PROTEIN TRANSPORT AND SECRETION

Dale L. Oxender, Organizer

April 23 — April 30

Plenary Sessions

Poster Sessions

Bacteriorhodopsin as a Model for Membrane Protein Structure Function Studies

1372 BACTERIORHODOPSIN H. Gobind Khorana. Department of Chemistry and Biology, Massachusetts Institute of Technology, Cambridge, MA

Bacteriorhodopsin, the only protein in the purple membrane of Halobactrerium halobium, carries out light-dependent vectorial translocation of protons.

A. Studies at the Protein Level.

(1) It is a very hydrophobic protein that is largely embedded in the membrane. Its carboxyl terminus is oriented towards the cytoplasm with about 20 amino acids exposed to the aqueous medium.

(2) It is a single polypeptide chain of 248 amino acids and its sequence is known both by protein and gene sequencing.

(3) Based on the amino acid sequence secondary and tertiary structure models have been derived to fit the electron diffraction data.

(4) The protein can be completely delipidated and the lipid-free protein can be reconstituted into defined lipids to give vesicles that pump protons efficiently. The direction of pumping now is completely reversed.

(5) Bacteriorhodopsin can be completely denatured by a variety of harsh treatments but it renatures to fully functional native state by addition of SDS, cholate, retinal and phospholipids.

(6) Chymotryptic fragments of Bacteriorhodopsin can similarly renature to regenerate the characteristic chromophore. This reconstitution provides a powerful tool for structure-function studies of Bacteriorhodopsin.

(7) Retinal in the protein is attached to lys-216 and crosslinking by photoaffinity labels shows that it is oriented at 30°-35° towards the outside of the membrane.

B. Characterization of a Precursor to Bacterio-Opsin.

A precursor molecule containing 13 additional amino acids at the amino terminus has been discovered and characterized. The precursor inserts into the membrane, evidently in proper orientation and conformation, without obligate cleavage of the precursor sequence.

C. Studies at the Gene Level.

For structure-function studies, we are taking a molecular biological approach that aims at specific amino acid replacements in the protein. Our recent work proceeds along the following lines:

(1) Bacteriorhodopsin gene has been characterized.

(2) The gene has been cloned into three different vectors that have been developed by Inouye and colleagues.

(3) The gene has been expressed, although at modest levels, in E. coli and the protein has been characterized.

(4) Three approaches are being used to generate site-specific mutations in the gene: These involve: (a), oligonucleotides containing nucleotide mismatches as mutagens for alterations at specific sites; (b), excision of gene fragments by specific restriction enzymes and replacement of the fragments by synthetic altered DNA's containing the desired nucleotide changes; (c), introduction and characterization of amber mutations in the gene and subsequent introduction of altered amino acids by using suppressor strains of E. coli.

D. Structure-function Studies Using Bacteriorhodopsin Mutants.

These studies which will be carried out in collaboration with Dr. Kenneth J. Rothschild of Boston University will include:

(1) Proton translocation.
(2) Photo-kinetics (at M.I.T. Laser Center).
(3) Fourier Transform Infra Red Spectroscopy.
(4) Resonance Raman Spectroscopy.

Model Systems for the Study of Protein Transport

1373 THE PROTEIN TRANSLOCATION MACHINERY OF THE ENDOPLASMIC RETICULUM, Günter Blobel, Peter Walter, Reid Gilmore, Matthias Müller, David Anderson and Ann Erickson, The Rockefeller University, 1230 York Avenue, New York, New York 10021.

Substantial experimental data has recently been provided on the cotranslational translocation of protein across and integration into the endoplasmic reticulum. So far, two components have been purified from dog pancreas and shown to be required for this translocation process. One of these is the so-called Signal Recognition Particle (SRP), an 11S ribonucleoprotein. SRP consists of six non-identical polypeptide chains (M_r=72,000, 68,000, 54,000, 19,000, 14,000 and 9,000 daltons) and one molecule of 7S RNA. The RNA has been identified by partial sequence analysis to be the previously described and recently sequenced small cytoplasmic 7SL RNA (7S RNA, ScL). Both, RNA and protein are required for SRP's activity. In dog pancreas at physiological salt concentration (150mM potassium ions) the bulk of SRP appears to be about equally distributed between a membrane-bound and a free or ribosome/polysome-associated form.

The other component, termed SRP-receptor, is a protein of 72,000 daltons that has been purified from detergent-solubilized microsomal membranes by SRP-affinity chromatography. The SRP-receptor is an integral membrane protein of the endoplasmic reticulum. It consists of a large cytoplasmic domain of 60,000 daltons that can be severed from the membrane in an intact form by treatment with a variety of proteases and can be added back to the proteolysed membranes to reconstitute activity.

