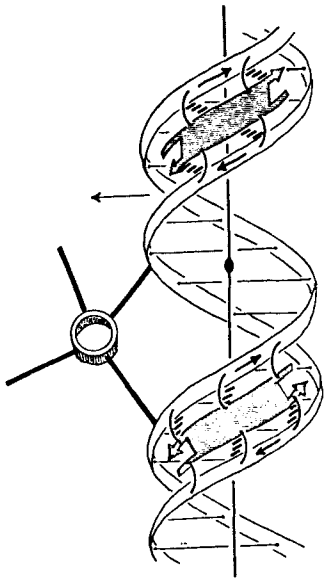


Figure 2

199 CRYSTAL STRUCTURE OF A COMPLEX BETWEEN A BASIC PROTEIN AND A NUCLEIC ACID, AND MODELS FOR PROTEIN-DNA INTERACTIONS, Sung-Hou Kim, Department of Biochemistry, Duke University, Durham, North Carolina 27710.



A circular dichroism spectrum of protamine, a small basic protein, shows that the molecule is in random coil state in solution. However, when nucleic acids are added to the solution the protein forms complex with nucleic acid and precipitates. The crystal structure of the protamine-tRNA complex at 5.4 angstrom resolution[1] indicates that the protamine becomes α helical structure in the presence of tRNA and these α helices are in contact with the minor groove of double helical portion of tRNA. This observation and careful model building suggest that there are structural complementarity between the secondary structures of protein and double helical structure of nucleic acids: α helix with basic residues are structurally complementary to the major groove of double helical DNA, and β-ribbon of protein is also structurally complementary to the minor groove of double helical DNA[2] as schematically shown in the figure.

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200 RNA SECONDARY STRUCTURES IN THE BIOSYNTHESIS AND FUNCTIONING OF RIBOSOMES, Joan Argetsinger Steitz, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06510

The current status of the evidence for base pairing between 16S ribosomal RNA and messenger RNA during the initiation of polypeptide chains in bacteria will be reviewed. The possible importance of comparable RNA·RNA interactions for the functioning of eukaryotic ribosomes will be considered. Also in ribosomal RNA biogenesis, RNA secondary structures are formed involving distant regions of the E. coli rRNA primary transcript; these are apparently essential for precise recognition and cleavage of the nascent RNA by RNase III.

201 LIMITED PROTEOLYSIS OF GLUTAMINE SYNTHETASE IS INHIBITED BY GLUTAMATE AND BY FEEDBACK INHIBITORS, David Eisenberg, Mary Lei, U. Aebi, and E.G. Heidner, University of California, Los Angeles, CA 90024.

Limited proteolysis of glutamine synthetase from E. coli has been studied under non-denaturing conditions (pH 7.6, 20° C). Trypsin cleaves the polypeptide chain of glutamine synthetase into two principal fragments of molecular weights about 32,000 and 18,000. The covalently bound AMP group is attached to the larger fragment and its presence does not affect cleavage. Although the cleaved polypeptide chain does not dissociate under non-denaturing conditions, catalytic activity is lost. Chymotrypsin and Staph. protease produce similar cleavages in glutamine synthetase. The substrate L-glutamate retards tryptic as well as chymotryptic digestion. Tryptic digestion is also retarded by some of the feedback inhibitors of glutamine synthetase including CTP, L-alanine, L-serine, L-histidine, and glucosamine-6-phosphate.

An implication of these findings is that there is a region of the glutamine synthetase polypeptide chain that is particularly susceptible to proteolysis. Either the glutamate and inhibitor sites are formed partly by this susceptible peptide, or the binding of glutamate and some inhibitors induces conformational changes within the GS molecule in the region of the susceptible peptide.

202 PACKING OF NUCLEIC ACIDS IN SPHERICAL VIRUSES. Stephen C. Harrison, Gibbs Laboratory, Harvard University, Cambridge, Mass. 02138.

DNA is wound uniformly into a smooth coil in the heads of isometric bacteriophages. The packing of adjacent turns of the coil becomes tighter, the more DNA there is in the head. Since DNA is added to preformed head precursors, these observations imply that energy is required to package DNA and that DNA must slide over the inner surface of the head during incorporation. Moreover, the absence of a precise geometrical relationship between DNA lying against this surface and the subunits themselves suggests the absence of specific interactions between head proteins and nucleic acid.

RNA in a spherical virus (tomato bushy stunt virus) is also not precisely located with respect to coat subunits. In this structure, however, a flexibly linked N-terminal arm of the protein may provide an "adjustable" binding site.

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Recognition Interactions in Contraction

203 PROTEIN SWITCHES IN MUSCLE CONTRACTION, Carolyn Cohen, Peter J. Vibert, Roger W. Craig and George N. Phillips, Jr., Rosenstiel Center, Brandeis University, Waltham, MA. 02154.

Two types of Ca^{2+}-sensitive protein complexes control the contraction of muscle: Troponin (TN) and tropomyosin (TM) are associated with the thin actin filaments, and a specific light chain is a regulatory subunit of myosin itself. Most muscles have both types of regulation. Notable exceptions are cross-striated vertebrate muscles and those of certain decapod crustaceans (regulated only by thin filaments); and molluscan and brachiopod muscles (regulated only by myosin thick filaments). Invertebrate muscles are especially suited for the X-ray diffraction analysis of both types of regulation. Thus the location of the TN complexes on the thin filaments and possible changes with Ca^{2+} have been revealed by X-ray studies of crustacean muscles (1). Diagrams from living scallop muscle show an unusual pattern of myosin cross-bridges (2), and removal of the regulatory light chain in the rigor state leads to a new pattern of cross-bridge attachment (3). The full interpretation of these X-ray diagrams is, however, often ambiguous, and structural studies of the purified proteins provide essential information. Recent crystallographic results (Phillips *et al.*, submitted for publication) reveal that the TM molecule has unusual local domains of marginal stability, leading to extensive motions of the tropomyosin filaments. Electron microscopy of negatively stained thin filaments decorated with subfragments of scallop myosin yield unusually detailed images that show marked conformational changes in myosin cross-bridges dependent on the presence of the regulatory light chain. These observations suggest that both the special dynamic design of tropomyosin and the striking structural changes in the myosin cross-bridges are significant clues for detailed models for the regulatory mechanisms.

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204 INITIATION SITES FOR MICROTUBULE ASSEMBLY, M. Kirschner, University of California, San Francisco, Department of Biochemistry & Biophysics, San Francisco, CA 94143

We have studied the kinetics of microtubule assembly by darkfield microscopy in real time and verified the polarity of microtubule growth. Using this approach we have examined the kinetics of microtubule assembly of metaphase chromosomes and show that they grow with their fast growing end pointing away from the kinetochore. Using indirect immunofluoreescence of microtubule regrowth after treatment with anti-microtubule drugs we have reexamined initiation sites for microtubule assembly in interphase mammalian fibroblast cells. We have found multiple sites, only one of which contains the centrioles. The distribution of sites is discussed and in particular changes in the distribution which accompany changes in cell morphology.

Recognition at the Cell Surface

205 INTERACTIONS AMONG SURFACE COMPONENTS OF CULTURED ANIMAL CELLS, Elliot Elson, Department of Chemistry, Cornell University, Ithaca, NY 14853

Surface components of animal cells must recognize and interact with each other as well as with molecules above and below the plasma membrane to carry out various physiological functions. These include transmission of signals and transport of material across the surface, regulation of the density and perhaps the sensitivity of receptors, and formation of specialized surface regions and structures. Measurements of the macroscopic lateral mobility of specific surface components provide information about the strength, persistence, and mechanisms of these interactions. Experimental studies in several laboratories have indicated significant differences in the mobilities of cell surface lipids and proteins, suggesting that specialized constraints may limit the motion of the latter. Studies of particular systems have also revealed many different types of interactions and behaviors. Strong, long-lived forces seem to stabilize some specialized surface structures such as the patches of acetylcholine receptor that form on developing myotubes and the strands of fibronectin that are apparently involved in the adherence of cells to each other and the substrate on which they grow. In contrast weak, transient interactions have been seen to influence the motions of several ganglioside analogs. Modulation of mobility has been observed to result from binding of concanavalin A to cell surfaces, from passage of neuroblastoma cells through their growth cycle, and from differentiation of neuroblastoma cells. Limited aggregation of immunoglobulin E receptors on mast cells and of receptors for insulin and epidermal growth factor (EGF) seems to be necessary for signal transmission and may be involved in regulation of the surface density of insulin and EGF receptors. In our laboratory we have recently been studying the behavior of glycoproteins of enveloped viruses on the surfaces of infected cells. These are useful systems in which to examine interactions among relatively well characterized surface components, to observe the formation of specific cell surface structures, and to gain some insights into mechanisms by which viral buds assemble.

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2. Unpublished work discussed here was carried out by S. W. deLaat and J. Schlessinger and by J. Reidler in collaboration with P. Keller, J. Lenard, D. Johnson, M. Schlesinger, and H. Wiegandt.

206 LIPOPROTEIN RECEPTORS: STRUCTURE-FUNCTION RELATIONS, Michael S. Brown, Richard G.W. Anderson, and Joseph L. Goldstein, Department of Molecular Genetics, University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

Mammalian cells supply themselves with cholesterol via receptor-mediated endocytosis of a cholesterol-carrying plasma lipoprotein, low density lipoprotein (LDL). Binding of LDL to its cell surface receptor is followed by endocytic uptake of the lipoprotein and its delivery to lysosomes wherein the protein and cholesteryl ester components are hydrolyzed. This liberated cholesterol is used by cells for membrane synthesis. The uptake process is extremely efficient - all of the receptor-bound LDL is internalized within ten minutes by the cells. This efficiency is achieved by a clustering of the LDL receptors over coated pits in the plasma membrane. The coated pits pinch off to form coated endocytic vesicles every ten minutes, carrying the receptor-bound LDL into the cells.

By immunocytochemical methods, we have shown that the coated pits contain clathrin, a 180,000 M.W. protein that was previously identified by Pearse as a component of the coat of coated vesicles in brain and other tissues. We have also demonstrated by immunoperoxidase studies that the clathrin-containing coated pits in human fibroblasts are localized over cytoplasmic stress fibers that are believed to contain actin and myosin. Endocytosis of LDL may be facilitated by an interaction between the LDL receptor, clathrin-containing coated pits, and actin and myosin-containing stress fibers. Human mutations disrupt this process in meaningful ways which will be discussed.

207 NERVE GROWTH FACTOR RECEPTORS, Eric M. Shooter, Earl W. Godfrey, Gary E. Landreth, Paul Layer, Arne Sutter and Bruce A. Yankner, Stanford Univ. Sch. Med., Stanford, CA 94305.

Nerve growth factor (NGF) is necessary for the survival, development and maintenance of sympathetic and certain sensory neurons and these activities of NGF are mediated by specific cell surface receptors. Two NGF receptors are found on 8-day-old chick embryo sensory neurons. Neither hormones which are structurally related to NGF, like insulin or proinsulin, nor somatomedin A and C or epidermal growth factor compete with the binding of NGF to either of these two receptors. The apparent equilibrium dissociation constant for the higher affinity receptor is $\leq 5 \times 10^{-12}$ M after correction for its cell concentration dependency. Since maximum neurite outgrowth is obtained at an NGF occupancy of 4% or higher of this receptor but only of 0.01% of the lower affinity receptor the former but not the latter is implicated in the mechanism controlling neurite growth. Sympathetic neurons from 11-day-old chick embryos also display the same two NGF receptors.
The fate of NGF after its binding to the high affinity receptor has been explored with the NGF-responsive clonal line of rat pheochromocytoma. These cells also possess two NGF receptors on the cell surface. The apparent equilibrium dissociation constant of the higher affinity receptor is at least 10-fold lower than that on sensory neurons and correspondingly at least 10-fold higher concentrations of NGF are required to initiate neurite outgrowth. Within one hour of exposure of PC12 cells to NGF significant amounts of intact NGF are found in the nucleus. Accumulation continues until two-thirds of the cell bound NGF is in the nucleus. No translocation of NGF to the nucleus occurs at low temperature. PC12 nuclei have two NGF receptors localized to the nuclear membrane. The internalization of NGF is accompanied by an initial decrease of cell surface receptors. A temperature dependent cell-mediated degradation of NGF is also observed.

Recognition and Structure in the Immune System

208 THREE-DIMENSIONAL STRUCTURES OF ANTIBODY COMBINING SITES, David R. Davies, NIH, Bethesda, Maryland 20014

The structures of the Fabs of several myeloma proteins have now been determined, as well as a number of V_L dimers and one light chain dimer. From these data a number of generalizations can be made:
1) The antibody combining site consists of hypervariable residues forming a surface of dimensions approximately 20 x 35A, located at the tip of the Fab. The loops containing the hypervariable residues, which vary in size and shape due to amino acid insertions and deletions, can be regarded as being grafted onto the nonhypervariable framework part of the molecule. The frameworks of different immunoglobulins remain structurally invariant.
2) Binding to haptens frequently involves a crevice or pocket in which the hapten is buried.
3) The pocket is not necessarily symmetrically disposed between the light and heavy chains and may involve predominantly the residues of one chain.
4) Hapten binding does not produce large conformational changes.
5) For those immunoglobulins whose three-dimensional structures have not been determined correlations can often be made between the amino acid composition of the combining site and the specificity of binding.
Data will be presented in support of these assertions.

209 ANTIBODY STRUCTURE AND DYNAMICS IN THE CRYSTALLINE STATE, Robert Huber, Max-Planck-Institut für Biochemie, D-8033 Martinsried, Germany

The intact Kol molecule (γ,λ) in the crystalline state is characterized by a disordered Fc part, an open elbow angle in the Fab arms with the consequence of lacking substantial nonbonded longitudinal V-C contacts, and a lack of non-bonded interaction between the Fab arms. These observations suggest a molecule in solution with segmental flexibility and define the hinge regions. The difference in quarternary structure between the Fab arms in intact Kol on one side and the Fab fragments New, McPC 603 and the Bence Jones dimer Mcg on the other side (open and closed elbow angle) led us to the hypothesis that Fc affects the elbow angle and a similar structural change might accompany antigen binding.
The crystal structure of the Kol Fab fragment provides evidence against this hypothesis as the elbow angle is also open here and only a few degrees more bent than in intact Kol. An intriguing observation, however, which might change the basis of the hypothesis mentioned above, is that the head to tail contact between molecules is conserved in both crystal structures. This contact involves the hypervariable loops of the V-modules and some segments of the C-modules ('crystalline antigen antibody complex'). Both, intact Kol and its Fab fragment are cryo-proteins.
The Fc fragment crystal structure revealed two unexpected features of this molecule. Unlike other domains C_H2 has no lateral interaction. It is essentially a single domain. Part of it is covered by the carbohydrate which plays an important structural role by protecting a hydrophobic patch. These unique structural features might be reflected in the functional properties ascribed to C_H2. The crystal structure of the complex formed by human Fc and fragment B of protein A from Staphylococcus Aureus may be regarded as a model for an interaction with a 'third partner'. Protein A fragment is remarkable as it exhibits a most simple folding scheme: three (anti-) parallel helices. Two fragments bind to Fc at the C_H2 and C_H3 interface, like ear ornaments on the rabbit head. In this complex a part of C_H2 (the upper third of the outer face) is disordered, demonstrating a type of disorder different from the segmental flexibility discussed: domain disorder.
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210 IS THE PRODUCT OF THE MAJOR HISTOCOMPATIBILITY COMPLEX A RECOGNITION MOLECULE?, J. Klein, Max-Planck-Institute fur Biologie, Abteilung Immungen., Corren. 42, 7400 Tubingen, Germany

The function of the major histocompatibility complex (MHC) is not known. The popular notion has it that MHC molecules function as recognition sites related to the receptor on thymus-derived (T) lymphocytes. In this presentation I shall argue that this notion is incorrect. Furthermore, I shall discuss evidence in favor of the concept that the original function of MHC was assistance in killing of self-cells.

Helical Viruses

211 THE STRUCTURE OF FILAMENTOUS BACTERIOPHAGE Pf1 AT 7 Å RESOLUTION, Lee Makowski, D.L.D. Caspar, Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA 02154

An electron density map of filamentous bacteriophage Pf1 has been determined to 7 Å resolution by analysis of X-ray diffraction data from partially oriented fibers of virus particles. The continous intensity distribution along layer lines was measured by numerically separating contributions from overlapping layer lines. The data were phased by an iterative technique that utilized the known outside diameter of the particle and its high α-helical content to refine a structural model. This refinement converges to a unique structural solution consistent with the X-ray data and with information derived from physical and chemical studies. The coat protein is made up of two α-helical segments: one tilted by 6° to the particle axis at a radius of about 15 Å; the other at a radius of 25 Å is tilted by 25° to the particle axis. the inner and outer α-helical segments interlock along most of their length with a crossing angle of 20.5°. The double layer of tightly packed, intricately interlocked α-helices forms a stable, 20 Å thick protein coat around the viral DNA.

212 DNA PACKING IN THREE FILAMENTOUS VIRUSES. L.A. Day, R.L. Wiseman, and C.J. Marzec. The Public Health Research Institute, New York, N.Y. 10016

Pf1 is 2.0μ long and Xf is 1.0μ long, but both their circular single stranded DNAs contain ∿7400 nucleotides. The fd virion is 0.9μ long and its DNA contains 6400 nucleotides. Chemical data indicate one nucleotide per protein subunit in Pf1 and two nucleotides per subunit in Xf, but about 2.3 or 2.4 nucleotides per subunit in fd. The amino acid sequences differ significantly, and CD results show 80% or more α-helicity in Pf1, only about 50% in Xf, and 80% or more in fd. One of the two tyrosyls per subunit in Pf1 can be reversibly deprotonated. There is one tryptophyl per subunit in Xf and in fd; about half in Xf but all in fd are accessible to N-bromosuccinimide. Spectral data indicate base-tyrosine stacking in Pf1, but base-base stacking in Xf and fd.

X-ray studies by D.A. Maryin and coworkers and by L. Makowski and D.L.D. Caspar indicate a helix of subunits with 15Å pitch for Pf1. The occurrence of one nucleotide per subunit suggests that the DNA in Pf1 is also in a helix of 15Å pitch. This and the axial nucleotide translation of 5.4 Å given by the physical data would require that the phosphates be 2.5 Å or less from the axis. Similarly, pitches of 15 Å for Xf and 16 Å for fd (Marvin and coworkers) and axial nucleotide translations of 2.7 Å and 2.8 Å, respectively, would require the phosphates to be less than 6.0 Å from the axes of these virions. DNA structure models being considered that conform to these constraints and to the spectral data are different for each virus, but in each model the phosphates are closer than the bases to the structure axis.

213 KINETICS OF ASSEMBLY OF TMV COAT PROTEIN. T. M. Schuster, R. B. Scheele, S. J. Shire, J. J. Steckert, L. H. Khairallah, Biological Sciences Group, University of Connecticut Storrs, CT 06268

Physical-chemical studies of the self-association of TMV coat protein (TMVP), the paradigm of helical assembly of subunits, continue to reveal new information about macromolecular interactions. Kinetics studies reveal a nucleation-controlled mechanism in which the 20S two-turn disk (or its helical conformer) is the nucleating species for the subsequent, more rapid, elongation reaction involving 4S protein. Such a mechanism predicts the existence of non-equilibrium polymerization states of various size rods which have been observed and characterized. In addition to this metastability above 17°C, there can exist metastable 20S protein (presumably disks) at 5-7°C, depending upon the pH. Initiation of TMVP assembly-disassembly by switching between these two temperatures at pH 6.5 results in reproducible "polymerization memory". In addition to clarifying the mechanism of TMVP polymerization, the results of these kinetics studies make possible new experiments that elucidate details of the mechanism of virus reconstitution, as described elsewhere in this symposium.

Supported by NIH grant AI-11573

214 SPECIFIC AND NON-SPECIFIC PROTEIN-NUCLEIC ACID INTERACTIONS IN THE SELF-ASSEMBLY OF PAPAYA MOSAIC VIRUS, John W.Erickson, M.AbouHaidar and J.B.Bancroft, University of Western Ontario, London, Ontario CANADA

Specific recognition of a viral nucleic acid by its coat protein is being studied for the in vitro self-assembly reaction of papaya mosaic virus(PMV), a helical, flexuous plant virus. The specificity of this reaction is pH-regulated. At or above pH 8.0, PMV coat protein initiates assembly with its own RNA(at or near its 5'-end) or with that of a closely related virus, but not with unrelated viral RNAs, mRNAs, or DNA. Below pH 8.0, PMV protein assembles with natural and synthetic RNAs, and with DNA. Assembly at the lower pHs is more rapid, but the products are faulty, having a "kinked" appearance. Studies with synthetic ploynucleotides showed that poly A and poly C, but not poly dA and poly dC, are recognized at specific and non-specific pHs, whereas poly I and poly U are encapsidated only at non-specific pHs. The initiation fragment of PMV-RNA is disproportionately rich in A. Based on these preliminary data, we can envisage a stabilizing role for the $-NH_2$ groups of adenine and cytosine in the base-amino acid interactions which must occur within the virus particle, and also conclude that the 2'-OH of the sugar moiety of RNA is important for assembly into the proper helical virus structure.

215 A MOLECULAR REPLACEMENT APPROACH TO THE STRUCTURE OF THE GENE 5 PROTEIN-DNA COMPLEX, Paula M.D. Fitzgerald*, Andrew A. Wang†, Frances Jurnak*, Ian Molineux‡, Frank Kolpak†, Alexander Rich†, and Alexander McPherson*, *Department of Biological Chemistry, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, PA 17033, †The Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, and ‡The Department of Microbiology, University of Texas, Austin, TX.

The gene 5 DNA unwinding protein has been complexed with various oligo-deoxynucleotides and crystallized in several different modifications. X-ray diffraction data to 5.0 Å resolution have been collected on a trigonal crystal form ($P3_1$,a=143 Å,c=83 Å) derived from a gene 5 protein/d-GGTAAT complex. These crystals, which exhibit a high degree of 32 pseudo symmetry likely contain 24 gene 5 protein monomers (each of 10,000 daltons) as the asymmetric unit. We have searched for and found non-crystallographic symmetry elements using the fast rotation function of Crowther. We have, in addition, used the native gene 5 dimer structure, which has been determined in the unliganded form by isomorphous replacement, to determine the orientation of the protein molecules in the complex crystals. It is our expectation that further application of the molecular replacement technique will yield the low resolution structure of the gene 5 protein/DNA complex and allow us to delineate the binding interactions which couple the two molecules.

216 THE EARLIEST STAGES OF TMV SELF-ASSEMBLY, Gary K. Ackers, and Leslie Katzel, The Johns Hopkins University, Baltimore, Md. 21218

Self-assembly of tobacco mosaic virus protein monomers into disks has been studied by high precision analytical gel chromatography as a function of temperature and pH. Resulting dissociation curves obtained over an 8000-fold range of concentration (0.4 mg/ml - 3.2 mg/ml) have been subjected to rigorous numerical analysis to test stoichiometric models and to evaluate thermodynamic quantities for the assembly process.

For the monomer→trimer stage of assembly the reaction proceeds at pH 7 with the formation of less than 5% dimers at any extent of reaction. The standard free energies of trimer formation range from -12.86 ± .25 kcal/mole at 5.5°C to -14.01 ± 0.3 kcal/mole at 15°C, yielding an enthalpy of formation of 6.13 kcal/mole. Data at the higher concentrations reflecting species larger than trimer permit a number of stoichiometric models to be excluded. The data at pH 6.5 indicate the existence of a slow equilibrium between monomers and higher complexes (smaller than disk), whereas the reactions at pH 7.0 are rapidly reversible.

217 RECOGNITION OF TRINUCLEOSIDE DIPHOSPHATES BY HELICAL RODS OF POLYMERIZED TOBACCO MOSIAC VIRUS PROTEIN, John J. Steckert & Todd M. Schuster, Biological Sciences Group, University of Connecticut, Storrs, CT 06268

The self-assembly of tobacco mosaic virus (TMV) has recently been shown to be bidirectional and to be initiated at an internal site on the single-stranded viral RNA. An examination of the nucleotide sequence of the initiation region and of the extents of RNA protection following reconstitutions with limited amounts of TMV coat protein (TMVP) has led Zimmern (Cell, 11, 463-482 (1977)) to propose that the subunits in the first nucleating disk protein aggregate can recognize a particular RNA reading frame. A triplet periodicity of this frame was chosen in accord with the fact that each of the two RNA binding surfaces on each coat protein subunit in TMV is associated with three nucleotides. In order to demonstrate the feasibility of triplet recongition, the K (apparent) values were measured for the binding of each of twenty-five tritium labelled trinucleoside diphosphates to helical TMVP rods by equilibrium microdialysis. These studies show that the binding interactions are strongly sequence dependent and that (5')AAG(3') has the highest binding constant (12,000 liter/mole of subunits). The location of two contiguous high affinity AAG's in the assembly initiation region suggests how the reading frame between the RNA and protein is aligned during the disk-RNA recognition event at the onset of TMV reconstitution. This RNA reading frame places several of the binding triplets adjacent to one another within the main non-bonded loop of the predicted hairpin-folded structure that figures prominently in recently proposed models of TMV self-assembly.

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218 DIRECT VISUALIZATION OF ADSORPTION PROTEIN OF fd PHAGE, C. W. Gray* R. S. Brown, and D. A. Marvin, European Molecular Biology Laboratory, Postfach 10.2209, Heidelberg 6900, West Germany

Using uranyl acetate stain on filamentous fd virus particles adsorbed to glow-discharge-activated thin carbons, we have been able to directly visualize the viral adsorption protein by electron microscopy. In favorably stained areas of the carbon support film, 75% of the fd virions exhibit a knobbed structure visible at one end of the virion only; no virions have knobs at both ends, consistent with published data indicating that the adsorption protein is located at one end of the virus particle. No knobby ends are seen on virions treated with subtilisin, which selectively degrades the adsorption protein as we have shown by SDS gel electrophoresis done on the virus preparations used for microscopy. As seen in electron micrographs, the adsorption protein of fd virus consists of a roughly globular element with an apparent diameter of 25-50 Angstroms (the variation probably depends on the manner of stain deposition); this rounded element is attached to the virus by a thin connection probably 40-70 Angstroms long. We see two, three, or sometimes possibly four globular units attached to one end of the virion, which frequently appears to be more tapered than the opposite end. Thus it appears that the fd adsorption protein shares features with the adsorption proteins of larger viruses, in that multiple copies are mounted on possibly flexible stalks, permitting variously oriented virions to attach to the receptor.

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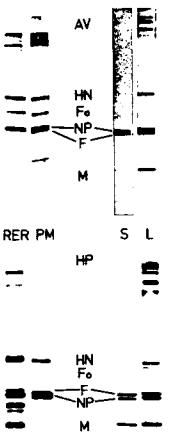
219 THE STRUCTURE AND ASSEMBLY OF ALFALFA MOSAIC VIRUS (AMV).

Jaap H.Kan, Jan E.Mellema, Gert T.Oostergetel, University of Leiden, The Netherlands.

The AMV structure has been studied by electron microscopy and low-angle neutron scattering. The virus particles have a cylindrical shape with hemispherical caps, contain RNA and are built from one type of polypeptide chain. The cylindrical symmetry is 3-fold and the surface lattice is based upon a P-6 net, in which the protein is clustered at the lattice dyads. The caps are formed by half-icosahedrons. The resulting structure in which the RNA is packaged, is relatively open; therefore the "coat" does not fulfill the classical protective function.
The dynamics of the assembly has been investigated by a variety of techniques and it has been shown that it is a cooperative process. A 2-state model has been put forward to explain the behaviour of the "coat" protein in the presence of nucleotides and protons, which favour polymerization.

220 ASSEMBLY OF THE F GLYCOPROTEIN OF BIOLOGICALLY DISTINCT STRAINS OF NEWCASTLE DISEASE VIRUS (NDV). Glenn W. Smith, James C. Schwalbe and Lawrence E. Hightower, University of Connecticut, Storrs, CT 06268

Proteolytic cleavage of a precursor glycoprotein (F_o) activates the fusion glycoprotein (F) of NDV. In chick cells F_o of most virulent strains (AV) is cleaved to produce infectious virions (penetration requires F) and to express cellular fusion activity on the cell surface (fusion from within, FFWI). In contrast F_o of avirulent strains is not usually cleaved in chick cells. Noninfectious virions containing F_o are produced and cells do not FFWI. However, some virulent strains (HP) present a mixed pattern. The F_o of HP is cleaved, F accumulates in the cell and on infectious virions, but infected cells do not FFWI. The accompanying electropherogram compares the intracellular sites of accumulation of proteins of AV and HP and their appearance in virions after a short (S) or long (L) chase period. The F of AV accumulates mainly in the plasma membrane (PM) and is efficiently incorporated into virions. In contrast F of HP accumulates mainly in the rough endoplasmic reticulum (RER). No detectable F accumulates in PM; however, the small amount of F which does reach the PM is efficiently incorporated into virions. Thus, HP-infected cells do not FFWI because F does not accumulate at the PM. Cleavage of F_o then is not the only determinant of FFWI; differential compartmentation of glycoproteins influences the expression of viral biological activities on the cell surface.



221 OBSERVATIONS CONCERNING THE PERMEABILITY OF A BACTERIOPHAGE T7 CAPSID, Philip Serwer, The University of Texas Health Science Center, San Antonio, Texas 78284.

It was previously shown that lysates of bacteriophage T7-infected E.coli contain two DNA-free capsids: capsid I has a thicker and rounder envelope than bacteriophage T7; capsid II has a bacteriophage-like envelope. Both these capsids and bacteriophage T7 have an internal, cylindrical core attached to the capsid envelope at the core base. Capsid I is a precursor to bacteriophage T7; DNA may enter capsid I through an axial hole in the core. In the present study capsid II has been separated into two components by buoyant density sedimentation in metrizamide density gradients. Metrizamide high density capsid II (MHD capsid II) has a density of 1.26 gm/ml and metrizamide low density capsid II (MLD capsid II) has a density of 1.086 gm/ml. The following properties of MHD and MLD capsid II are the same: the buoyant density in cesium chloride density gradients (±.015 gm/ml), the electron microscope appearance of the capsid envelope, and the protein composition of the capsid envelope. These data indicate that the lower density of MLD capsid II is the result of a higher preferential solvation, Γ, in MLD capsid II (2.4 gm H_2O/gm protein) than in MHD capsid II. The comparatively high Γ of MLD capsid II probably is caused by impermeability of the capsid II envelope to metrizamide. MLD capsid II particles have cores and most MHD capsid particles do not, suggesting that the largest hole in the capsid II envelope is at the core base and is obstructed by the core. During kinetic labeling experiments MLD capsid II appears after capsid I, after MHD capsid II, and before bacteriophage T7 , suggesting that MLD capsid II is either (a) a bacteriophage precursor occurring just before DNA packaging or (b) a degradation product of a precursor occurring just before or during DNA packaging. (Supported by National Institutes of Health Grant GM-24365-01)

222 HELICAL PROTEIN ASSEMBLIES RELATED TO THE PROHEAD CORE OF BACTERIOPHAGE T4. Alasdair C. Steven, Laboratory of Physical Biology, National Institute of Arthritis, Metabolism, and Digestive Diseases, Bethesda, Maryland 20014.

T4 prohead core proteins can self-assemble in vitro into structures which resemble authentic cores (1), implying that solution of the core geometry may hold the key to the form determination mechanism of the T4 head. Helical aggregates of core proteins with giant proheads and polyheads assembled both in vitro have been studied by electron microscopy and image processing. Such aggregates are presumably polymorphic variants of the ellipsoidal prohead core. For giant proheads, we find these cores to comprise six strands, spaced 9.3 nm apart, with a pitch angle of 36°, in agreement with (2). From a systematic analysis of the differential flattening of these particles on the electron microscope grid, we conclude that (a) the core helices are left-handed, like the hexagonal surface lattice folding; (b) that the model for the core-capsoid ensemble as two nested icosahedra is improbable. In contrast, polyhead cores are mainly right-handed, and exhibit a range of pitch angles (45-55°) as do their surface lattices. The constraints which these and other observations impose on possible core models will be discussed.

(1) Van Driel, R. and Couture, E. (1978). J. Mol. Biol. (in press)
(2) Paulson, J. and Laemmli, U. K. (1977). J. Mol. Biol. 111:459.

223 THE MOLECULAR ENVELOPE OF ADENOVIRUS TYPE 2 HEXON AND ITS INTERACTIONS IN THE VIRAL CAPSID, Roger M. Burnett, Markus G. Grütter, Zora Markovic, Janice L. White, Biozentrum der Universität Basel, Basel, Switzerland.

Hexon, a trimer with 3 subunits each of MW 120,000, is the major coat protein of adenoviruses. The icosahedral viral shell is formed from 240 hexons at the faces and 12 pentons and fibres at the vertices.

The molecular envelope of hexon has been traced from an electron density map determined by X-ray crystallography. Hexon has a solid pseudo-hexagonal "base" and a less dense triangular "top", with the apices of the triangle roughly above the mid-points of three basal edges. A small hole at the top of the molecule widens to form a cavity extending to a larger opening at the base.

The hexagonal shape of the base permits packing of hexons to form the flat continuous surface seen on a face of the viral capsid. The imperfect hexagonal symmetry probably has implications for the dissimilar interactions at the capsid edges. The hole could be an example of structural economy. Electron micrographs of the intact virus showing a spiky appearance are consistent with an array of triangular hexon tops. This evidence orients the isolated molecule, the structure of which we have determined, with respect to the complete virus found in nature.

224 STRUCTURES OF POLYMORPHIC FORMS OF TOBACCO MOSAIC VIRUS PROTEIN, Gerald Stubbs, Stephen Warren and Eckhard Mandelkow, Brandeis University, Waltham, MA 02154

Tobacco mosaic virus protein forms several aggregates, including a disk of 34 subunits in two layers (17 subunits per turn) and the virus itself, which contains about 2200 subunits in a helix with 16 1/3 subunits per turn, enveloping the RNA. In the presence of the RNA, the disk switches to the helical form. This is a key step in the assembly of the virus. At low pH the protein can form a helix without the RNA, having either 16 1/3 or 17 1/3 subunits per turn. Both of these forms have been studied and compared with the intact virus by calculating difference Fourier maps using X-ray diffraction from oriented gels. The helical protein forms have structures very similar to each other and to the protein part of the virus, including the low radius region which has a very different structure in the crystalline disk form of the protein. The RNA is replaced by at least one bound anion. The difference between the protein forms may possibly be related to the conformation of one or more arginine groups.

225 MECHANISM OF TOBACCO MOSIAC VIRUS ASSEMBLY: INCORPORATION OF 4S AND 20S PROTEIN AGGREGATES, S. J. Shire, J. J. Steckert, M. L. Adams and T. M. Schuster, Biological Sciences Group, University of Connecticut, Storrs, CT 06268.

Helical virus assembly may generally proceed as a nucleation controlled process involving stable disk-like protein aggregates as in tobacco mosiac virus where it has been unequivocally demonstrated that a two-layer 20S disk serves as the obligate nucleus for assembly. The direct participation of this preformed aggregate in the subsequent elongation phase has not been demonstrated as clearly. Kinetics experiments designed to test various models of assembly have relied solely on measurements of the rate of formation of reconstituted virus using mixtures of 4S and 20S protein. We have investigated by analytical ultracentrifugation directly the formation of reconstituted virus as well as the uptake of 4S and 20S protein components under two different assembly conditions: pH 6.5, 6.5°C and at pH 7.0, 20°C. The pH 6.5 condition involves assembly with metastable 20S aggregate and the low temperature affords the advantage of slowing the assembly reaction sufficiently to study the kinetics of assembly and to detect assembly intermediates by sedimentation analysis. Similar experiments at pH 7.0 take advantage of the slow overall rate of conversion between the 4S and 20S protein components. Our results under both sets of conditions indicate that the 4S component incorporates preferentially during elongation. Supported by NIH grants AI-11573 and AI-05266.

Viral Membrane Glycoproteins

226 SYNTHESIS AND PROCESSING OF SINDBIS VIRUS STRUCTURAL PROTEINS D.F. Wirth, C. Mann, R.A. VanEtten, H.F. Lodish, P.W. Robbins, M.I.T. Cambridge, MA 02139

Previous work has shown that the Sindbis structural proteins, core, the internal protein, and PE_2 and E_1, the integral membrane glycoproteins are synthesized as a polyprotein from a 26S mRNA; core PE_2 and E_1 are derived by proteolytic cleavage of a nascent chain. Newly synthesized core protein remains on the cytoplasmic side of the endoplasmic reticulum while newly synthesized PE_2 and E_1 are inserted into the lipid bilayer, presumably via their amino termini. We examined a temperature sensitive mutant of Sindbis virus which fails to cleave the structural proteins, resulting in the production of a polyprotein of 130,000 molecular weight in which the amino termini of PE_2 and E_1 are internal to the protein. Although the envelope sequences are present in this protein, it is not inserted into the endoplasmic reticulum bilayer, but remains on the cytoplasmic side as does the core protein in cells infected with wild type Sindbis virus. We have also examined the fate of PE_2 and E_1 in cells treated with tunicamycin, an inhibitor of glycosylation. Unglycosylated PE_2 and E_1 are inserted normally into the lipid bilayer as are the glycosylated proteins. These results are consistent with the notion that a specific amino terminal sequence is required for the proper insertion of membrane proteins into the endoplasmic reticulum bilayer, but that glycosylation is not required for this insertion. PE_2 and E_1 are glycosylated by the addition of precursor oligosaccharides which are subsequently processed. We have examined two classes of temperature sensitive mutants of Sindbis virus which each appear to be defective in glycoprotein processing.

227 STEM MICROSCOPY OF POLYSACCHARIDES AND GLYCOPROTEINS
James Resch, Robert Hyland, David Tunkel, Chris Stoeckert, Wendell Wiggins, Michael Beer
The Johns Hopkins University, Department of Biophysics, Baltimore, Maryland 21218
With the objective of localizing cell surface polysaccharides the reactions of several osmium (VI)-ligand complexes with glycols have been applied to sugar residues in mono- and polysaccharides and glycoproteins. Water soluble products are obtained with the ligands 4,4'-dicarboxy-2,2'-bipyridine and NN N'N'-tetramethyldiethyhemediamine. Galoctose reacts with two moles of reagent. Cyclodextrins with seven glucose residues can react with no more than three moles indicating possible nearest neighbor exclusion. When at least some charged ligands are used in reacting amylose, the macromolecule can be extended on a grid and high resolution reveals rows of osmium atoms. Isolated glycophorin binds about 50 moles of the reagent. The labeled glycoproteins are clearly detected in our high resolution STEM, suggesting a basis for identification.