The function of these components in the protein translocation process was deduced from in vitro reconstituted assay systems. Using such assays, SRP was found to function in decoding the information contained in the signal peptide of nascent secretory, lysosomal, and membrane proteins to the effect that it mediates the specific attachment of the translating ribosomes to the microsomal membrane. In the absence of microsomal membranes SRP specifically arrests the elongation of secretory protein synthesis in vitro just after the signal peptide has emerged from the ribosome, thereby preventing the completion of pre-secretory proteins (many of which could be potentially harmful to the cell) in the cytoplasmic compartment. Upon interaction of these arrested ribosomes with a specific integral membrane protein, the SRP-receptor, on the microsomal membrane this elongation arrest is released and the nascent chain is translocated across or - as in the case of integral membrane proteins - integrated into the lipid bilayer.

1374 INTRACELLULAR PROTEOLYTIC MECHANISMS: RELATIONSHIP TO HORMONE AND NEUROTRANSMITTER BIOSYNTHESIS, K. Docherty, J. Marsh, S. Chan, D. Nielsen, S. Kwok, H.S. Tager, and D.F. Steiner, Univ. of Chicago, Chicago, IL 60637

Proteolytic processing of secretory proteins may occur at several stages during their intracellular transport, storage and secretion. The responsible enzymes are closely integrated topographically with the secretory pathway and this discrete localization poses challenging problems in their identification and isolation. At least two major levels of processing have been distinguished on the basis of their timing, subcellular localization and cleavage mechanisms (Docherty, K. and Steiner, D.F. Ann. Rev. Physiol. 44, 625, 1981). The first is the signal cleavage mechanism which operates very rapidly, probably mainly cotranslationally, at the level of the R.E.R. (rough endoplasmic reticulum) membrane. Although a single endoprotease located on the luminal side of the R.E.R. may accomplish this, other evidence suggests that additional proteases associated with the R.E.R. may generate further cleavages within the signal sequence, thereby facilitating the rapid degradation of the prepeptide.

The second major site of limited intracellular proteolysis is the Golgi/granule apparatus. Studies on the formation and processing of many proproteins including proinsulin, POMC and proparathyroid hormone have shown that processing of these forms begins only after their arrival in the Golgi complex and then continues for many hours subsequently as these products are stored in cytosolic granules. In the case of some neuropeptides, maturation may occur as the vesicles move along nerve processes. These products are almost always processed at pairs of basic residues by enzymes having cleavage specificity similar but not identical to that of trypsin and carboxypeptidase B. Several laboratories have recently described broken cell systems which process precursors such as proinsulin or POMC in vitro. The endoprotease appears to be trypsin-like in specificity but with a thiol rather than serine in the active site. Its inhibitory profile most closely resembles that of cathepsin B. However, several earlier studies have shown that cathepsin B has too broad a specificity, with significant dipeptidyl carboxypeptidase activity against peptides such as glucagon and aldolase. We have recently identified a 31.5K enzyme in impure islet granule fractions which is labeled with ALACK (Ala-Lys-Arg-chloromethyl ketone), an active site titrant of trypsin-like and cathepsin B-like enzymes. This enzyme is also precipitable with antibodies to cathepsin B (kindly provided by H. Kirschke) and has other similarities. Further studies are now in progress to determine its substrate specificity and cleavage activity against various prohormonal peptides. Our results taken together with those of others clearly implicate this or similar enzymes in the endoproteolytic processing of prohormones. The carboxypeptidase B-like enzyme also exhibits properties more characteristic of catheptic enzymes, i.e. a more acid pH optimum, and other features which tend to distinguish it from the exocrine pancreatic enzyme. It is of interest to note that while the distribution of signal peptidase activity is extremely widespread, not all cells (e.g. Xenopus oocytes, monkey kidney cells, bacteria) appear to be able to process proproteins. Nonetheless, this form of processing appears to be of ancient phylogenetic origin, occurring in primitive vertebrates as well as molluscs and yeast (USPHS AM 13914).

1375 RECYCLING OF TRANSFERRIN AND THE TRANSFERRIN AND ASIALOGLYCOPROTEIN RECEPTORS IN HEPATOMA HEPG2 CELLS, Harvey F. Lodish, Aaron J. Ciechanover, Alice Dautry-Varsat, and Alan L. Schwartz*, Department of Biology, Massachusetts Institute of Technology, Cambridge MA, 02139 and *Sidney Farber Cancer Institute, 44 Binney St., Boston, MA 02115

Receptor mediated endocytosis of transferrin or asialoglycoproteins is mediated through clustering of receptor-ligand complexes in coated pits on the cell surface, followed by internalization of the complex into endocytic vesicles. We have shown that internalization of asialoglycoprotein by HepG2 hepatoma cells is accompanied by a rapid ($t_{1/2}$ = 1/2 to 1 min) depletion of surface asialoglycoprotein receptors. This is followed by a rapid ($t_{1/2}$ = 2-4 min) reappearance of surface receptors; most of these originate from endocytosed cell surface receptors. Internalized asialoglycoprotein, by contrast, is dissociated from receptor in the CURL organelle, and is degraded within lysosomes.