228 GLYCOSYLATION OF A gag POLYPROTEIN IN MOLONEY MURINE LEUKEMIA VIRUS INFECTED FIBROBLASTS. Steven A Edwards and Hung Fan Tumor Virology Laboratory, The Salk Institute San Diego California 92112

Immunoprecipitation of pulse labelled cytoplasm from M-MuLV infected cells reveals two major gag related polyproteins; one of 80,000 daltons and one of 65,000 daltons (Pr65^{gag}). Pr65^{gag} has been shown to be the immediate precursor of the internal structural (gag) proteins of MuLV. Our studies on the 80,000 dalton polyprotein indicate a) that it contains mannose, b) that it is further glycosylated to form a 95,000 dalton glycoprotein, and c) that its unglycosylated form has a molecular weight of 75,000 daltons. Cell-free translation of M-MuLV genomic RNA yields two major products of 75,000 and 65,000 daltons which comigrate with gag polyproteins made in cells in which glycosylation has been inhibited with tunicamycin. The cell-free translation products can be labelled with ^{35}S-formyl-methionine, indicating that they contain an intact amino-terminus. Since the 80,000 and 65,000 dalton polyproteins made in the cell are known to differ in their N-terminal peptides, these results suggest that there are two initiation sites used for translation of the gag gene of MuLV, and possibly two mRNAs as well.

229 MORPHOGENESIS OF THE MEMBRANE OF BACTERIOPHAGE PM2 FROM THE CYTOPLASMIC MEMBRANE OF ALTEROMONAS ESPEJIANNA, Gregory J. Brewer, Dept. Microbiology, USC Sch. Med., Los Angeles, CA. 90033.
How is a new biological membrane assembled? What controls its size, shape and lipid composition? These questions are addressed in studying the formation of the lipid-bilayer membrane of bacteriophage PM2 inside its host Alteromonas espejianna, a Gram-negative marine bacterium. Host cells were stripped of their outer membrane and reacted with 2,4,6-trinitrobenzene sulfonic acid (TNBS). Under appropriate conditions, 65% of the total phosphatidylethanolamine (PE) of the cytoplasmic membrane was modified suggesting that 65% of the total PE is in the outer lamella. Since the host cytoplasmic membrane is 72% PE and 24% phosphatidylglycerol (PG) and the experiment indicates twice as much PE on the outside as on the inside, the rest of the lipid bilayer must be composed of PG, 95% of which is localized in the inner lamella. In the virus, the lipid composition is 27% PE and 64% PG, with PG asymmetrically localized in the outer lamella (Schäfer et al., Eur. J. Biochem. 50: 15-27 (1974)). To reconcile PG on the inner lamella of the host membrane becoming PG on the outer lamella of the viral membrane, we propose a model of viral membrane morphogenesis by invagination from the cytoplasmic membrane of the host. The stimulation of membrane invagination and specific binding of PG by viral structural proteins will be discussed.

230 SYNTHESIS, CLEAVAGE AND GLYCOSYLATION OF THE PRECURSOR POLYPROTEIN TO THE MOUSE MAMMARY TUMOR VIRUS GLYCOPROTEINS, C.Dickson & M.Atterwill, I.C.R.F., London, U.K.

Mouse mammary tumor virus (MuMTV) glycoproteins (gp52 and gp36) are derived by cleavage of a precursor polyprotein $Pr73^{env}$. The mechanism involved in the maturation of the precursor is still obscure. To elucidate some of the cellular processes involved in the maturation of these glycoproteins, we have examined the subcellular localization, composition and degree of glycosylation of the precursor and its products. In crude subcellular fractions, all the $Pr73^{env}$ and most of the other major viral precursor $Pr77/75^{gag}$ are associated with the microsomal fraction. The $Pr73^{env}$, however, is resistant to digestion with trypsin while the $Pr77/75^{gag}$ is not, indicating that $Pr73^{env}$ is a true transmembrane protein. ^{3}H-labeled sugar precursors shows that $Pr73^{env}$ contains an abundant amount of mannose and glucosamine and little fucose and galactose. The cleavage products gp52 and gp36 contain both the latter two sugars, while the mannose segregates with the gp52. These results suggest that cleavage of the precursor occurs prior to secondary glycosylation. Discontinuous density gradient fractionation of microsomes allows separation of three vesicle populations: a minor fraction of predominantly rough endoplasmic reticulum which contains $Pr73^{env}$ and $Pr77/75^{gag}$, a mixed population of less dense membrane vesicles and a third fraction of plasma membrane. The latter two fractions contain the cleaved products gp52 and gp36. However, the fucose:methionine ratio is greater in the plasma membrane fraction, suggesting that some cleaved products are associated with smooth membranes in less fucosylated form. Cell surface labeling with galactose oxidase-KBH_4, indicates that there may be a related glycoprotein in the plasma membrane which is not incorporated into virions.

231 ASSEMBLY, RELEASE, AND MATURATION OF MOLONEY MURINE LEUKEMIA VIRUS, John A. Ptak and Paul K. Y. Wong, University of Illinois, Urbana, Ill. 61801. The processing and recognition events necessary for assembly, release, and maturation of Mol-MuLV have been studied using a number of ts mutants. In addition, various antimetabolites have been employed to selectively block specific processing events in an attempt to determine the types of protein-protein and protein-nucleic acid interactions occurring during viral assembly and to delineate the proteolytic processing requirements for release and subsequent extracellular maturation. The role of actin in viral assembly will also be discussed.

232 STUDIES ON THE FUSION PROTEIN OF SENDAI VIRUS Judy White and Don Wiley Harvard University Cambridge, Mass. 02138

Proteolytic activation of the fusion protein of Sendai virus ($F_o \rightarrow F_1\text{-SS-}F_2$) and the hemagglutinin of Influenza ($HA_o \rightarrow HA_1\text{-SS-}HA_2$) confer infectivity upon both viruses and, upon Sendai, the ability to cause cell fusion. The newly generated amino terminal sequences of F_1 and HA_2 are homologous and very hydrophobic (Gething, M., J. White and M. Waterfield. 1978 PNAS 75: 2737-2740). Lipid binding studies suggest that these sequences may allow the viruses to interact with hydrophobic components of cell membranes.

To find other regions of the fusion protein involved in its activity we have conducted chemical modification and limited proteolysis experiments on biologically active vesicles containing Sendai lipids and HN and F glycoproteins. Reaction with the arginine reagent phenylglyoxal causes complete loss of hemolytic activity(a consequence of fusion) without affecting hemagglutinating activity. Mild trypsin treatment results in loss of ~2,000 M.W.from F_1 with loss of all hemolytic activity; the HN protein and activities remain unaffected. We are currently locating the site of phenylglyoxal modification and of the trypsin sensitive peptide.

233 PREPARATION AND CHARACTERIZATION OF RECONSTITUTED G PROTEIN-LIPID VESICLES FROM VESICULAR STOMATITIS VIRUS, John Lenard and Bernice I. Feuer, CMDNJ - Rutgers Medical School, Dept. of Physiology & Biophysics, Piscataway, N.J. 08854.

The single glycoprotein (G) of vesicular stomatitis virus (VSV) has been isolated in high yield by extraction of the purified virions with 0.05 M. octyl-β-D-glucoside (OG) in 0.01 M phosphate buffer, pH 8.0. The extract contains 60-80% of viral phospholipids, and is essentially free of other viral proteins. The efficiency of extraction of G protein decreases with decreasing pH, although the specificity of extraction is not affected. Dialysis to remove OG results in formation of G protein-lipid vesicles of 300-800 nm diameter with at least 80% of the G protein susceptible to externally added protease. Addition of increasing amounts of soybean phospholipid to the viral extract before dialysis resulted in vesicles of decreasing protein:lipid ratios, as indicated by decreases in buoyant density. The G protein-lipid vesicles were effective in eliciting specific anti-G antibodies that neutralized viral infectivity. Addition of G protein-lipid vesicles to BHK 21 cells prior to infection by VSV inhibited viral infectivity by up to 80%, as determined from a neutral red assay of cell viability measured 11 hours after infection.

234 VIRAL MEMBRANE GLYCOPROTEIN: SYNTHESIS, ASSEMBLY AND TOPOLOGY, Hara P. Ghosh, Robert Irving, Frances Toneguzzo, John Capone and Theo Hofmann†, Dept. Biochemistry, McMaster University, Hamilton, Ontario, Canada L8S 4J9 and †Dept. Biochemistry, University of Toronto, Toronto, Ontario, Canada.

In vitro studies using the enveloped vesicular stomatitis virus (VSV) transmembrane glycoprotein G (M.W. 69,000) show that the primary translation product of the G mRNA is a non-glycosylated protein G_1 (M.W. 63,000). In the presence of added microsomal membrane vesicles G_1 is converted to a partially glycosylated protein G_2 (M.W. 67,000) which is inserted into membrane vesicles with carboxy terminus on the cytoplasmic side of the vesicle (Toneguzzo and Ghosh, P.N.A.S. 74, 1516 (1977); 75, 715 (1978)). Determination of the NH_2 terminal sequence of G_1, G_2 and G shows that a leader sequence of 16 amino acids is present in G_1 but absent from the glycosylated proteins G_2 and G.

G_1: MetLysCysLeuLeuTyrLeu - PheLeuPheProVal - Gly↓
- Phe - - ValPhePro - - - LysGly - - - - Val -

G_2 and G: - Phe - IleuValPheProTyr - - LysGly - - Lys - ValPro - -

This leader peptide contains at least 62% hydrophobic amino acids and is removed during insertion of G_1 into the membrane (Irving et al., P.N.A.S., in press). Digestion of the VSV virion with carboxypeptidase or aminopeptidase or glycosidases shows that both the COOH- and NH_2 termini are protected from peptidases by the virion envelope. In contrast the sugar residues are readily accessible to the glycosidases.

235 THE NONGLYCOSYLATED GLYCOPROTEIN OF VSV IS TEMPERATURE-SENSITIVE AND UNDERGOES AGGREGATION AT ELEVATED TEMPERATURES. Ron Gibson, Sondra Schlesinger, and Stuart Kornfeld, Washington University School of Medicine, St. Louis, Mo. 63110.

We have studied the effect of tunicamycin (TM), an antibiotic which prevents the glycosylation of nascent proteins, on the replication of two isolates of vesicular stomatitis virus (VSV). At 38°, TM inhibited the assembly of VSV (San Juan) 97-99.9% and the assembly of VSV (Orsay) 85-95%. At 30°, in the presence of TM the assembly of VSV (San Juan) was inhibited 90-95%, while the assembly of VSV (Orsay) was inhibited only 30-55%. We have observed differences in the physical properties of the nonglycosylated G protein of VSV (Orsay) depending on the temperature at which it was synthesized. The nonglycosylated G protein synthesized at 38° pelleted to the bottom of a sucrose gradient following extraction from infected cell membranes with Triton X-100. In contrast, the nonglycosylated G protein synthesized at 30° stayed at the top of the gradient as did the glycosylated protein. The nonglycosylated G protein of VSV (San Juan) synthesized at either 30° or 38° pelleted to the bottom of the gradient. When the nonglycosylated G protein of VSV (Orsay) synthesized at either 30° or 38° was extracted with guanidine:HCl containing Triton X-100 and then dialyzed to remove the guanidine, the G protein dialyzed at 4° remained in solution while those molecules dialyzed at 38° aggregated. Glycosylated G protein treated in a similar fashion remained in solution whether dialyzed at 4° or 38°. We conclude that (1) nonglycosylated G proteins are temperature-sensitive and undergo aggregation at elevated temperatures, (2) G proteins with different amino acid sequences have different requirements for glycosylation and (3) glycosylation probably influences the ultimate conformation of the glycoprotein.

236 VARIATIONS OF OLIGOSACCHARIDE STRUCTURES ON THE VESICULAR STOMATITIS VIRUS GLYCOPROTEIN FROM VIRUS GROWN IN DIFFERENT HOST CELLS, James R. Etchison and D.F. Summers, Dept. Micro., Univ. of Utah, SLC, UT. 84132

The oligosaccharide moieties of the vesicular stomatitis virus (VSV) glycoprotein from virus grown in HeLa, L929, and MDCK cells have been compared to those from VSV grown in BHK21 cells by sequential degradation followed by high resolution gel filtration analysis. Several differences in the oligosaccharide structures are indicated by differential sensitivity to hydrolysis by endo- and exoglycosidases. Approximately 15-25 mole % of the oligosaccharide structures from VSV grown in L929 cells are resistant to a mixture of exoglycosidases and endo-β-N-acetylglucosaminidase D (Endo D) from pneumococcus and these structures are the same size as the asialo structures (S_3) from BHK-grown VSV. These resistant structures are resistant to the pneumococcus β-galactosidase but are sensitive to hydrolysis by β-galactosidase from aspergillus. An additional 30-40 mole % of the oligosaccharides from L929-grown VSV glycoprotein are also resistant to complete digestion by the glycosidase mixture and are the same size as the structures from BHK-grown VSV that have been digested with neuraminidase, galactosidase, and glucosaminidase. Based on the size, therefore, these structures appear to have been digested by the exoglycosidases in the mixture but are resistant to cleavage by Endo D. Other differences have been characterized and will be presented.

237 TUNICAMYCIN ALTERS HERPES VIRUS GLYCOPROTEIN MATURATION, L.I. Pizer, G.H. Cohen, and R.J. Eisenberg, University of Colorado, Denver, Colorado 80262 and University of Pennsylvania, Philadelphia, Pennsylvania 19174

The antibiotic tunicamycin (TM) inhibits the incorporation of glycosamine into Herpes Simplex Virus (HSV) infected BHK cells. From PAGE analyses of ^{35}S-Met labeled polypeptides we conclude that TM acts specifically on viral glycoproteins, since only the polypeptides corresponding to the HSV-glycoproteins (Molecular Wt. Classes, 50-60,000 daltons and 110-130,000 daltons) fail to appear. By use of monospecific antisera against a purified viral glycoprotein and viral envelopes, polypeptides can be found in "pulse" labeled extracts of TM treated cells that are antigenically related to glycosylated proteins. Instead of maturing into molecules of higher molecular weight, as found in untreated cells, the polypeptides in the TM treated cells cannot be detected when the initial "pulse" is "chased" for 5 hrs. It appears that when the addition of polysaccharide to HSV-glycoprotein precursor molecules is blocked, the precursor molecules are unstable.

238 RECONSTITUTION OF THE GLYCOPROTEINS OF SENDAI VIRUS. Mary-Jane Gething. Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PX, England.

A detergent dilution procedure has been utilized for the reconstitution of vesicles from an Empigen BB detergent solubilized mixture of purified Sendai virus glycoproteins and purified lipids. Electron micrographs of the vesicles obtained when the fusion protein (F) is reconstituted show that they are closed unilamellar structures of diameter 200-300 nm (cf. virus particle diameter 150-250 nm) with clearly defined glycoprotein spikes of 10-15 nm in length. When these vesicles are treated with trypsin, papain or pronase, 90-95% of F is cleaved indicating that the spikes are on the external surface of the vesicles. However none of these protease treatments remove the spikes from the lipid membrane because the cleaved fragments are held together by disulphide linkages. Reconstitution of a mixture of purified F and haemagglutinin-neuraminidase glycoprotein yields similar vesicles which exhibit haemagglutinating activity but are not active in haemolysis or cell-cell fusion. Conditions for preparing fusion active vesicles are being explored.

239 RESTRICTION BY VIRAL AND *H-2* ANTIGENS OF T KILLER CELL ACTIVITY SPECIFIC FOR MURINE LEUKEMIA VIRUSES. Fernando Plata and Frank Lilly, Albert Einstein College of Medicine, Bronx, New York 10461.

Tumor cell lines were induced by Gross leukemia virus (GV) and Friend erythroleukemia virus (FV) in a variety of mouse strains differing at *H-2* and congenic to BALB/c, including BALB/c ($H\text{-}2^d$), BALB.B ($H\text{-}2^b$), BALB.K ($H\text{-}2^k$), BALB.G ($H\text{-}2^g$), BALB.5R ($H\text{-}2^{i5}$) and (BALB/c × BALB.B)F_1 ($H\text{-}2^{d/b}$). Comparative experiments showed that variable levels of GV-specific and FV-specific cytolytic T lymphocytes (CTL) could be generated in these strains of mice after immunization with syngeneic tumor cells. Cytotoxicity assays on panels of ^{51}Cr-labeled tumor cells showed that CTL generated in each case were tumor-specific; for example, GV-immune CTL from BALB.B mice would only kill efficiently target cells induced by GV and not by other murine leukemia viruses including FV, Rauscher leukemia virus (RV), and Moloney leukemia virus (MV). In contrast, FV-immune CTL from BALB.B mice were incapable of destroying GV-induced tumor cells, but did kill tumor cells induced by FV, MV and RV (which express the FMR antigen in common). Furthermore, the tumor-specific cytotoxic activity of GV-specific or FV-specific CTL was restricted to tumor target cells sharing identity with the killer cells at least one antigenic determinant coded by the *H-2K* or *H-2D* gene. The finer analysis of these restrictions by *H-2* and by virus-induced antigens will be discussed.

Immune Electron Microscopy of Enzyme Complexes and Cellular Organelles

240 HIGH RESOLUTION EM STUDIES OF IgM, Craig A. Smith and George W. Seegan, Molecular Biology Institute, UCLA, Los Angeles, Ca. 90024

High resolution EM studies of the pentameric immunoglobulin IgM reveal it to be a very compact conformationally flexible structure, much different than the star-shaped planar structure previously suggested. Native, unaggregated equine anti-dansyl IgM (uncomplexed with antigen) appears in the micrographs as a compact amoeboid-like structure about 235A in diameter, with only a few of the expected ten Fab arms visible at the periphery and showing relatively little stain penetration into the Fc-containing central region. In contrast, cyclic pentamers of IgG when co-mounted with IgM are clearly planar and show routine resolution down to the domain level. This further suggests a compact and non-planar structure for native IgM. The Fab arm-depleted $(Fc_\mu)_5$ fragment of IgM contains only 35% of the mass of native IgM and yet still appears as a compact, nearly spherical structure with a diameter roughly 70% of the native molecule. Acid treated IgM, known from fluorescence depolarization studies to have undergone an irreversible conformational change, appears as a more open structure characterized by greater stain penetration into the central region and increased visibility of the Fab arms. These findings suggest the inability of native, unaggregated IgM to trigger secondary immunological processes such as complement activation may be due to simple steric hindrance of the secondary binding sites in the Fc portions of the molecule.

241 LOCALIZATION OF THE SITE OF ADENYLYLATION OF GLUTAMINE SYNTHETASE BY IMMUNOELECTRON-MICROSCOPY, Raymond J. Frink, Dohn G. Glitz and David S. Eisenberg, University of California, Los Angeles, CA 90024.

Antibodies to $1,N^6$-ethenoadenosine have been used to localize the adenylylated sites of E.coli glutamine synthetase. The antibodies were induced in rabbits by injection of an etheno-adenosine-bovine serum albumin complex; they precipitated both bovine and rabbit albumin complexes of ethenoadenosine or etheno-AMP, but not rabbit albumin-adenosine or AMP complexes. Antibody binding of [^{3}H] ethenoadenosine is inhibited by both ethenoadenosine and etheno-AMP; 50% inhibition occurs at concentrations of 5×10^{-8} and 1.3×10^{-7} M respectively, while ca 10^{-1} M adenosine or 5×10^{-2} M AMP are needed for an equivalent effect. Ethenoadenylyl glutamine synthetase, prepared using etheno-ATP and the E.coli adenylyltransferase, is also quantitatively precipitated by antibodies to ethenoadenosine in conjunction with goat anti-rabbit gamma globulin. Electron micrographs of antibody-glutamine synthetase mixtures show IgG molecules bound to individual glutamine synthetase dodecamers, pairs of enzyme molecules cross-linked by a single antibody, and short glutamine synthetase cables with bound antibody molecules. The adenylylation site, estimated from the apparent point of contact of enzyme and antibody, has been localized to the outer edge of the hexagonal disc offset by 15 ± 10° from the two-fold axis of symmetry through a vertex of the hexagon and 20 ± 10Å from the plane between the layers of the disc.

242 IMMUNOELECTRONMICROSCOPY OF THE 30S RIBOSOMAL SUBUNIT OF ESCHERICHIA COLI: LOCALIZATION OF THE 3'-END OF THE RNA. Helen M. Olson and Dohn G. Glitz, University of California, Los Angeles, California.

The 3'-terminus of the 16S ribosomal RNA in the intact 30S ribosomal subunit has been oxidized with sodium periodate, and the resulting dialdehyde has been conjugated with a mono 2,4-dinitrophenyl derivative of ethylene diamine. The Schiff's base addition product was then stabilized by borohydride reduction; from 0.6 to 1.1 moles of ^{3}H dinitrophenyl derivative were incorporated per mole of 30S subunit. Antibodies to dinitrophenyl-bovine serum albumin were induced in rabbits, and anti-DNP gamma globulins were purified by ammonium sulfate fractionation and gel filtration on Ultrogel ACA 22. Reaction mixtures of modified ribosomal subunits plus anti-dinitrophenyl globulins were freed of unbound antibodies by Sepharose 6B chromatography, and ribosome-antibody complexes were then examined by electronmicroscopy of negatively stained preparations. Subunit dimers, linked through a single IgG molecule, as well as individual antibodies bound to a single ribosomal subunit were observed. The predominant antibody binding site has been localized to the platform region of the subunit in the model proposed by Lake (Lake, J.A. (1976) J. Mol. Biol. 105, 131-159).

243 CONFORMATIONAL CHANGES OF PROTEINS DURING RIBOSOME ASSEMBLY: K.P. Wong and J.M. Dunn, Dept. of Biochem., Univ. of Kansas Med. Ctr., Kansas City, KS 66103.

Difference circular dichroism and chemical iodination were employed to study the conformational changes of the proteins during the assembly of the 30 S ribosomal subunit. The initial binding of the reconstituted intermediate (RI) proteins to the 16 S RNA at 5°C causes a decrease in the helical contents of the proteins. Heat activation of this RI particle to the RI* particle results in a further decrease in helical contents. This temperature dependent conformational change of the proteins in the ribosomal particles is completely irreversible upon cooling. Chemical iodinations indicate that the RI proteins upon binding to the RNA take up 30% less iodine than those in the free state. However, proteins S5 and/or S6 and S12 and/or S13, actually experience increased iodination. After the heat activation the proteins bound in the RI* particle incorporate only 60% as much iodine as the proteins free in solution. However, a number of the proteins in this RI* particle experience an increase in iodination relative to the proteins in the RI particle. These results show that the initial binding process causes conformational changes in all of the RI proteins, which are due to their interaction with the 16 S RNA and each other. The heat activation also involves irreversible conformational changes in all of the proteins. In addition, the split proteins interact directly with proteins which experience increased iodination during the heat activation step. This suggests that the key transformation of assembly is an irreversible conformational change of the proteins. These alterations may provide the binding sites for the split proteins to form the 30 S ribosomal subunit. (Supported by NIH grants GM 22962, GM 70628 and HL 18905).

244 RECOGNITION IN THE INTERACTION OF RNA POLYMERASE WITH A DNA TEMPLATE, Zaharia Hillel, Chan S. Park and Cheng-Wen Wu, Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York 10462.

We have been dissecting the interaction of E. coli RNA polymerase (RNAP) with a native DNA in order to identify elementary units involved in the recognition. This has been approached from both the protein and the DNA side of the system. RNAP (subunit structure $\alpha_2\beta\beta'\sigma$) was photocross-linked to T7 phage DNA homogeneously labeled with radioactivity, in two different types of binary complexes. In non-specific complexes which occur in all regions of the DNA, subunits β,β' and σ were identified as interacting with the DNA. In specific binary complexes and in initiation complexes (holoenzyme + T7 DNA + three nucleoside triphosphates) in which RNAP recognizes promoter sites on T7 DNA, subunits σ and β were found to photocross-link to the DNA while β' was not (Biochemistry 17, 2954 (1978)). These results suggest that while σ subunit plays a direct role in promoter recognition, subunit α is not involved in the enzyme-template interaction and indicate different modes of interaction in non-specific DNA binding and promoter recognition. To identify which DNA strand is recognized by RNAP in promoter complexes, photocross-linking was performed with T7 DNA whose major promoter region was labeled enzymatically in vitro with ^{32}P in the sense strand and ^{3}H in the non-sense strand. Analysis of the products indicated that at promoter sites, σ subunit discriminates between the two DNA strands and interacts almost exclusively with the non-sense strand. Since the β subunit most probably interacts with the sense strand in the same region, a picture emerges where two different subunits each are "holding on to" a different DNA strand possibly generating an "open" region in the promoter complex. Further studies on strand recognition by β and β' subunits are underway.

245 ACCESSIBILITY OF ANTIGENIC DETERMINANTS OF SMALL RIBOSOMAL SUBUNIT PROTEIN S4 ON ASSEMBLY INTERMEDIATES, Donald Winkelmann and Lawrence Kahan, University of Wisconsin, Madison, Wisconsin 53706

Antibody to E. coli ribosomal protein S4 reacts with intermediates of in vitro 30S subunit assembly, but not with intact 30S subunits (Winkelmann, D. and Kahan, L. (1978) Fed. Proc. 37, 1739). The accessibility of S4 in assembly intermediates is affected by the protein composition of the intermediates. S4 is very accessible to antibody in a reconstitution intermediate containing S4, S7, S8, S15, S16, S17, S19 and 16S rRNA (RI[4,7,8,15,16,17,19]). S4 is much less reactive in the RI* particle which has eight additional proteins (S5, S6, S9, S11, S12, S13, S18 and S20). This difference is due to the presence of proteins S12 and S20. The inclusion of S12 in the RI[4,7,8,15,16,17,19,12] particle results in a 70% decrease in reactivity with Anti-S4 compared to the RI[4,7,8,15,16,17,19] particle. Similarly, the RI* particle is 40% less reactive than the RI* with S12 omitted. In contrast, the inclusion of S20 in the RI[4,7,8,15,16,17,19,20] particle or its presence in the RI* particle slightly increases the reactivity of these particles with Anti-S4 when compared to the corresponding particles lacking S20. These effects may be brought about either by direct interaction of S4 with S12 and/or S20 or by differences in the conformations of the intermediates caused by the presence of the latter two proteins. The interaction of S4 and S12 is consistent with the phenotypic interdependence of mutations in the rpxD (ram) and rpxL (str) genes and with the reported crosslinking of S4 with S12 (Sommer, A. and Traut, R. (1975) J. Mol. Biol. 97, 471-481).

246 RNA-PROTEIN CROSS-LINKING WITHIN RIBOSOMAL SUBUNITS OF E. coli UTILIZING A NOVEL, REVERSIBLE, BIFUNCTIONAL REAGENT, Linda A. Brewer and Susan Goelz, Thimann Laboratories, University of California, Santa Cruz, CA. 95064

With a view toward obtaining detailed topographical information concerning the macromolecular components of the ribosome, a reagent capable of covalently and reversibly joining RNA to protein, has been synthesized. The reaction is reversible in the sense that the RNA-protein cross-link can be cleaved by treatment with mild base, allowing characterization of the involved macromolecules by standard techniques. This reagent, ethylene glycol-bis-3-(2-ketobutyraldehyde), possessing two reactive groups similar to kethoxal, reacts selectively with guanine in nucleic acid and arginine in protein. Further, since sites of kethoxal modification of RNA in both subunits have been sequenced in this laboratory, the task of identifying the components of cross-linked complexes is considerably simplified.

We have demonstrated that this compound cross-links RNA to protein within both subunits of the E. coli ribosome. Following incubation with the reagent, ^{35}S-labelled ribosomal proteins co-migrate with rRNA on SDS-agarose gels and urea-sepharose columns. Cross-linked 30S proteins were identified by two-dimensional polyacrylamide gel electrophoresis, following removal from 16S RNA by subsequent treatment with T1 ribonuclease and/or mild base cleavage of the reagent. The major 30S proteins found in cross-linked complexes under these conditions are: S4, S5, S6, S7, S8, S9(or S11), S13, S16, and S18; the minor ones are: S12, S14, and S17.

Regulation of Glutamine Synthetase

247 ANALYSIS OF GLUTAMINE AUXOTROPHS OF BACILLUS SUBTILIS, Dennis Dean and Arthur I. Aronson, Dept. Biol. Sciences, Purdue Univ., W. LAFAYETTE, IN. 47907

Approximately 25% of the glutamine auxotrophs isolated after mutagenesis and penicillin enrichment overproduce the glutamine synthetase (GS) protein 10-20 fold. They all have mutations in the structural gene for GS and are very closely linked. This pattern has been found with or without mutagens and employing a variety of selective media. Enrichment for those capable of utilizing D-histidine results in some GS mutants and these are exclusively overproducers. Several hundred auxotrophs have been screened and they all contain some enzymatic activity and considerable antigen. Alteration of the kinetic properties of GS can account for the glutamine requirement. The GS from several auxotrophs was purified and found to be the same size as the wild type by electrophoresis on SDS gels. One overproducer contained a somewhat smaller subunit. Since this mutant was revertible, a nonsense mutation near the carboxyl end is suspected although attempts to suppress the mutation have not been successful. Three factor crosses are being done in order to construct a fine structure map of the gln A locus. The pattern to date shows a clustering of the GS overproducers probably at the region coding for the carboxyl end of the polypeptide. None of the mutants is markedly altered in the rate or extent of sporulation. One mutant studied in detail is not altered in the regulation of formation of histidase, arginase nor glutamine transport. The inability to isolate auxotrophs totally lacking GS activity or antigen and the overproduction of GS in a substantial fraction of mutants implies however that the enzyme may have essential regulatory functions.

248 INITIATION RATE OF THE UV5/L8 LAC PROMOTER, J.E.Stefano, J.D.Gralla, University of California, Los Angeles, CA 90024

The rate of initiation of preformed RNA polymerase-promoter complexes has been determined for the lac mutant promoter UV5/L8 by a direct method and by the rifamycin challenge assay. This rate is very different from those measured on whole phage DNA templates, having a half-time of 35 seconds at 200uM NTP. Accordingly, complexes are extremely sensitive to rifamycin. Thus, the rate-limiting step for the overall process of UV5/L8 promoter utilization is not necessarily the rate of formation of stable preinitiation complexes. This property of the promoter contrasts with its rapid rate of complex formation, a proposed criterion for promoter strength. This observation raises the possibility of the existence of a class of promoters regulated by intrinsic initiation rates.

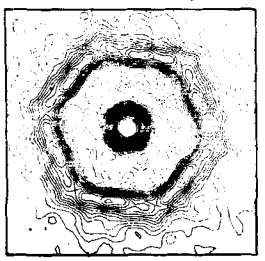
249 MUTATIONS AFFECTING GLUTAMINE SYNTHETASE REGULATION AND NITROGEN UTILIZATION IN SALMONELLA TYPHIMURIUM, Jean E. Brenchley, Susan M. Dendinger, and John M. Kuchta, Purdue University, W. Lafayette, IN. 47907.

Several Salmonella typhimurium mutants selected for altered regulation of glutamine synthetase have been characterized as to their ability to use various nitrogen sources. Two strains have elevated glutamine synthetase levels and the mutations are 83% and 66% cotransducible with glnA1010, a mutation which causes the loss of glutamine synthetase activity. Although the levels of glutamine synthetase in these mutants are always elevated when compared to those in a control strain, the activities can be further increased or decreased in response to different growth conditions. The mutants grow more rapidly than a control with arginine as a nitrogen source but grow more slowly with proline. The transport of these amino acids is correlated with their growth properties in that arginine uptake is elevated and proline uptake is decreased. Furthermore, glutamine uptake is increased 4 to 5 fold in the mutants with elevated glutamine synthetase levels. A revertant of one mutant selected for the loss of the glutamine synthetase overproduction carries a second mutation closely linked with the first. This revertant produces approximately wild type levels of glutamine synthetase, can not use arginine or L-glutamate as a nitrogen source, acquired the ability to use D-glutamate and D-glutamine as nitrogen sources, and has lowered glutamine transport. Although the regulation of glutamine synthetase in this revertant appears normal, it no longer requires the glnF gene product for expression. The combination of these and other results demonstrates the existence of nitrogen control for amino acid transport and suggests that the expression of glutamine synthetase is central to that control.

250 CONFORMATIONAL DIFFERENCES BETWEEN UNADENYLYLATED AND ADENYLYLATED *E. COLI* GLUTAMINE SYNTHETASE ON BINDING L-METHIONINE SULFOXIMINE, Ann Ginsburg, Andrew Shrake, John B. Hunt, and Edward J. Whitley, Jr., NHLBI, NIH, Bethesda, MD 20014

L-Methionine (SR)-sulfoximine (a transition state analog of glutamine synthetases) produces a tyrosyl residue perturbation when it binds to unadenylylated but not to adenylylated glutamine synthetase from E. coli, as evidenced by UV difference spectra. Hill plots of spectrophotometric titrations of the unadenylylated enzyme with L-met (SR)-sulfoximine (assuming one binding site per subunit) give: $[S]_{0.5} \simeq 45$ μM and $n_H = 0.9$ with the Mn-enzyme; $[S]_{0.5} = 9$ μM and $n_H \simeq 1.0$ with the Mn-ADP-enzyme complex; $[S]_{0.5} = 1.6$ mM and $n_H = 0.5$ with the Mg-enzyme. Binding L-met (SR)-sulfoximine to the unadenylylated enzyme species given above produces a red-shifted, UV difference spectrum consistent with the burial of one tyrosyl residue per unadenylylated subunit. This tyrosyl residue may be the site of adenylylation since the binding of L-met (SR)-sulfoximine to the unadenylylated enzyme blocks the adenylyltransferase-catalyzed adenylylation reaction. Furthermore, binding L-met (SR)-sulfoximine to fully adenylylated Mn-enzyme ($[S]_{0.5} = 180$ μM and $n_H = 0.7$) produces a red-shifted spectral perturbation of covalently bound 5'-adenylate groups. These observations and other data on Mn^{2+} binding to the enzyme demonstrate that the environments at the high-affinity metal ion site and at the adenylylation site of the glutamine synthetase subunit change on binding L-met (SR)-sulfoximine or L-glutamate at the catalytic site.

251 GLUTAMINE SYNTHETASE STRUCTURE AS DETERMINED BY VARIOUS EM STAIN AND ELECTRON DOSE CONDITIONS, J. Frank and W. Goldfarb, Division of Laboratories and Research, New York State Department of Health, Albany, NY 12201, and M. Kessel, The Hebrew University-Hadassah Medical School, Jerusalem, Israel.



The structure of Glutamine Synthetase (GS) in axial projection has been reconstructed by combining the images of separate particles using correlation search and averaging. Reconstructions of the molecules from micrographs made under conditions of different stain and electron dose show consistently related features. In each case the averaged image is a six-sided rosette without any twofold lines. The apparent handedness of the reconstructions is contrary to the probable 622 three-dimensional symmetry of the GS molecule and may be due to nonuniform stain distribution. Micrographs of negatively stained material made with very low values of electron exposure (<2 e/Å^2) yield reconstructions showing more structural features than those made with conventional doses. In the low dose images the six peripheral lobes of the figure are found to be made up of two distinct parts [fig. 1]. Positively stained low dose particle reconstructions also show this feature. The bipartate subunit structure seen in these reconstructions is consistent with the tentative model for the GS subunit proposed by Lei et al. [1] who call on a two part subunit to explain the behavior of the enzyme when exposed to proteolytic agents.

[1] M. Lei, U. Aebi, E. G. Heidner and D. Eisenberg, J. Biol. Chem., in press, 1978.

252 SPATIAL RELATIONSHIPS BETWEEN THE ALLOSTERIC AND CATALYTIC SITE OF E. coli GLUTAMINE SYNTHETASE. J. J. Villafranca, S. G. Rhee and P. B. Chock, Dept. of Chem. Penna State University, University Park, PA 16802 and NIH, NHLBI, Bethesda, MD 20014.

The activity of glutamine synthetase from E. coli is known to be affected by various feedback modifiers. Studies were conducted to test whether two inhibitors of the enzyme, viz., L-ala and D-val, bind at separate enzyme sites or overlap with the catalytic site. NMR measurements were made of the paramagnetic effects of enzyme bound Mn(II) on the protons of L-gln, L-ala and D-val. When the enzyme complex E-$(Mn)_2$-ADP (with Mn(II) bound to the n_1 metal ion site and Mn(II)-ADP to the catalytic site) was titrated with L-gln there was a monotonic increase in relaxation times of the methylene protons ($K_D^{L\text{-}Gln}$ = 10 mM). When L-ala was added the apparent binding constant of L-gln increased but the data were not consistent with competition of L-ala and L-gln for the same site. With L-ala and D-val as titrants, biphasic titration curves were obtained suggesting two binding sites for each inhibitor. The tight binding site ($K_D^{L\text{-}ala}$ = 0.6 mM; $K_D^{D\text{-}val}$ = 0.8 mM) in both cases seems to be the allosteric site. Weak binding of these two inhibitors at the active site is suggested for the observed biphasic nmr titration data and this weaker binding can be largely overcome by the presence of L-gln. Thus these results confirm our previous conclusions (Fed Proc 36, 3083 (1977)) that the allosteric sites detected by kinetic experiments are remote sites on the enzyme surface $\geq$ 10 Å from the active site. Supported in part by PHS Grant GM-23529 (J.J.V.)

253 PURIFICATION AND PROPERTIES OF GRO E PROTEIN FROM E. COLI, Zachary Burton, Ueli Aebi, Mary Lei, and David Eisenberg, University of California, Los Angeles, CA 90024.

The principal contaminant of glutamine synthetase as isolated by the procedure of Woolfolk and Stadtman [Methods in Enzymology, XVII, 915 (1970)] has been identified as gro E protein. This protein has been isolated to near homogeneity by a variation of the Woolfolk-Stadtman method, and its properties have been studied. In our procedure, bacteria are grown under conditions which repress glutamine synthetase levels. The preparation involves the following steps: (1) Cell disruption; (2) Streptomycin sulfate precipitation; (3) Polyethylemine precipitation; (4) Heating; (5) Ammonium sulfate precipitation; (6) Acetone precipitation; (7) Gel filtration. Gro E protein purified in this manner is soluble and has identical mobility on native gels to gro E purified by the technique of Hendrix [R.W. Hendrix and L. Tsui, Proc. Nat. Acad. Sci. US, 75, 137 (1978)]. Identity of the protein was established by gel mobility compared to a gro E standard and reaction with anti gro E antibody (standards and antibody were the kind gift of Dr. R. Hendrix).

254 REGULATION OF E. COLI AND THERMOPHILIC GLUTAMINE SYNTHETASE BY SUBSTRATE AND MODIFIER SITES. Frederick C. Wedler, Dept. of Biochemistry & Biophysics, Penn State University, University Park, PA 16802

Glutamine synthetase (E_o) from E. coli binds substrate L-glutamate and the transition state analog, L-methionine sulfoximine (MSOX) with either negative cooperativity or half-of-the-sites reactivity. In biosynthetic assays the binding of L-glutamate is biphasic, as is the binding of L-glutamine in transferase assays. In competition vs L-glutamate, MSOX exhibits K_i = 1 μM, but in the reaction of MSOX + MgATP the half-saturation, $I_{0.5}$, occurs near 100 μM. [^{3}H]MSOX binds to E•MgAMP-PNP in a biphasic manner ($K_1 \leq 20$ μM, r = 0.5 MSOX/SU; K_2 = 200 μM, r = 1.0 MSOX/SU).