The loss and reappearance of asialoglycoprotein receptors is specific and depends on prebinding of ligand to its receptors. HepG2 cells also contain abundant receptors for both insulin and transferrin. Endocytosis of asialoglycoprotein and its receptor has no effect on the number of surface binding sites for transferrin or insulin. We conclude that binding of asialoglycoprotein to its surface receptor triggers a rapid and specific endocytosis of the receptor ligand complex, probably due to a clustering in clathrin-coated pits or vesicles.

The acidic pH of the endocytic vesicle or CURL causes dissociation of asialoglycoprotein from its receptor, and also causes dissociation of iron from receptor-bound transferrin. Apotransferrin, however, remains bound to its receptor at the acidic pH of these vesicles. Apotransferrin dissociates from its receptor at neutral pH. Thus, when the receptor-apotransferrin complex reaches the cell surface, the apotransferrin dissociates, and is released into the medium.

Signal Sequence and Processing I

1376 ALTERATIONS OF B. LICHENIFORMIS PENICILLINASE GENE AFFECTING TRANSPORT AND MODIFICATION; J. Oliver Lampen, Jennifer B. K. Nielsen, Peter Mezes, Wu Wang, Yue Qin Yang, and Edward C. Yeh; Waksman Institute of Microbiology; Rutgers, The State University; Piscataway, N.J. 08854

Cultures of B. licheniformis 749 contain two forms of exopenicillinase, exo-large (30.5 kd) subsequently processed to exo-small (29.5 kd), and a hydrophobic membrane penicillinase (32 kd) which can be cleaved to exoenzyme but apparently is not an obligatory precursor. Membrane penicillinase carries a lipophilic glyceride-cysteine modification at its N-terminus similar to that of the E. coli outer membrane lipoprotein. The in-vitro translation product of the penP gene carries 42 residues beyond the N-terminus of exo-small; 26 are removed by signal peptidase of B. licheniformis membrane vessels. For the signal peptides of various glyceride-cysteine lipoproteins a tentative consensus sequence around the cleavage site is L-A-G-C-a-S-N (a, neutral or nonpolar residue). Cleavage occurs N-terminal to the cysteine that is modified in the eventual membrane form. In contrast to B. licheniformis, B. cereus strains 569 and 5B produce almost exclusively exopenicillinase (28 kd and 30 kd, respectively). All three genes have been cloned in E. coli and there yield only membrane bound forms. The sequences of the B. cereus signal peptides should indicate whether differences in efficiency of processing or of lipophilic modification are responsible.

The penP gene of B. licheniformis 749/C has been treated with FnuDII and HhaI to remove 15 bp with consequent deletion of residues A-L-A-G-C at the usual site of glyceride-thioether modification. With the modified gene (penPΔ1) cloned in E. coli, the yield of active penicillinase is 3 - 5 times that with unmodified 749/C. No lipoprotein form is detectable. About one-fourth of the modified product is processed to the approximate size of exo-large and is in the periplasm. The remainder appears to be loosely attached to the plasma membrane with at least half of it on the outer surface. When the two genes were cloned in B. subtilis, total penicillinase production with penPΔ1 was similar to that with wild-type; however, only 5% was secreted vs. 30 to 50% with wild-type. A significant portion of the cellbound enzyme appeared to be cytoplasmic.

1377 *FUNCTION OF THE SIGNAL PEPTIDE FOR PROTEIN SECRETION ACROSS THE MEMBRANE*

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The outermembrane of gram negative bacteria provides an ideal model system to study protein secretion across the membrane since it contains only a few major proteins, many of which have been characterized extensively (1). These proteins are initially synthesized in the cytoplasm as higher molecular weight precursors which are secreted across the cytoplasmic membrane and processed prior to assembly in the outer membrane (2). In particular, we have chosen to study the major outer membrane lipoprotein (Lp) of E. coli (3). Like all other secreted proteins in E. coli, Lp is initially synthesized in the cytoplasm as a higher molecular weight precursor prolipoprotein (pLp) which contains an NH_2-terminal "signal sequence". There are many structural homologies which exist between the pLp signal peptide and those of other secreted proteins. To investigate the function of these regions in the secretion of pLp across the cytoplasmic membrane, we have constructed many site-specific mutations within the NH_2-terminus, hydrophobic core and cleavage site of the pLp signal peptide using synthetic-oligonucleotide directed mutagenesis and analyzed the effect of these mutations on the secretion of pLp across the cytoplasmic membrane.