Glutamine synthetase from the extreme thermophile Bacillus caldolyticus is found to exist as two enzymes, distinctly different in their physical, chemical, kinetic, and regulatory properties. Their separate responses to reaction products (L-Gln and ADP) and to feedback metabolic endproducts derived from L-Gln are complementary, so as to provide rigorous regulation of glutamine production by the full spectrum of crucial modifiers. Responses to modifiers depend on pH, divalent metal ions, and substrate levels. E_I is sensitive to Gly, L-Ala, L- or D-Ser, and L-Trp but not L-Gln or AMP; whereas E_{II} is insensitive to end-product amino acids but very sensitive to L-Gln and AMP. Both enzymes are inhibited significantly by ADP, GDP, GTP, IDP, ITP, CTP and NAD at or below 2 mM. There is no evidence of adenylylation-deadenylylation with either enzyme. (Supported in part by grants from NSF, NASA, and NIH).

255 REGULATION OF GLUTAMINE SYNTHETASE IN KLEBSIELLA AEROGENES, Forrest Foor, Ziva Reuveny, David Rothstein and Boris Magasanik, Massachusetts Institute of Technology, Cambridge, MA 02139

The level and adenylylation state of glutamine synthetase (GS) are regulated by the availability of ammonia. Excess ammonia leads to a low level of highly adenylylated GS (the adenylylated form is inactive), while limiting ammonia leads to a high level of unadenylylated GS. Studies on regulatory mutations at the structural gene (glnA) have led to a model of autogenous control. A second gene product (glnF) is required for expression of glnA. The properties of mutations suppressing glnF suggest that GS can be a positive activator of glnA transcription in the presence of the glnF product, and a repressor in its absence. The isolation of a probable operator constitutive mutation at the glnA site supports the idea of positive and negative control of glnA, since the mutation results in glnA that can be activated, but not repressed. Studies on new mutations in the GS adenylylation system show that the response of glnA transcription to the availability of ammonia is not mediated by the adenylylation state of GS itself, but rather directly by the P_{II} regulatory protein of the GS adenylylation system. The evidence suggests that the P_{IIA} form of P_{II} acts as anti-activator of GS stimulated transcription of glnA.

256 REGULATION OF NITROGEN METABOLISM IN ESCHERICHIA COLI, Bonnie Tyler, Fredric R. Bloom, and Greg Pahel, Massachusetts Institute of Technology, Cambridge, MA 02139

The formation of glutamine synthetase (GS) from the glnA gene increases when growth is limited by the availability of nitrogen. Mutants at an unlinked gene, glnF, synthesize such low levels of GS when grown on glucose in the presence of excess or growth-rate limiting quantities of nitrogen that they are glutamine auxotrophs. In E. coli (but not in K. aerogenes, K. pneumoniae, or S. typhimurium) the level of GS in glnF mutants increases sufficiently under conditions of carbon limitation to allow glutamine-independent growth. We have isolated two classes of mutants in which phage Mu has inserted in the glnA region of the chromosome. The first class fails to synthesize GS under any conditions. The second class is Gln^+, but produces a low level of GS regardless of the availability of nitrogen, or the presence of the glnF gene product. Analysis of mutants with these two phenotypes indicate that they result from Mu insertions in different cistrons.

GS also plays a role in the nitrogen-regulated synthesis of proteins necessary for the utilization of a variety of nitrogenous compounds. Not only mutants devoid of GS, but also glnF mutants fail to synthesize these proteins in response to nitrogen limitation. Therefore, the question arises whether the glnF gene product is only necessary for the synthesis of GS or whether it is also directly involved in the synthesis of other proteins required for the assimilation of nitrogen. Our experiments indicate that the glnF gene product is necessary for the nitrogen-regulated synthesis of the Hut (histidine utilization) enzymes.

Protein-Nucleic Acid Interactions

257 TOPOGRAPHY OF THE RNA POLYMERASE-lac UV5 PROMOTER INTERACTION
Robert B. Simpson, Biological Laboratories, Harvard University, Cambridge, MA 02138

A central transcriptional control point in bacteria is the recognition of a promoter by RNA polymerase. I have identified the thymines in the lac UV5 promoter whose methyl groups are close to bound RNA polymerase. The experiment is based on the ability of ultraviolet light to cleave 5-bromouracil-substituted DNA. In collaboration with U. Siebenlist, I have constructed a CPK model of this promoter. The contact points displayed on this model cluster and reveal critical regions in the promoter sequence for polymerase recognition. In addition, I have photochemically crosslinked the RNA polymerase to the promoter. The results place the subunits beta and sigma, respectively, two bases upstream and three bases downstream from the startpoint of transcription. The location of the sigma subunit suggests its direct involvement in promoter recognition and unwinding the DNA prior to initiation of transcription.

258 DNA POLYMORPHISM: A BASIS FOR RECOGNITION, Stanley Bram,† Alexander Rimsky,* and Hubert de Lacavier,*† †Institut Curie, *Faculte des Sciences, Paris 5, France.

Recent "alternative" non-double helical models are found not to be compatible with wide angle X-ray scattering on solutions of DNA. These experiments furnish strong support for a Watson-Crick, double, coaxial helical structure. Studies on fibers and solutions of various DNAs require that the helical parameters (i.e. the pitch) and base pair geometry depend on the base content and sequence (1,2,3). The results show that calf thymus DNA has about 10.5 b.p./turn in solution and 9.7 in chromatin, while A-T rich DNA in solution has 11 to 11.5 b.p./turn. We have considered the possible structural variations in a Watson-Crick double helix and compared them to our fiber and solution X-ray data as well as to minimum energy calculations. Base pair specific structural changes and their possible roles in the recognition of DNA sequences by proteins will be discussed.

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259 SEQUENCE RECOGNITION BY ECORI DNA RESTRICTION & MODIFICATION ENZYMES, Paul Modrich, Dept. of Biochemistry, Duke University Medical Center, Durham, N. C. 27710.

The major interest of our laboratory is in specific DNA sequence recognition by proteins. Utilizing the EcoRI enzymes as a model system, we have demonstrated that:

a) The endonuclease exists as dimers and tetramers of a 28,500 dalton subunit, with the equilibrium constant for tetramer dissociation being about 4×10^{-7} M. The dimer is catalytically active. In contrast the catalytically active form of the modification enzyme is a 39,000 dalton monomer.

b) Double strand cleavage by the endonuclease proceeds via an intermediate containing a single strand break in the EcoRI sequence. In most cases this intermediate remains enzyme bound indicating that the endonuclease dimer can interact with both DNA strands of the recognition sequence during a single binding event. In contrast, the modification enzyme transfers methyl groups to the EcoRI site one at a time with dissociation from the DNA substrate being a prerequisite for subsequent methyl transfer.

c) The endonuclease and methylase make different contacts within the EcoRI sequence during recognition and catalysis. It therefore seems likely that distinct mechanisms are employed for sequence recognition by the two proteins.

d) In collaboration with Dr. S. H. Kim we have obtained crystals of the endonuclease suitable for preliminary diffraction analysis.

This work was supported by USPHS Grant GM23719.

260 THE STRUCTURES OF THE PROTEIN-NUCLEIC ACID INTERACTION MODELS THY^2-C_3-IND^3 AND ADE^9-C_3-IND^3, Donald Voet, Gerard Bunick and William Armistead, University of Pennsylvania, Philadelphia, Pa. 19104

The title compounds consist of the nucleic acid bases thymine, cytosine or adenine linked via a propyl bridge to the indole side chain of tryptophan. The molecular interactions in these crystal structures may be taken as models for protein-nucleic acid interactions between tryptophan and the above nucleic acid bases.

Thy^1-C_3-Ind^3 crystallizes in an extended conformation. The indole group interacts with the thymine moiety of a neighboring molecule through an IN(1)-H···TO(2) hydrogen bond. In addition the indole and thymine rings of differing molecules associate through stacking interactions.

The molecules of Ade^9-C_3-Ind^3 also crystallize in an extended conformation. The indole group interacts with the adenine ring of a neighboring molecule in what can at best be described as a tenuous IN(1)-H···AN(7) hydrogen bond. There are no stacking interactions among the ring molecules of this structure. This is in constrast to the determination that in aqueous solution Ade^9-C_3-Ind^3 forms an internally stacked complex that is somewhat stronger than that of Thy^2-C_3-Ind^3. Furthermore the comparison of the Ade^9-C_3-Ind^3 structure with other crystal structures containing both adenine and indole moieties indicates that indole···adenine hydrogen bonding interactions are quite weak.

The structure of Cyt^2-C_3-Ind^3 has been solved and is being refined. Its structural features will be compared to those of Thy^1-C_3-Ind^3 and Ade^9-C_3-Ind^3.

261 THE SURFACE OF $tRNA^{Met}$ IN CONTACT WITH E. COLI METHIONINE:tRNA LIGASE, P.B. Sigler*, E. Ackerman*, J.J. Rosa*, M.D. Rosa*, and R.J. Feldmant *Dept. of Biophysics, University of Chicago, Chicago, IL 60637, †Div. of Computer Research and Technology, NIH, Bethesda, MD 20014.

Photo-induced covalent crosslinking has been used to identify a common surface of four methionine accepting tRNAs which interacts specifically with the E. coli methionine:tRNA ligase. tRNA-ligase mixtures were irradiated and the covalently linked complexes isolated and digested with T1 RNase. Only specific cognate tRNA-ligase pairs produce covalently linked complexes. The fragments lost from the elution profile of the T1 RNase digest were considered crosslinked to the protein and therefore in intimate contact with the enzyme. The four substrate tRNAs have substantially different sequences, but showed a common crosslinking pattern indicating the functionally common contact surface. The three-dimensional interpretation of these attachment points has been made in terms of the canonical conformation of crystalline yeast $tRNA_f^{Met}$ determined in this lab. The crosslinked contact surface is comprised of three regions:(1) the narrow groove of the anticodon stem and its extension into the anticodon loop; (2) the 3'-terminal residues; (3) the 3'side of the 'T-arm'. Unlike previous studies with other tRNAs, the D-arm is not involved and significant radiation damage is suffered by the tRNA which must be taken into account in the analysis. The results are consistent with and complement chemical modification studies. The crosslinked peptides of the ligase and 3-dimensional structure of the ligase are required to complete the picture of the recognition of methionine accepting tRNAs by the changing enzyme.

262 CATIONS IN tRNA: THEIR ROLE IN FOLDING AND RECOGNITION, Martha M. Teeter, Boston University, Boston, Ma. 02215 and M.I.T., Gary J. Quigley and Alexander Rich, Mass. Inst. Tech., Cambridge, Ma. 02140

For the polyanionic nucleic acids, recognition and folding into the native conformation are ionic processes to a large degree. Thus the precise location of cations along the chain becomes important for a description of the recognition surface. In crystals of yeast $tRNA^{Phe}$ and most probably in solution (the crystals are 75% solvent), cations bind tightly at or near each sharp turn the backbone makes. Weak ion binding is needed to stabilize the double helical regions, but where a cationic polyamine such as spermine binds specifically, a kink is produced in the anticodon helix. The cationic control of the tRNA conformation creates a unique surface for the recognition of it by the synthetase, ribosomal proteins and the modifying enzymes. Finally, the specific ion binding sites serve as models for the interaction of tRNA with charged amino acids which might be found on the protein surface.

263 THE USE OF A MERCURATED POLYNUCLEOTIDE IN AFFINITY LABELLING THE P-SITE of Drosophila melanogaster RIBOSOMES. STEVEN FABIJANSKI and MARIA C. PELLEGRINI. Dept. of Molecular Biology, U.S.C., L.A., CA. 90007

A mercurated polynucleotide derived from the 3' terminus of a charged leucyl-tRNA covalently labels a small, basic ribosomal protein at the P-site of peptidyl transferase in Drosophila melanogaster ribosomes. The tRNA fragment, which has the sequence N-Ac-leu-$^{3'}$A-C-C-A-C(U)$^{5'}$, has three pyrimidines which can be mercurated at the C-5 position. When bound to the ribosomes, the mercurated fragment can quantitatively transfer N-Ac-(^{3}H)-leu to puromycin; this reaction can be effectively competed with non-mercurated fragment. These data indicate P-site specific binding for the mercurated fragment. Following this binding and subsequent disassembly of the ribosomes, the mercurated fragment is found to co-purify with the r-protein fraction. Under identical conditions, non-mercurated fragment is not associated with either r-protein or rRNA. The attachment of an r-protein(s) to mercurated fragment can be cleaved by DTT reduction indicating that this linkage is a mercury-sulfer bond. The mechanism of this attachment is consistent with the observation Carrasco and Vazquez (Eur.J.Biochem. 50,317-323 (1975)) indicating the involvement of sulphydryls in eukaryotic peptidityl transferase. The use of ^{203}Hg in these affinity labelling experiments allowed autoradiographic identification of labelled r-proteins following separation by 2D gel electrophoresis. The major portion of radioactivity on these gels is located with a small, basic protein, while a minor amount of labelling may occur on one or two other proteins. A striking similarity in electrophoretic behavior exists between P-site proteins of Drosophila melanogaster ribosomes and those of E. coli.

264 THE DNA BINDING INTERFACE OF THE GENE 5 DNA UNWINDING PROTEIN
Alexander McPherson*, Andrew H.J. Wang†, Frances A. Jurnak*, Tan Molineux‡. and Alexander Rich†, *The Department of Biological Chemistry, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, PA 17033, †The Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, and ‡Department of Microbiology, University of Texas, Austin, Texas.

The three dimensional structure of the native Gene 5 DNA unwinding protein has been determined to 2.3 Å resolution by isomorphous replacement. The 87 amino acid residues are arranged in a compact unit composed of a three stranded antiparallel β sheet and two antiparallel β ribbons, there is no α helix. On one surface of the molecule is a lengthy indentation or cradle region that we believe to be the DNA binding site. The distribution of amino acid residues in this region, which includes most of those implicated in binding to DNA by physical-chemical studies, will be presented. In particular we have positioned the three tyrosine side chains, 26, 41 and 56 and phenylalanine 13, which are thought to stack upon or intercalate between the bases of the DNA. By fitting model oligo deoxynucleotides to this interface, we are hopeful that the atomic interactions involved in protein-nucleic acid binding, for this particular case, can be deduced with some precision.

Ciliary and Microtubular Organization

265 DYNAMIC AND EQUILIBRIUM PROPERTIES OF TUBULIN RING AND MONOMER ASSOCIATED GUANINE NUCLEOTIDES, Michael Caplow and Barry Zeeberg, Dept. of Biochem., Univ. of North Carolina, Chapel Hill, N. C. 27514

A recently described column centrifugation procedure (J. Biol. Chem. 252, 2891-2899, 1977) has been used to determine the stoichiometry for GDP binding at the tubulin E-site. The stoichiometry with unfractionated depolymerized protein has been related to the binding properties of ring and monomer fraction. The dissociation constant for binding of GDP at the E-site, determined by the Hummel-Dryer procedure, is equal to 6.2×10^{-8} M. Previous determinations of the dissociation constant are incorrect and the errors involved have been analyzed. Using the column centrifugation procedure it was established that GDP and GTP bind to the same site. GTP is bound 2.8 fold tighter than GDP and the dissociation constant is 2.2×10^{-8} M. The amount of guanine nucleotide incorporation during polymerization mediated by [^{3}H]GTP or by [^{3}H]GDP and ATP (where a transphosphorylation reaction is involved) has been determined. A significant fraction of the radioactive nucleotide incorporated is at a site which is transiently available to added nucleotide during the assembly process. This radioactive nucleotide is not at the N-site, although it cannot be displaced by added excess nonradioactive nucleotide; a fraction of the radioactive nucleotide incorporated during polymerization is at the E-site. The nondisplaceable radioactive nucleotide can be in either ring or monomer fractions, depending upon the conditions for depolymerization. No significant nondisplaceable nucleotide is incorporated during tubule disassembly. The kinetic properties of nondisplaceable nucleotide at the transient E-site have been analyzed.

266 VANADATE INDUCED ARREST OF MUSSEL GILL CILIA, Jacobo Wais, Anthony Paparo and Peter Satir, Dept. of Anatomy, Albert Einstein College of Medicine, Bronx, N.Y. 10461

Lateral (L) cilia of freshwater mussels beat metachronally when perfused with 12.5mM $CaCl_2$. Perfusion of these gills with ca. 20mM sodium orthovanadate (V) causes arrest of the L cilia within 5 minutes. Metachronal beat is restored when vanadate is removed from the solution within 5 minutes following cessation of beat. Pretreatment of these gills with $5x10^{-5}M$ serotonin (5HT) protects against vanadate-induced arrest, prolonging the time prior to stoppage to approximately 30 minutes. L cilia of exfoliated cells are rendered motionless within 5 minutes using ca. 0.5mM V, while detergent treated L cilia stop with ca. 5μM V. Addition of 25mM L-norepinephrine to demembranated, but morphologically intact L cilia arrested with vanadate causes the cilia to resume beating. L cilia of intact gills arrested with vanadate stop in a specific position, the "hands down" position, i.e. pointing abfrontally. This is opposite in sense to the usual "hands up" position, where the cilia point frontally, for example induced by perfusion with 12.5mM $CaCl_2$ and $10^{-5}M$ A23187. Upon addition of a solution containing 10mM V, 12.5mM $CaCl_2$ and $10^{-5}M$ A23187 to L cilia arrested briefly with vanadate, L cilia move to and arrest at an intermediate position lying in between the "hands up" and "hands down" positions. Removal of the vanadate from this solution causes some of the L cilia to move towards the "hands up" position. Conversely, upon addition of both inhibitors to L cilia arrested with Ca-ionophore, L cilia move to and arrest at an intermediate position and removal of the Ca-ionophore from the solution causes some cilia to move towards the "hands down" position. These observations indicate that the effects of Ca-ionophore and vanadate are on different mechanisms responsible for motility.

267 TETRAHYMENA CILIARY MEMBRANES: PROTEIN COMPOSITION AND MECHANISM OF BINDING TO CILIARY MICROTUBULES, William L. Dentler, University of Kansas, Lawrence, KS. 66044

Ciliary membranes were prepared using 4 different techniques and their protein composition was analyzed by SDS-polyacrylamide gel electrophoresis. Both intact ciliary membranes, prepared by 3 different methods, and membranes solubilized by detergent treatment of cilia have similar compositions: the major proteins are a 55,000 dalton protein, composing 50-60% of the membrane protein, and high molecular weight dynein-like proteins, which compose 1-13% of the membrane protein, depending on the isolation method employed. In addition, 10-20 other proteins appear to be associated with the membranes. The 55,000 dalton protein is identified as tubulin by (a) co-migration with axonemal tubulin on Tris-acetate gels, (b) splitting into α and β bands on Tris-glycine and Laemmli gels, and (c) by its similarity to other tubulins when analyzed by two-dimensional peptide maps. The membrane tubulin is weakly glycosylated, as determined by the PAS reaction. The dynein-like membrane proteins form a class of bands which co-migrate with the lower molecular weight axonemal dynein bands. The membrane dynein may be glycosylated. Studies using the photoactivatible cross-linking reagent 4,4' dithiobisphenylazide demonstrate that the membrane-associated dynein may interact with the membrane tubulin to link ciliary membranes to the outer doublet microtubules and that the membrane-associated dynein forms the outer doublet microtubule-membrane bridge.

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268 CONTROL OF CILIARY MOTION IN METAZOAN CILIA

Marika F. Walter and Peter Satir. Inst.f.Pharmazeutische Biochemie, Universität Tübingen, Germany, and Dept. of Anatomy, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Cilia were obtained from isolated ciliated gill cells of the fresh-water mussel Elliptio. The removal of the axonemal membrane with 1% Triton X-100 was followed by a brief treatment with trypsin. Samples were observed in the light microscope under dark field. Upon addition of ATP,Mg^{2+} to axonemes which were only treated with detergent, normal beat was resumed. However, addition of ATP,Mg^{2+} to trypsin-treated axonemes resulted in desintegration and sliding of the microtubules. Beat was inhibited when Ca^{2+} was added to reactivated and only detergent-treated axonemes, whereas desintegration of trypsin-treated axonemes took place to the same extent as without Ca^{2+}

We conclude that Calcium ions do not inhibit directly the sliding of microtubules and thus do not interfere with the interaction of the dynein-tubulin system. The axonemal component involved in regulating the ciliary beat is trypsin sensitive and will be studied in further detail.

269 THE ROLES OF MICROTUBULES AND MICROFILAMENTS IN MITOCHONDRIAL MOVEMENT, Timothy J. Bradley and Peter Satir, Dept. of Anatomy, Albert Einstein Coll. of Med., Bronx, N.Y. The lower Malpighian tubule of the insect Rhodnius prolixus is a simple, cuboidal, ion transporting epithelium, composed of one cell type. The surface of the cells possesses two types of cytoplasmic extensions: 1) axopods, containing arrays of microtubules and peripheral microfilaments, and 2) smaller and more numerous microvilli, primarily containing centrally located bundles of microfilaments. In vitro application of serotonin (5-HT) induces extension of the mitochondria previously located in the cell interior to a more apical position inside the microvilli and axopods. When examined in the electron microscope, the mitochondria are intimately associated with microtubules and microfilaments during 5-HT-stimulated movement. Application of 10mM colchicine at 0 C for 10 min, followed by 10 mM colchicine at 22 C for 20 min greatly reduces the number of microtubules both in the cell interior and in the axopods. When subsequent to this treatment, 5-HT is applied in the presence of colchicine, or if 5-HT stimulation precedes and continues during colchicine treatment, the mitochondria move normally. In contrast, treatment with 20 M cytochalasin B for 15 min or removal of carbon energy sources from the bathing medium entirely blocks the movement of mitochondria in response to 5-HT application. This suggests that the final stages of mitochondrial movement into the microvilli in association with stimulation by 5-HT depend on cytochalasin-sensitive elements (microfilaments?) and do not depend on microtubules. Supported by USPHS grant HL22560 and NIH postdoctoral fellowship AM 05499.

270 THE STABILITY OF SMALL SHEETS AND THEIR ROLE IN NUCLEATION OF MICROTUBULE ASSEMBLY, H. P. Erickson, Anatomy Department, Duke University, Durham, N. C. 27710

Assembly of the microtubule wall is essentially a phenomonon of two dimensional polymerization. Small sheets are formed and grow larger by stepwise additon of individual subunits or short filaments. Since the smaller sheets are less stable thermodynamically than large sheets and intact microtubules their formation is the limiting step in nucleation of assembly. For assembly to occur the "critical embryo" (the least stable sheet in the pathway) must exist as an equilibrium structure at a concentration on the order of the final yield of microtubules. The concentrations of the small sheets may be calculated if one can estimate (a) the energies of the inter-subunit bonds; and (b) the energy, corresponding to the "intrinsic entropy" of the free subunit, required to immobilize the subunit in the polymer. A reasonable model of microtubule assembly can be obtained in which the critical embryo is a sheet of two protofilaments, 6 to 12 subunits long. It is formed at a concentration of 10^{-10} M in equilibrium with a subunit concentration 3 to 4 times the final equilibrium or critical concentration. A novel and crucial point in this calculation, and one that is relevant to a broad range of assembly reactions for two dimensional and helical polymers, is the explicit use of the intrinsic entropy. Reasonable assembly models appear to require a value of about 10 e.u. (3 kcal/mol), much lower than previously published estimates. If larger values (40 e.u.) are assumed for the loss of intrinsic entropy of the polymerized subunits special mechanisms must be proposed to explain the stability of small sheets.

Assembly Interactions: Thermodynamic Aspects

271 INTERFERON INDUCED ALTERATION OF MuLV ASSEMBLY, P. M. Pitha, B. Fernie, and F. Maldarelli, Johns Hopkins University, School of Medicine, Baltimore, MD 21205

Interferon treatment of JLSV-6 cells chronically infected with Rauscher MuLV leads to the formation of noninfectious particles (interferon virions) containing in addition to the structural proteins of env and gag genes additional viral polypeptides. While in the control virions the major glycoprotein detected is gp71 interferon virions contains in addition to gp71 and 85k dalton (gp85) glucosamine-containing, fucose-deficient glycoprotein which is recognized by antiserum to gp71 and may be related to env pr85. The surface iodination of the intact virions indicates that both gp71 and gp85 are the major components of the external virions envelope. However, unlike the control virions in which gp71 associates with p15E (gp90), the gp71-p15E complex was not detected in interferon virions. The analysis of ^{3}H-amino acid labeled proteins or iodinated proteins of the disrupted interferon virions revealed the presence of 65k, 55k, 40k, 20k, and 12k dalton proteins precipitable with antiserum to MuLV.

A distinct difference in the patterns of incorporation of pulse labeled (^{3}H) amino acid polypeptides into virions was observed in the presence and absence of interferon. The virion polypeptides labeled in the presence of interferon and recovered in the extracellular virions in a chase with interferon appeared to have substantially less copies of p30 and more 55k polypeptide than the controls. These results indicate that the assembly associated proteolytic cleavage is altered in the presence of interferon.

272 MECHANISM OF SICKLE CELL HEMOGLOBIN SELF ASSEMBLY, William A. Eaton, James Hofrichter, Frank A. Ferrone and Helen R. Sunshine, NIH, Bethesda, Md. 20014

Deoxygenated sickle cell hemoglobin (Hb S) in concentrated solutions polymerizes into multi-stranded helical fibers, which spontaneously align to form an ordered phase. At equilibrium the system is well-described by a simple crystallization model. When polymerization is initiated by a temperature increase there is a pronounced delay prior to the appearance of polymer. The delay time depends upon a very high power (30-50) of both the initial Hb S concentration and the equilibrium solubility. These derivatives are well-explained by an equilibrium nucleation - growth model, which includes the large effects of solution non-ideality. The model is readily extended to multiple components and can quantitatively account for the composition, concentration, and temperature dependence of the delay time in mixtures of Hb S with normal adult or fetal hemoglobins, and in solutions partially saturated with carbon monoxide. The model fails, however, to predict the extreme autocatalytic shape (10-20th order time dependence) of the progress curves with delay times between 10 sec. (the shortest time that has been investigated with a temperature-jump technique) and 10^5 sec. To induce polymerization more rapidly, laser photolysis of carbonmonoxy Hb S has been employed to prepare Hb S at arbitrary fractional saturation with CO in about 5 msec. Polymerization is detected by measuring the scattered laser light. Progress curves with delay times in the subsecond regime exhibit as little as a 3rd order time dependence, much closer to predictions of the equilibrium nucleation - growth model. These results suggest that the high order time dependence for more slowly polymerizing samples results from some additional process, such as the fragmentation of polymers or the nucleation of domains of aligned polymers.

273 ROLE OF OLIGOMERS IN MICROTUBULE ASSEMBLY AND DISASSEMBLY, Richard Weisenberg, Department of Biology, Temple University, Philadelphia, PA 19122

Tubulin can aggregate into a variety of oligomers in addition to microtubules. The formation of oligomers is dependent upon the presence of microtubule associated proteins (MAPs) divalent cations, temperature and other parameters. To study the role of oligomers we have studied the rates of nucleotide exchange during polymerization and depolymerization, the kinetic and steady state behavior of tubulin by analytical centrifugation, the reversibility of microtubule assembly in colchicine and GDP and the effect of MAPs and temperature on each of the above. Our results suggest that microtubule assembly involves small oligomers (not rings) and as a consequence microtubule assembly and disassembly can display cooperative behavior. Direct evidence for cooperative disassembly of microtubules by Ca has been obtained from measurements of disassembly kinetics, length distributions, and release of labeled subunits. The rate of microtubule disassembly by Ca is several times faster than that obtained by dilution, while no change is observed in the length distribution of tubules after Ca treatment. No change is observed following Ca addition in the specific radioactivity of microtubules which have been labeled at their ends. These results suggest a cooperative all-or-none response to Ca. Apparent irreversible behavior of microtubules in GDP and colchicine may also be explained by cooperative interactions, which are probably mediated by MAPs.
Supported by NSF grant PCM 76-02307.

274 HIGH AFFINITY CATION BINDING SITE OF LIPOPOLYSACCHARIDE FROM GRAM-NEGATIVE BACTERIA, Melvin Schindler and M. J. Osborn, Dept. of Microbiology, UCONN Health Center, Farmington, CT 06032

Lipopolysaccharides (LPS) were isolated from Salmonella typhimurium G30A (rough), G30, and LT2 (smooth). These variants differed in the amount of carbohydrate attached to the lipid A backbone but shared as structural elements the lipid A moiety and three acidic sugars (3-deoxy-D-manno-octulosonic acid or KDO). To investigate Ca^{++} and Mg^{++} binding, these LPS molecules were derivatized with the fluorescent chromophore dimethyl-aminonaphthalene sulfonyl chloride (DNS-Cl) and subjected to fluorimetric titrations. Both Ca^{++} and Mg^{++} demonstrated a shift in the emission maximum and an increase in the relative fluorescence of the dansylated derivatives. These effects were completely reversible with EDTA. The dissociation constants (Kd) were 6μM for Ca^{++} and 15μM for Mg^{++} in G30A and G30 LPS. Equilibrium dialysis gave a stoichiometry of approximately 1.0 cation per mole LPS in each case. Acid hydrolysis (0.1N acetic acid, 100°C for 1h) of G30 afforded a lipid A free of carbohydrate side chains and KDO. In this case, a much lower Kd ~80μM and a stoichiometry of 0.5 moles Ca^{++} per mole LPS was obtained. A precursor of LPS biosynthesis without KDO residues gave similar results to the pure lipid A. LPS therefore has a unique high affinity carbohydrate site for cations. These results in conjunction with other data suggest that this site plays a major role in a postulated LPS catalyzed assembly of the outer membrane of gram negative bacteria. (Supported by USPHS Grant AI-08650).

275 THERMODYNAMICS OF POLYMERIZATION AND POLYMORPHISM OF TUBULIN. M.-F. Carlier,* D. Pantaloni, J. Laporte, T. Soussi, C. Simon. Laboratoire d'Enzymologie, CNRS 91190 Gif-Sur-Yvette, France.

In vitro polymerization of tubulin free from MAPS was monitored by turbidimetry in the presence of glycerol, GTP and 2-10 mM $MgCl_2$. Kinetic analysis of the polymerization process showed that an initial slow nucleation is followed by a growth process. The elongation rate constant varied cooperatively with tubulin concentration. The cooperativity indicated the size n of the nucleus, which is defined as the shortest photofilament able to undergo stable lateral interaction (Carlier and Pantaloni, 1978, Biochemistry 17, 1908). The value of n is determined by the ratio of longitudinal to lateral energies between the tubulin subunits in a microtubule wall. Its dependence on temperature (n = 24, 14, 8, 4, 2 at 25, 30, 35, 40, 45°C, respectively) is due to the different signs of the Van t'Hoff enthalpies of longitudinal and lateral bonds. The extent of inhibition of polymerization by vinblastine has the same temperature pattern as n, showing that vinblastine inhibits the nucleation process. Mg^{2+} and ^{+}H ions do not affect the value of n, but change the polymorphism of polymers: the ratio microtubules/sheets increases with increasing Mg^{2+} and pH. Statistical studies of sections of embedded sheets and microtubules show that the lateral growth of microtubule walls is bidirectional and that the frequency of inversion of a photofilament in the wall is 2.33% in 5 mM $MgCl_2$.

276 THE SELECTIVE BINDING OF E. COLI RNA POLYMERASE TO T7 PROMOTERS, Harlee Strauss, R. R. Burgess and M. Thomas Record, Jr., University of Wisconsin, Madison, WI 53706

We have investigated the selectivity of binding of E coli RNA polymerase to promoter sites over a range of solution conditions. We used a double label nitrocellulose filter binding assay involving a ^{32}P-labelled T7 DNA restriction fragment containing two promoters for E coli polymerase (A1 and D) and ^{3}H-labelled nonpromoter restriction fragments of comparable size. Approximately equimolar amounts of the ^{32}P and ^{3}H fragments were incubated with sigma saturated holoenzyme and filtered through a nitrocellulose membrane. Selectivity was evidenced by greater fractional retention of the promoter-containing fragment on the membrane. We studied binding as a function of $[Na^+]$, $[Mg^{2+}]$, pH and temperature. We find selective binding in the absence of divalent cations and at low temperature (0-17°C) as well as high temperature in the presence of Mg^{2+}. The stability of RNA polymerase-promoter complexes is a very sensitive function of the pH of the solution, while RNA polymerase-nonpromoter complexes are only mildly sensitive to pH [deHaseth, P. L., Lohman, T. M., Burgess, R. R. and Record, M. T. (1978), Biochemistry 17, 1612-1622]. Consequently selectivity of binding is lost at high pH (>7.5 at 0°C and 8.5 at 37°C). The temperature dependence of binding shows an abrupt, twofold increase in selectivity near 20°C. The selective binding at low temperature and the transition at 20°C support the closed complex-open complex model of RNA polymerase binding proposed by Chamberlin [Chamberlin, M. (1974) Ann. Rev. Biochem. 43, 721-775].

Non-Muscle Motility

277 BRUSH BORDER α-ACTININ? Mark S. Mooseker and Raymond E. Stephens. Marine Biological Laboratory, Woods Hole, Ma. 02543.

The cytoskeleton of nonmuscle cells may contain a protein similar to the Z line component, α-actinin, based on results of antibody localization studies. From such studies on the brush border of intestinal epithelial cells we have suggested a role for α-actinin in the attachment of actin filaments to each other and to the plasma membrane. SDS gels of brush borders isolated from chicken intestine contain two prominant polypeptides with molecular weights similar to that of α-actinin (95,000 and 105,000 daltons). These two polypeptides were compared to α-actinins isolated from chicken smooth and skeletal muscle by the technique of tryptic peptide mapping. The brush border proteins and chicken gizzard and breast α-actinins were purified by preparative SDS gel electrophoresis. Tryptic digests were compared by chromatography-electrophoresis on silica gel thin layer plates (Stephens, Anal. Biochem. 84:116). The two brush border sub-units and both α-actinins each yielded 90-95 discrete peptide spots/ n mole of protein. The homology among these proteins was estimated by determining the degree of coincidence on composite maps. The two brush border sub units showed 85-90% coincidence indicating a high level of relatedness. Skeletal and smooth α-actinins were 65-70% coincident; both brush border proteins were 55-60% coincident with skeletal α-actinin but only 45-50% coincident with smooth muscle α-actinin.
These results indicate that there may be sufficient homology between the brush border protein(s) and α-actinin to explain immunological similarities, but the functional similarity between them is unknown because the role of α-actinin in muscle is not understood .

278 CALCIUM-ATP BUFFERS REACTIVATE CHROMOSOME MOVEMENTS IN VITRO. E.D. Salmon and R. Segal. Department of Zoology, University of North Carolina, Chapel Hill, NC 27514.

We have been able to induce shortening of kinetochore fibers and poleward movement of chromosomes at physiological rates (3 μm/min) in detergent-extracted spindles of the sea urchin Lytechinus variegatus. Spindles were isolated at 25°C using a calcium-chelating lysis buffer: 5.0-EMP (5.0 mM EGTA, 0.5 mM $MgCl_2$, 10 mM PIPES, pH 6.8) plus 0.5% Nonidet P-40 and 25% glycerol. For reactivation experiments the spindles were resuspended in 5.0-EMP plus 50% glycerol at 22°C. When the spindles were perfused with 0.2-EMP buffer (0.2 mM EGTA, 0.5 mM $MgCl_2$, 10 mM PIPES, pH 6.8) followed by the same buffer to which had been added 1 mM ATP, pH 6.8, the spindles' birefringence and morphology remained largely unchanged. When spindles were perfused with 0.2-EMP buffer plus 0.13-0.16 mM $CaCl_2$ (no ATP), BR decayed but kinetochore fibers did not shorten. If, however, the spindles were perfused with 0.2-EMP buffer containing both calcium and ATP, spindle birefringence decayed, kinetochore fibers shortened, and the chromosomes moved poleward, the rates all being increasing functions of $[Ca^{++}]$. If $[Ca^{++}] > 5$-10 μM, spindle birefringence disappeared within 10-20 sec, but the chromosomes hardly moved. It appears that, in living cells, local control of calcium ion levels may govern two processes simultaneously: (1) the rate of depolymerization and shortening of the kinetochore fiber microtubules, which "rate-limits" chromosomal velocity, and (2) the activation of poleward forces that are transmitted along the kinetochore fiber microtubules. Supported by NSF 77-07113 and NIH GM-24364-1.

279 DOUBLE LABEL IMMUNOFLUORESCENT STUDIES OF ACTIN, MYOSIN, TROPOMYOSIN, α-ACTININ AND FILAMIN IN A NONMUSCLE CELL TYPE. William E. Gordon III and Stephen H. Blose, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724.

Double label immunofluorescence was used to study the organization of the actomyosin system in gerbil fibroma cells (CCL 146). Actin, myosin, tropomyosin, α-actinin and filamin were all localized individually and in pairs relative to stress fibers, polygonal microfilament networks, ruffling membranes and regions of cell-cell contact. In stress fibers and polygonal networks myosin, tropomyosin and α-actinin are localized in discrete units. Our results provide direct confirmation that the units of both myosin and tropomyosin alternate with the units of α-actinin. In addition, they provide the first demonstration of the exact coincidence of the myosin and tropomyosin units. Finally, they show the distribution of these periodic units relative to actin, which has a mostly uniform distribution. The distribution of filamin is the most intriguing because of its variability; it is coincident with actin in stress fibers, whereas in polygonal networks it is present only in some connecting fibers but in all foci. In the foci filamin is excluded from a small region at the center where actin and α-actinin are both present. Localizations in membrane ruffles and cell-cell contact areas varied. In some cases actin and filamin were found to be present with the other proteins apparently excluded. Yet, numerous examples were noted where the other proteins were localized in such areas as well.

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280 GLUTAMINE SYNTHETASE AND THE REGULATION OF NITROGEN FIXATION IN RHIZOBIUM. Robert A. Ludwig, Dept. of Biology, M.I.T, Cambridge, Mass. 02139.

Present evidence implies that glutamine synthetase I (GS I) is involved in the regulation of nitrogenase in Rhizobium both in culture and in symbiosis. Rhizobium 32H1 assimilates ammonium very poorly and does so solely via the glutamine synthetase - glutamate synthase pathway. Mutant analysis suggests that of the two GS activities present, it is GS II which is primarily responsible for ammonium assimilation (i.e. growth of the cell on ammonium as sole nitrogen source). Nevertheless GS II is highly sensitive to repression by ammonium, its substrate! A mutant which results in highly adenylylated GS I and which is also GS II$^-$ fails to induce nitrogenase under appropriate conditions both in culture and in symbiosis. Revertants which maintain a highly adenylylated GS I but are GS II$^+$ still fail to induce nitrogenase, wherease revertants which possess a constitutively deadenylylated GS I and which are still GS II$^-$ overproduce nitrogenase. Whether or not this latter class of revertants expresses nitrogenase activity constitutively (i.e. is not susceptible to ammonium repression) during symbiosis is presently being studied.

In Rhizobia nitrogen fixation is normally a process which is discrete from those of cell growth, and this occurs in a terminally differentiated form of the organism, the "bacteroid". However evidence suggests that revertants of the aforementioned latter class can under appropriate conditions assimilate ammonium produced by nitrogenase for cell growth. This would allow direct selection for nitrogen fixation-defective mutants in Rhizobium.

281 GLIDING MYCOPLASMAS CONTAIN A POLYMERIZING PROTEIN FRACTION, Jack Maniloff and Utpal Chaudhuri, Dept. of Microbiology, Univ. of Rochester, Rochester, NY 14642.

Mycoplasma gallisepticum has subcellular organelles which may function as a primitive "mitotic-like" apparatus. Cytochalasin B (CB) inhibits division of these cells, but does not inhibit their glucose or macromolecule precursor uptake (Cell 13:57, 1978). Since these data suggested that CB may affect some sort of actin-like protein structures, we have attempted to isolate such a protein from M. gallisepticum. An isolation protocol similar to that used to isolate actin yields a reversibly polymerizing protein fraction. Polymerization (as measured by viscometry) occurs in high salt (0.6 M KCl) and depolymerization in low salt. Electron microscopy of the polymerization product shows 6-9 nm fibers. Polyacrylamide gel electrophoresis of the depolymerized protein shows three bands; prominant bands at 46,000 and 60,000 daltons and a faint band at 95,000 daltons. Further studies of the effect of CB on growth of other mycoplasmas show that only M. pneumoniae and M. pulmonis are also inhibited. These two plus M. gallisepticum are the only mycoplasmas which have terminal structures and are known to exhibit gliding motility. The CB effect on these three mycoplasma species is static, not cidal, and is reversible.

282 ANKYRIN: THE MEMBRANE PROTEIN WHICH ATTACHES HUMAN ERYTHROCYTE SPECTRIN TO THE CYTOPLASMIC SURFACE OF THE PLASMA MEMBRANE, Vann Bennett, The Wellcome Research Laboratories, Research Triangle Park, N.C. 27709

The membrane attachment site for spectrin has been examined by measuring reassociation of pure human erythrocyte [^{32}P]spectrin with inside-out vesicles which were depleted of spectrin and actin. [^{32}P]spectrin binds specifically to a single class of protein sites localized on the inner surface of the membrane with a k_D of 10^{-7}-10^{-8}M (Bennett, V., Branton, D. (1977) J.B.C. 252 2753). A 72,000 M_R proteolytic fragment of this attachment site has been purified and characterized, and shown to bind to spectrin in solution and to compete for binding of spectrin to vesicles (Bennett, V. (1978) J.B.C. 253, 2292). Antisera have been raised against this fragment which had been purified further by SDS PAGE. Monospecific IgG, prepared by affinity chromatography with immobilized fragment, is a potent inhibitor of binding of spectrin to vesicles. A 200,000 M_r protein (band 2.1) is the only membrane polypeptide which cross-reacts with the IgG, and this reaction is abolished in the presence of the 72,000 M_r fragment. A specific complex of spectrin and band 2.1 was isolated with anti-fragment IgG following reassociation of spectrin with inverted vesicles. Band 2.1 has been partially purified from detergent extracts of spectrin-depleted vesicles and is a competitive antagonist of binding of [^{32}P]spectrin to membranes with a K_i of 2 μg/ml. It is concluded that band 2.1 is the protein responsible for the high affinity association of spectrin with the inner surface of the membrane. This polypeptide has been named "ankyrin" from the Greek ankyra which means anchor.

Electron Diffraction and Membrane Proteins

283 CRYSTALLINE CYTOCHROME OXIDASE VESICLES PREPARED BY A CHOLATE METHOD, W. Goldfarb, and J. Frank, Division of Laboratories and Research, New York State Department of Health, Albany, NY 12201, J. C. Hsung, C. H. Kim and T. E. King, Laboratory of Bioenergetics, State University of New York at Albany, Albany, NY 12222.

We have formed cytochrome oxidase crystalline membranes starting with the highly purified enzyme and reconstituting it into liposomal form with azolectin, a soybean phospholipid mixture, using cholate for solubilization. These crystals are suitable for low dose high resolution electron microscopy and image reconstruction. They appear to be similar to the crystalline membranous sheets formed from Triton preparation of mitochondrial fragments [1]. However, the enzyme when solubilized by deoxycholate or Triton shows very low respiratory control when reconstituted into liposomes while, in contrast, cytochrome oxidase prepared with cholate has high respiratory control and oxidative phosphorylation in the presence of other factors. Our results show that negatively stained normal projections of the cytochrome oxidase crystal lattice have p22 symmetry and unit vectors of $\underline{a}$ = 91 ± 4 Å and $\underline{b}$ = 117 ± 5 Å. Computer averaged reconstructions of the electron microscope images are presented.

[1] Vanderkooi, G., Senior, A. E., Capaldi, R. A. and Hayshi, H. (1972) Biochim. Biophys. Acta 274, 38-48.

284 THE EFFECT OF OPTICAL ABERRATIONS ON IMAGING OF PERIODIC BIOLOGICAL STRUCTURES, J.W. Wiggins and M.E. Dumont, Department of Biophysics, The Johns Hopkins University Baltimore, Maryland 21218.

When examining biological structures by electron irradiation, damage to the specimen by the electrons limits the cumulative dose to about one electron per sq. Angstrom. With this limitation, high resolution information has been obtained only from periodic structures; most notably the purple membrane. In at least four cases, periodic specimens have been obtained that yield electron diffraction patterns to about 3.5 Angstroms resolution. Direct imaging of the same specimens yield only about 7 Angstroms resolution. After pondering the cause of the poorer resolution obtained in imaging, we have concluded that radial distortion of the image (generally classified as barrel or pincushion distortion) is a major factor. A simple estimate for imaging purple membrane in the best current microscopes implies a resolution limit of about six Angstroms. There are several approaches to correcting the distortion. The simplest is to make a direct measurement of the distortion in a given microscope and to correct the image accordingly. The distortion may change or be difficult to measure with sufficient accuracy. We are investigating a separation of the Fourier transform of the image into the product of two terms: one containing the distortion factors and the other containing the structure factors. This separation would allow one to determine the distortion directly from a given image and account for it. The procedure would be somewhat analogous to the determination of the temperature factors in X-ray diffraction measurements.

285 STRUCTURE OF CYTOCHROME OXIDASE IN TWO CRYSTAL FORMS Stephen D. Fuller and Roderick A. Capaldi, Univ. of Oregon, Eugene, OR 97403

A model for the structure of cytochrome oxidase, based on reconstructions of the molecule in two crystal forms, has been developed. Three dimensional reconstruction of the $P2_1$ deoxycholate derived form reveals the molecular boundry of the 180 kd monomer. Reconstructions of the $P2_12_12$ Triton X-100 derived form, which contains a dimer, in media of different densities disclose the location of the lipid bilayer.

The shape of the enzyme can be discerned by a comparison of the two forms. In overall shape the monomer is a lop-sided "Y",dividing naturally into three domains. The stem of the "Y", domain C, projects 60 A into the solution from the cytoplasmic side of the mitochondrial inner membrane. The arms of the "Y", domains M_1 and M_2, cross the 40 A bilayer in which they are seperated by lipid. These two domains extend approximately 15 A from the other side of the membrane The whole molecule is approximately 115 A in length. The inter-monomer contact is along the flat of the "Y".

This work was done in collaboration with Richard Henderson and James F. Deatherage at the MRC Laboratory of Molecular Biology, Cambridge, England.

286 STRUCTURAL STUDIES OF PURPLE MEMBRANE AT LOW TEMPERATURE, Steven B. Hayward, Donner Laboratory, University of California, Berkeley,CA 94720

The orientation of the purple membrane electron diffraction pattern with respect to the extracellular and cytoplasmic sides of the cell membrane has been determined using membranes adsorbed to polylysine in a preferred orientation. High resolution order of the membranes was preserved by a new method of specimen preparation. Grids with adsorbed membranes were withdrawn through a Ca-stearate film at an air-water interface. The resulting hydrated specimens, devoid of bulk water, were frozen in liquid nitrogen, and observed on a low temperature electron microscope stage. Low dose imaging and diffraction of glucose-embedded membranes at room and low temperature respectively was used to relate the diffraction pattern orientation to the structure of the membrane at 7Å resolution. The α-helical segments were found to fan outwards towards the cytoplasmic side of the membrane.

Electron diffraction pattern fading experiments have shown that the critical dose for radiation damage of purple membrane is five times greater at -120°C than at room temperature. A mechanically stable low temperature stage has been designed and constructed, and has been shown to be capable of imaging to 3.4Å resolution. This stage should be useful for imaging purple membrane to higher than 7Å resolution.

287 THE STRUCTURE OF CYTOCHROME c OXIDASE: USE OF SPECIFIC LABELING TECHNIQUES IN ELECTRON MICROSCOPY OF TWO-DIMENSIONAL CRYSTALS. T.G. Frey* and S.H.P. Chan+, *Johnson Foundation, University of Pennsylvania and +Dept. of Biology, Syracuse University.
Chemical and antibody labeling of whole mitochondria and of membrane-inverted submitochondrial particles clearly show that cytochrome c oxidase spans the inner mitochondrial membrane asymmetrically with different subunits exposed on the two sides (Eytan et. al., JBC 250, 8598, 1975; Chan et. al., Eur. J. Biochem. 89, 595, 1978). Structural studies by electron microscopy of two-dimensional crystals of cytochrome c oxidase reveal an asymmetric distribution of stain-excluding material across the lipid bilayer in the collapsed vesicles from which the crystals are derived; most of the material appears to be on the inside of the vesicle (Henderson et. al. JMB 112, 631, 1977). We have combined both techniques, antibody labeling and electron microscopy, and we have determined that antibodies against subunits II and III will not bind to "crystalline" cytochrome c oxidase vesicles, but antibodies against subunit IV will bind to the external surface of the crystals. Thus, the orientation of the enzyme in these crystalline vesicles is opposite to its orientation in the mitochondrion; the matrix or inner side of the mitochondrial inner membrane is the external surface of cytochrome oxidase crystals. From this work and that of Henderson et. al., one can conclude that in the intact mitochondrion, cytochrome c oxidase protrudes 60-70 Å beyond the lipid bilayer on the outer or cytoplasmic side of the mitochondrial inner membrane and very little beyond the lipid bilayer on the inner or matrix side. Preliminary results will be presented on the use of subunit-specific Fab (antibody) fragments and heavy atom clusters to label cytochrome oxidase crystals.

288 ACETYLCHOLINESTRASE (AchE) AND ACETYLCHOLINE RECEPTOR (AchR) LOCALIZATION IN ADULT MAMMALIAN SKELETAL MUSCLE, U.R. Tipnis and S.K. Malhotra, Biological Sciences Electron Microscopy Laboratory, University of Alberta, Edmonton, Alberta T6G 2E9.
Denervation of adult skeletal muscle leads to spread of Ach sensitivity to extra-junctional sarcolemma and this is attributed to the incorporation of AchR molecules in this region of the sarcolemma following denervation (Devreotes and Fambrough, 1976, Cold Spring Harbor Symp., Quant. Biol. 40: 237). By using ^{125}I-α-bungartoxin and Ferritin-α-bungarotoxin conjugates, we have attempted localization of AchR molecules in innervated and denervated sarcolemma by autoradiography (^{125}I-α-bungarotoxin) and freeze-fracturing-etching (Ferritin-α-bungarotoxin) at electron microscopic level. By using specific inhibitor for AchE and histochemical localization, we have obtained results that indicate that of the three known molecular forms (Hall, 1973, J. Neurobiol. 4: 236), 16s form of AchE undergoes markedly detectable loss in denervated muscle sarcolemma (Malhotra and Tipnis, 1978, Proc. Roy. Soc. Lond. B., in press). (Supported by grants from the National Research Council of Canada.)

289 CHEMICAL MODIFICATION OF BACTERIORHODOPSIN FOR DIFFRACTION. M.E. Dumont and J.W. Wiggins, Jenkins Department of Biophysics. The Johns Hopkins Univ., Baltimore, Md. 21218
Chemical modification of bacteriorhodopsin is being used to produce and characterize heavy atom derivatives for electron diffraction. A complex of the dipeptide glycyl-L-methionine with platinum binds to between two and six sites per bacteriorhodopsin, depending on the reaction conditions. Because it is uncharged at neutral pH and has a hydrophobic surface, the complex is attractive for use in membranes. Oxidation of purple membrane with performic acid causes a large decrease in subsequent platinum binding, suggesting methionine as the primary platinum-binding site, since purple membranes contain no cysteine. Similar results were obtained with sodium azide, a compound commonly used to prevent bacterial growth in purple membrane suspensions, but which has also been reported to attack methionine. Extensive modification of bacteriorhodopsin with ethyl acetimidate, a lysine reagent, caused only a slight decrease in platinum binding. Also, trinitrobenzene sulfonate quantitation of purple membrane free amines shows a small decrease (from 3.5 to 3.0) in the number accessible amines per protein when platinum is bound. Temperature, salt concentration, and pH all also influence platinum binding.

Thus, chemical manipulations can be used to prepare different platinum derivatives of purple membrane. Electron diffraction of one such derivative has already been performed. A difference map between labelled and native membranes has been obtained, and the heavy atom positions are now being refined. In addition, isomorphous replacement is being used to phase the high resolution purple membrane diffraction obtained by Steve Hayward (PNAS, 75, p. 4320 (1978)). We acknowledge support by USPHS grants GM24237 and 5SO7RR07041-12.

290 TOPOGRAPHICAL ASPECTS OF THE NICOTINIC ACETYLCHOLINE RECEPTOR IN POST-SYNAPTIC MEMBRANES, Vejᵗ Witzemann, and Michael A.Raftery, California INSTITUTE OF TECHNOLOGY, Pasadena, Ca.91125

The nicotinic acetylcholine receptor complex purified from Torpedo californica was shown to consist of several polypeptides of different molecular weights. Since solubilization and purification could cause major changes in the quaternary assembly of subunits it is important to perform structural and topographical studies on the acetylcholine receptor only in its membrane environment. For that reason photolabile bifunctional reagents were synthesized, which could be selectively linked to ^{125}I labeled α-bungarotoxin via disulfidebridges. Following binding of the modified toxin to the receptor crosslinking was achieved by photo lysis. This resulted in the formation of radiolabeled polypeptides. Analysis on sodiumdodecylsulfate polyacrylamide gel electrophoresis showed that α-bungarotoxin could be crosslinked to a 40,000 dalton polypeptide, to which it is known to bind, and in addition to another polypeptide of 65,000 dalton. The implications of these findings are that the nicotinic acetylcholine receptor is composed of different polypeptides and that some of the subunits interact with each other or are in close proximity on the exterior surface of the postsynaptic membrane.

291 ELECTRON DIFFRACTION OF CROTOXIN, W. Chiu[1] and T. W. Jeng[2], Donner Laboratory[1] and Dept. of Molecular Biology[2], Unviersity of California, Berkeley, CA 94720

Crotoxin, a neurotoxin isolated from Brazilian rattlesnake, consists of two protein components with total molecular weight of 22,000. Its high neurotoxicity is observed only when both components are administered. The mechanism of synergistic interaction in crotoxin complex has been proposed that the acidic component serves as a carrier to deliver the phospholipase A2 active (basic) component to the specific target site in the neuromuscular junction. After the basic component is bound to the receptor, the acidic component would be released from the complex. Low dose electron microscopy has been used to study the structure of a thin crystal of the crotoxin complex as a condition has not been found to grow the crystal big enough for x ray diffraction study. An electron diffraction pattern of the crotoxin complex embedded in 0.5% glucose has been obtained to a resolution ∿2.5 Å. The two dimensional crystal lattice constant is ∿38 Å x 38Å, and the crystal thickness varies from 170Å to 700Å. The density of the crystal is measured to be 1.3 gm/cc by density gradient method. It is estimated to have 8 molecules in each unit cell. The strong intensities at reflections ∿4.7Å suggests that the crotoxin complex would have a high content of β sheet conformation. By visual inspection, the electron diffraction pattern shows good mm symmetry along the orthogonal axes and along the lines intersecting at 45° with the axes. A model of the molecular packing in a unit cell has also been deduced. (We thank Profs. R. M. Glaeser and H. Fraenkel-Conrat for generous support).

Mechanisms of Endocytic and Exocytic Protein Transport

292 INFLUENCE OF BUFFER IONS, DIVALENT CATIONS, & CLATHRIN CONCENTRATION ON COATED VESICLE DISASSEMBLY AND REASSEMBLY, Michael P. Woodward and Thomas F. Roth, Department of Biological Sciences, University of Maryland Baltimore County, Catonsville, Maryland 21228

Treatment of coated vesicles with a variety of reagents results in disruption of the coat lattice and solubilization of clathrin, as well as other proteins(1). Coated vesicles dissociated in 10mM Tris bufffer at pH8 reassemble on addition of 10mM $MgCl_2$. If dissociated preparations are centrifugated, the supernatant contains few coats and approximately 90% of the total protein. Then: A) If 10mM $MgCl_2$ is added particles reassembling intact coats are observed. However, after centrifugation sufficient to pellet coats 65% of the total protein is still in the supernatant as are the particles. When this supernatant is dialysed to pH6.7 against 0.1M Mes--10uM $MgCl_2$ the particles becoming sedimentable. This suggest that there may be stable intermediates in the assembly-disassembly equilibrium. B) If 100mM NaCl is included in the initial dissociation buffer the supernatant reassembles upon the addition of 10mM $MgCl_2$ at protein concentrations greater than 500ug/mL. Reassembly also occurs when the 100mM NaCl containing supernatants are dialysed to pH6.7 against 0.1M Mes--10M $MgCl_2$. C) Replacing 100mM NaCl with 0.1M TRIS decreases the amount of clathrin solubilized, and following centrifugation the supernatants do not reassemble either upon the addition of 10mM $MgCl_2$ or dialysis to pH6.7. Our results suggest that divalent cations, buffer composition, and clathrin concentration play pivotal roles in the disassembly-reassembly equilibrium. 1. Woodward, M.P. & Roth, T.F. (1978). PNAS 75, 4394-4398.

293 DIRECT EVIDENCE FOR BINDING OF A LYSOSOMAL ENZYME TO FIBROBLAST RECEPTORS, Leonard H. Rome and Elizabeth F. Neufeld, NIAMDD, Bethesda, MD 20014, and Bernard Weissmann, U. of Illinois College of Medicine, Chicago, IL 60612.

Binding of lysosomal enzymes to surface receptors of cultured human fibroblasts has been previously postulated from the Michaelis-Menten kinetics of uptake (Sando and Neufeld, *Cell* 12, 619, 1977). Such binding has now been demonstrated using a sensitive assay to detect bound enzyme. Human fibroblasts deficient in α-L-iduronidase were prelabeled with tracer ^{75}selenomethionine, detached from culture dishes by mild trypsinization and used in suspension after recovery. The binding of urinary α-L-iduronidase was determined by incubation of cells with enzyme at 0°, followed by centrifugation of the cells through a layer of concentrated bovine serum albumin; the bound enzyme was assayed with 4-methylumbelliferyl α-L-iduronide and related to the radioactivity (a measure of protein concentration) in the sample. Binding was completed within 90 min, saturable, and specific for the high uptake form of the enzyme. Analysis of the Scatchard plot showed 14,000 receptors/cell and a single K_D of 1 nM. Binding was competitively inhibited by mannose 6-phosphate (K_i = 0.1 mM), a known competitive inhibitor of uptake. Bound enzyme dissociated upon dilution in the presence of mannose 6-phosphate; by contrast, enzyme that had been internalized at 37° could not be released. The rate of enzyme uptake at 37° was not reduced by cycloheximide; in a one hour experiment over 200,000 molecules of iduronidase had been internalized per cell even though new protein synthesis had been completely halted. The results suggest that, barring the existence of a very large internal pool, surface receptors are rapidly recycled.

294 FLUIDITY CHANGES OF PLASMA MEMBRANES DURING MYOGENESIS, Hannah F. Elson and Juan Yguerabide, Department of Biology, University of California, La Jolla CA 92093.

One naturally occurring instance in which membranes of two separate cells fuse to form a multinucleated syncytium is during muscle development when myoblasts fuse to form a myotube. The process of fusion could require very fluid membranes, which may fuse upon encounter much the way lipid vesicles do. On the other hand, the membranes may take on a more rigid, lattice-like structure to ensure appropriate cell-cell recognition and fit. Measurements of membrane fluidity during myogenesis would supply evidence favoring one of these possibilities or an intermediate one.

Other workers have reported that the microviscosity of muscle cells decreased during the period of active fusion, suggesting greater fluidity (Nature 268: 761 (1977)). Their studies, however, were performed on suspensions of muscle cells contaminated by fibroblasts, which had been removed from tissue culture dishes by trypsinizing the cells. Since trypsin digestion may produce effects upon membrane fluidity, we undertook to examine this quesion using fluorescence photobleach recovery. The advantage of this technique, in which a fluorescent probe is incorporated into the membrane, a small spot is bleached with a laser and the rate of diffusion of fluorescent molecules back into the spot is measured, is that the membrane fluidity of single, identifiable attached cells can be determined. Using a lipid probe, a carbocyanine dye, we found that myoblast membrane fluidity increased during fusion. These and other results will be presented.

295 FORMATION OF INTRA- AND INTERCHAIN DISULFIDE BONDS OCCURS ON NASCENT IMMUNOGLOBULIN POLYPEPTIDES. Lawrence W. Bergman and W. Michael Kuehl, Univ. of Virginia, Charlottesville, VA. 22908

The initial step of covalent assembly of immunoglobulin (Ig) molecules occurs on nascent polypeptides as isolated by QAE-Sephadex chromatography. In MPC 11 (IgG_{2b}), this involves the covalent assembly of a mature light (L) chain onto a nascent heavy (H) chain, whereas in MOPC 21 (IgG_1) a mature H chain is assembled with a nascent H chain. This is consistent with the H-L half molecule being the major intermediate in the assembly of MPC 11 IgG_{2b} and H-H being the first intermediate formed in assembly of MOPC 21 IgG_1. Pulse-chase labeling studies of MPC 11 have shown that the L chain assembly with the nascent H chain must occur within 2-4 min of the synthesis of the L chain even though MPC 11 cells synthesize a 1.5-2.0 molar excess of L chains over H chains. In MPC 11 the nascent H chain must be at least 38000 daltons in size before assembly with the L chain occurs, even though the residue on the H chain involved in the disulfide bond is 131 residues from the amino terminus. Using radiolabeled iodoacetic acid to specifically label only those cysteines involved in disulfide bonds, we have shown that some intrachain disulfide bonds are formed prior to the release of the polypeptide from the ribosome. These data provide direct experimental evidence that both secondary and quaternary structure formation occurs on nascent polypeptides synthesized *in vivo*.

296 FLUORESCENT CHEMOTACTIC PEPTIDE: VISUALIZATION OF BINDING AND INTERNALIZATION BY HUMAN NEUTROPHILS, James E. Niedel, Itzhak Kahane and Pedro Cuatrecasas, Wellcome Research Laboratories, Research Triangle Park, N.C. and Division of Hematology-Oncology, Duke University Medical Center, Durham, N.C.

N-formyl-nle-leu-phe-nle-try-lys, felt to be a structural analog of naturally occuring bacterial products, is a potent chemoattractant for human neutrophils, having an ED_{50} of 0.4nM for chemotaxis and a K_D of 1.3nM for binding. The purified tetramethyl rhodamine conjugate of this peptide (TMR-pep) displays an EC_{50} of 0.7nM and an IC_{50} of 1.5nM in a competitive binding assay. Direct visualization of the binding of this TMR-pep to human neutrophils is possible using a silicon intensifier target TV camera attached to a fluorescent microscope. At 37°, in Gey's balanced salt solution with 2% ovalbumin, diffuse staining of the cells is evident within one minute. At two minutes the TMR-pep has begun to aggregate into patches and by five minutes many of these patches have been internalized into small endocytic vesicles which display the saltatory motion characteristic of cytoplasmic organelles. At 15 minutes, all of the patched TMR-pep has been internalized and has coalesced into one to three large vesicles per cell which are visible by both fluorescent and phase contrast microscopy. These fluorescent events appear to be dependent upon specific binding to the formyl peptide chemotactic receptors for the following reasons. 1) The fluorescent staining is markedly attenuated by simultaneous exposure to 18nM non-fluorescent peptide and abolished by pre-incubation with 100nM non-fluorescent peptide. 2) The fluorescent events occur at biologically relevant times (0-5 min) and peptide concentrations (0.2-5.0nM). 3) The staining is saturable. 4) Red blood cells, lymphocytes, platelets and 3T3 fibroblasts which lack the receptor, do not stain.

297 ATP-EVOKED RELEASE FROM RAT PAROTID SECRETORY GRANULES. Stephen G. Oberg and Murray R. Robinovitch, Univ. of Washington, Seattle, Wa. 98195.

Secretory vesicles isolated from a variety of mammalian tissues are known to lyse and thereby release their secretory products when exposed to ATP. This release process has been studied most extensively using adrenal chromaffin granules and has been termed 'ATP-evoked release'. (Mol. Pharmacol. 3, 561). We report here that ATP-evoked release occurs from a highly purified preparation of rat parotid granules (Anal. Biochem 52, 589). ATP-evoked release was characterized with respect to pH, osmolarity and temperature. The rate of granule lysis was measured spectrophotometrically (Mol. Pharmacol. 3, 572). ATP-evoked release is expressed as the increase in the rate of granule lysis (r=% lysis/min.) when ATP is added. All assays were performed at 37°C in media containing 110mM KCl, 5mM $MgCl_2$ and 25mM maleic acid titrated to the desired pH. Parotid granules incubated under optimal conditions were totally lysed during a 15 minute exposure to 5mm ATP. We found the parotid process to have the following properties in common with the chromaffin granule process (Arch Biochem Biophys. 176, 375): (1) It is a saturable function of ATP, with K1/2=0.5mM ; (2) it is inhibited in hyperosmotic media e.g. r=8.60 at 300mOsM vs. 1.60 at 400mOsM ; (3) it has a marked temperature dependence e.g. r=6.0 at 30°C vs. r=13.0 at 37°C and (4) it is very pH dependent e.g. r=13.2 at pH 6.8 vs. $r \leq 1.7$ at pH 6.5 and 7.0. These findings combined with preliminary reports of ATP-evoked release from insulin secretory granules (J. Cell. Biol. 47, 289) posterior pituitary granules (Mol. Pharmacol. 4, 531) and cholinergic vesicles (Biochim. Biophys. Acta, 150, 739) imply that ATP-evoked release may be an ATP-dependent property of all secretory vesicles and as such could play a similar role in all mammalian processes of exocytotic release.

298 ENERGY-DEPENDENT REGIONAL MOBILIZATION OF CONTRACTILE PROTEINS TO PARTICLE ADHERENCE SITES DURING PHAGOCYTOSIS, R.G. Painter & A.T. McIntosh, Scripps Clin. & Res. Fdn. La Jolla, CA

Using immunofluorescent and immunoferritin techniques, we have simultaneously followed the location of actin and myosin in mouse peritoneal macrophages during phagocytosis of zymosan particles. We find that these two intracellular proteins rapidly associate under cell membrane sites with which particles are associated within five minutes of particle challenge. Kinetic analysis suggested that actin accumulates more rapidly than myosin. The observed accumulation of actomyosin components is maximal prior to any detectable particle engulfment. The observed changes in actin and myosin distribution with respect to adherent particles and their subsequent engulfment was found to require cellular energy. With 10 mM sodium azide, for example, less than 0.03% of the adherent particles had associated actin and less than 0.01% of the particles were completely engulfed by the cells. Thirty minutes after removal of the inhibitor, however, these values rose to 66% and 39%, respectively, showing that the inhibition by azide is completely reversible. These values are comparable to those obtained in untreated controls. In contrast, pretreatment with cytochalasin B (10 µg/ml) did not prevent actomyosin accumulation at particle attachment sites even though the drug completely blocked particle engulfment and radically altered actin and myosin distribution patterns of resting macrophages. We conclude that particle binding at the cell surface generates a transmembrane signal which results in a regional energy-dependent mobilization of contractile proteins to that site.

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299 DIPHTHERIA TOXIN INHIBITORY ACTIVITY FROM RABBIT LIVER PLASMA MEMBRANES, Daniel Chin, Rockford K. Draper, Paul Gardner, and Melvin I. Simon, Dept. of Biology, University of California at San Diego, La Jolla, California 92093

A Diphtheria toxin inhibitory activity (DTIA) has been isolated and partially characterized from rabbit liver plasma membranes. The biological activity was determined by the ability to inhibit Diphtheria toxin cytotoxicity on Chinese hamster ovary cells. Specific binding activity was followed by two assays utilizing ^{125}I-Diphtheria toxin; the ability of DTIA to inhibit ^{125}I-DT binding to Vero cells and the direct binding of ^{125}I-DT to purified liver plasma membranes. DTIA co-purifies with plasma membranes and can be extracted with a non-detergent buffer in the presence of 1 mM EDTA and a host of proteolytic inhibitors. A high speed supernatant of the extract comprises 80% of the plasma membrane's biological activity and about 60% of the specific binding activity in the Vero binding assay; in addition, the pellet has less than 1% of the membrane's biological activity and retains less than 5% of the membrane associated ^{125}I-DT binding activity. These results are consistent with the quantitative removal of DTIA from plasma membranes. After fractionation on DEAE cellulose, the biological activity appears to be heat stable and sensitive to trypsin and carboxyaminomethylation.

While the mechanism of DT entry to the cytoplasm is unknown, two hypotheses have been suggested: direct penetration through the plasma membrane or entry via an endocytotic pathway. Further characterization of the interaction of DTIA with Diphtheria toxin may help to elucidate the entry mechanism(s).

300 EVIDENCE FOR THE SEPARATION OF PINOCYTOSIS INTO FREE-ENERGY DEPENDENT AND METABOLIC ENERGY DEPENDENT COMPONENTS, Robert J. Sturm and Sorell L. Schwartz, Georgetown University, Washington, D.C.

Mouse peritoneal macrophages were exposed to medium containing ^{3}H-sucrose and the total uptake of isotope (A) determined. Cells were then post-incubated for 15 min. in isotope-free medium (B). Post incubation resulted in a loss of radioactivity, only a part of which was due to labile binding of isotope to cell or vessel surfaces (LS). The rest was due to an apparent labile pinocytic pool (LP). Further experiments with time variables and metabolic inhibitors demonstrated the following relationships:

$$A(cpm) = P + LP + S + LS \qquad B(cpm) = P + S$$

where P and S are stable pinocytic and binding pools, respectively. From plots of A or B vs. time of initial incubation (t), S + LS = A - (slope x t) and S = B - (slope x t). P and LP were reduced if the initial incubation with isotope was done at 4°C but only P was reduced by iodoacetate. LP could be "stabilized" if post-incubation in isotope-free medium was done at 4°C. LP may represent a free-energy dependent antecedent to a metabolically dependent shift of pinosomes to the cell interior. LP may also reflect what has been previously referred to as micropinocytosis. Further studies are being done using membrane fluidizing agents to alter surface free energy and determining effects on P and LP.

Lipoproteins

301 A CRYSTALLOGRAPHIC STRUCTURE ANALYSIS OF A DIMERIC PHOSPHOLIPASE AT 2.5Å RESOLUTION, C. Keith, D. Feldman, E. O. Jones, S. Deganello, A. Yonath & P. B. Sigler, Dept. of Biophysics & Theoretical Biology, University of Chicago, Chicago, IL 60637.

The structure of the dimeric phospholipid A_2 from the venom of C. atrox has been examined by single crystal X-ray diffraction. The enzyme is a dimer both in solution and in the crystalline asymmetric unit. It derives its special interest from the fact that phospholipid substrates are hydrolyzed far more readily when they are in a condensed state such as a monolayer or micelle.

A 2.5Å electron density map has been prepared by the method of multiple isomorphous replacement and analyzed in terms of the primary structure. The critical questions under study are a) the organization of the dimer in relation to its catalytic function, in particular the putative asymmetric roles of the protomers, b) a comparison of the structure with that of the monomeric bovine enzyme (Dijkstra et al., J. Mol. Biol. 124, 53 (1978)) with particular attention to the highly homologous and clearly nonhomologous sequences, c) mode of phospholipid recognition and the basis for enhanced interaction with substrates in the condensed phase, d) the role of Ca^{2+} ion in membrane interactive proteins.

302 PHOSVITIN RECEPTOR FROM CHICKEN OOCYTES: EVIDENCE FOR SOLUBILIZATION BY TRITON X-100. John W. Woods and Thomas F. Roth. Department of Biological Sciences, University of Maryland Baltimore County, Catonsville, Maryland 21228.

Vitellogenin, a female specific serum protein, is selectively sequestered by developing chicken oocytes. We have found that both vitellogenin and phosvitin (PV), a subunit of vitellogenin, are specifically bound to oocyte membranes with an apparent Kd of 10^{-6}M. Competition studies suggest that both proteins bind to the same receptor.

We attempted to solubilize ^{125}I-PV-receptor complexes from the membrane with Triton X-100. Solubilized ^{125}I-PV labeled membranes yielded an ^{125}I-PV containing complex which had a MW of ~ 500,000 daltons as indicated by gel exclusion chromatography. This complex completely dissociated when incubated with unlabeled PV. However, when incubated with: 1) no addition, 2) IgG or 3) serum albumin, the complex only partially dissociated, suggesting that bound ^{125}I-PV would only be displaced by unlabeled PV.

In order to determine if solubilized receptors were able to bind free ^{125}I-PV, we first solubilized the membranes with Triton. The solubilized material was then incubated with ^{125}I-PV in the presence or absence of unlabeled PV. Chromatography of these samples indicated that a 500,000 dalton complex formed only in absence of unlabeled PV. Further experiments in which this complex was incubated with 1) no addition, 2) unlabeled PV, 3) IgG or 4) serum albumin indicated that only unlabeled PV will displace bound ^{125}I-PV from the complex.

These results indicate that the 500,000 dalton species represents a soluble PV-receptor complex and that it is possible to solubilize the PV receptor in an active form.

303 INTERACTIONS BETWEEN PM2 VIRAL POLYPEPTIDES AND HOST CYTOPLASMIC MEMBRANES, Laura V. Grobovsky and Gregory J. Brewer. The University of Southern California Medical School, Los Angeles, CA 90033.

We are concerned with interactions between membrane proteins and membrane lipids. PM2, a lipid membrane containing bacteriophage, makes new membranes inside its host Alteromonas espejiana. We have synthesized viral proteins in vitro, using a transcription-translation system directed by PM2 DNA. Under the controlled conditions of the in vitro system, several of the newly synthesized viral polypeptides insert into suitably prepared cytoplasmic membranes of the host.

304 MARKING OF PROTEIN SUBUNITS IN CONTACT WITH LIPID ENVIRONMENTS IN ACETYLCHOLINE RECEPTOR-RICH MEMBRANE PREPARATIONS. M. Martinez-Carrion, V. Sator, J.M. Gonzalez-Ros and P. Calvo-Fernandez. Medical College of Virginia Commonwealth University, Richmond, VA 23298

A lipophylic photolabel, [^{3}H]pyrenesulfonyl azide, has been synthesized and employed to detect which portions of the acetylcholine receptor (AcChR) molecule make contact with its lipid environment in Torpedo californica electroplax membranes. The probe preferentially partitions into the hydrophobic regions of membrane lipids or Triton X-100 micelles of detergent solubilized membranes. When irradiated by UV light the nitrene generated binds covalently to membrane proteins. In detergent solubilized AcChR, the 40,000 and 48,000 molecular weight subunits are preferentially labeled, whereas in situ membrane labeling produces incorporation of the radioactive photoproduct into the 48,000 and 55,000 subunits of the AcChR isolated from the membrane. The results suggest that in the membrane environment the 48,000 and 55,000 molecular weight subunits have a pronounced exposure to the membrane lipids, whereas the 68,000 subunit is protected from the label, possibly being partially enveloped by the other polypeptide chains from AcChR. The in situ labeling of AcChR does not perturb α-bungaratoxin binding to the membrane fragments. Furthermore, the well-known effect of prolonged exposure of AcChR to carbamylcholine in the affinity state for cholinergic ligands is also unperturbed by the covalent labeling of the membranes. These time dependent carbamylcholine induced effects are similar to in vivo pharmacological sensitization changes which until now were thought to be eliminated with chemical or enzymic perturbations of the protein or lipid components of the membrane. (Supported by NSF grant BNS 77-24715)

305 LIPOPROTEIN RECEPTORS OF PRIMARY OLFACTORY NEURONS, Dale M. Norris, Judith Hageman and Joseph Piotrowski, *The University of Wisconsin, Madison, WI 53706*

Biological recognition involves energy (i.e., electron or proton) transfer or sharing between a receptor macromolecule and a ligand. In olfaction the ligand is an odorant, and in our insect model, Periplaneta americana, the receptor lipoproteins are in dendritic membranes of primary sensory neurons in specialized sensilla on the animal's antennae. Using classical methodologies lipoprotein receptors for especially 1,4-naphthoquinone repellents were isolated and purified from the membranes of involved neurons. Triton X-100 extracted receptor retains its abilities to interact electrochemically with odorant species. This interaction was quantitated in vitro at pH 7.0 using dropping-mercury-electrode polarography. Amino acid analysis and functional group studies of the receptor protein clearly implicated sulfhydryls as major components of the binding site(s) for the odorant naphthoquinones. Alteration in vitro or in vivo of protein-sulfur chemistry in the receptor using classical reagents (e.g., PCMB, NEM or dithiothreitol) predictably changed the receptor's sensitivity in recognizing the biologically repellent naphthoquinones. Within the species of insect, genetically distinct biotypes with regard to sensitivity to the test odorants were clearly identified. Studies of the pertinent electrochemistry of the involved lipoprotein receptor in each biotype showed that this chemistry differs significantly among the biotypes, and these differences correlate in seemingly reasonable manners with data from our behavioral and neurophysiological analyses of the sensory neuron recognition of the odorants. Presented combined data from several experimental approaches strongly support the conclusion that a lipoprotein entity by which a sensory neuron quantitatively recognizes odorant ligands has been identified for the first time.

306 STRUCTURAL STUDIES OF RECOMBINANT LIPOPROTEINS, David Atkinson, G. Graham Shipley and Donald M. Small, Biophysics Division, Boston University School of Medicine, Boston, MA. 02118.

Plasma high density lipoprotein (HDL) is secreted by the liver as a phospholipid (PL), apoprotein (apoHDL), free cholesterol (C) rich "nascent" particle deficient in cholesterol ester(CE), the major neutral lipid component of normal HDL. Esterification of free cholesterol by the plasma enzyme lecithin-cholesterol acyl-transferase converts these "nascent" lipoproteins into native HDL. Native HDL has a spherical morphology and a structural organization of a neutral lipid core, surface stabilised by PL, C and apoHDL. In contrast, the morphology of "nascent" HDL is discoidal. Recombinant lipoprotein complexes formed from apoHDL and PL have been used as models for this "nascent" form of HDL. Electron mocroscopy and x-ray scattering studies have shown these recombinants to be segments of phospholipid bilayer solubilised by apoprotein. Thus the discoidal morphology reflects the fundamental bilayer organization of the phospholipid and the spherical shape of native HDL is generated as a consequence of solubilising the CE. We are using neutron scattering methods to probe the structural organization of these particles in more detail. Utilising complexes formed from apoHDL and($N^{+}(CD_3)_3$) dimyristoyl lecithin, the protein moiety may be structurally distinguished from the lipid polar groups at the particle surface. *Different organizations of apoHDL on the particle may be investigated by* following changes in the radius of gyration and scattering profile as a function of contrast together with model calculations. The organization of apoHDL on these discoidal particles will be discussed together with the implications of the organization in the transformation of a lipid bilayer "nascent" particle to a native spherical HDL on incorporation of CE.