We have also inserted EcoRI linkers into four different sites within the Lp structural gene. Using these new restriction sites, we have constructed a variety of fusion proteins which contain an internalized signal sequence. Similarly, we have inserted the DNA coding for the mature β-lactamase into these sites. From the analysis of these proteins, we were able to determine the role of the structural regions in the Lp sequence for its secretion across the membrane, its proper localization and assembly in the outer membrane, and the function of the signal peptide.

Analysis of this wide variety of mutants has led us to novel insights into the secretion and assembly of Lp in the outer membrane of E. coli.

1) DiRienzo, J.M. (1978) Ann. Rev. Biochem. 47:481; 2) Inouye, M., and Halegoua, S. (1980) CRC Crit. Rev. Biochem. 7:339; and 3) Inouye, M. Membranes and Transport (Martonasi, A.N. ed.) Plenum Press, 1982, p 289.

1378 DETERGENT-SOLUBILIZED HEN OVIDUCT SIGNAL PEPTIDASE, Mark O. Lively and Kenneth A. Walsh, Bowman Gray School of Medicine, Winston-Salem, NC 27103 and University of Washington, Seattle, WA 98195. When present during cell-free protein synthesis, isolated hen oviduct rough endoplasmic membrane vesicles transport and process nascent secretory proteins. A "signal peptidase" activity in the membranes removes the signal peptide from the nascent precursor of human placental lactogen and segregates the native form of the hormone within the microsomal vesicles. The signal peptidase has the properties of an integral membrane protein. Conditions required for its solubilization are those that completely disrupt the lipid bilayer of the vesicles. We have taken advantage of the immobilized state of the peptidase in sedimentable vesicles and developed a procedure to strip oviduct microsomes of their extrinsic proteins prior to solubilization with detergent. Membranes are effectively stripped of all but their integral proteins by treatment with ice-cold 0.1 M sodium carbonate, pH 11.5 (1). Application of this technique to oviduct microsomes removes as much as 90% of the protein and more than 97% of the RNA isolated with crude microsomes. Signal peptidase remains associated with the denuded vesicles and can be solubilized in an active state using non-denaturing detergents such as Triton X-100, sodium deoxycholate, or octyl-β,D-glucopyranoside. The partially purified signal peptidase solubilized in 60 mM octyl glucoside (approx 10 mol detergent/mol phospholipid) retains its activity and specificity. Full-length pre-placental lactogen is processed by the peptidase to a form that comigrates with native placental lactogen during electrophoresis in polyacrylamide gels in the presence of sodium dodecyl sulfate and during high performance liquid chromatography in C-18 μBondapak reversed phase columns. The purified product of post-translational cleavage has the amino terminal sequence of secreted placental lactogen. The solubilized peptidase is inhibited by the presence of excess detergent, presumably because of removal from the enzyme of phospholipids necessary for activity. The addition of phosphatidyl choline or, to a lesser extent, phosphatidyl ethanolamine to the detergent-inhibited peptidase results in partial reactivation. Therefore we concluded that hen oviduct signal peptidase requires phospholipid for activity, just as has been shown for the dog pancreas peptidase (2). Hen oviduct is a convenient and rich source of the enzyme that proteolytically processes nascent preproteins as they are transported into the lumen of the endoplasmic reticulum. (This research has been supported by the National Institutes of Health research grant GM-15371 and National Research Service Award GM-07250 from the Institute of General Medical Sciences.)

(1) Fujiki, Y., Hubbard, A.L., Fowler, S. and Lazarow, P.M. (1982) J. Cell Biol. 93, 97-102.
(2) Jackson, R.C. and White, W.R. (1981) J. Biol. Chem. 256, 2545-2550.

Signal Sequence and Processing II

1379 CHARACTERIZATION OF COMPONENTS REQUIRED FOR PROTEIN SECRETION IN ESCHERICHIA COLI, Donald Oliver, Carol Kumamoto, and Jon Beckwith, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Ma. 02115.

By using a fusion between a secreted protein and a cytoplasmic enzyme, we have devised a genetic selection for mutants defective in the early steps of protein secretion(1). Mutants in two new loci, secA and secB, have been found. Such mutants appear to define cellular components necessary for protein secretion. The mutants are pleiotropically defective in localization of certain periplasmic and outer membrane proteins, accumulating stable cytoplasmic precursors to these normally secreted proteins.

Using antibody to the secA gene product we have identified the protein as a 92kd cytoplasmic membrane component(2). The antibody is also useful for identifying other components tightly associated with secA protein. The synthesis of secA protein is greatly derepressed by at least two methods which block cellular protein secretion. Therefore, we have suggested that components of the secretion machinery of E. coli, such as secA protein, may be regulated in response to the secretion needs of the cell.

Extragenic suppressors of the sec mutants have been isolated. One such suppressor maps in or very near the prlA gene, a locus previously shown to be important in protein secretion(3). Characterization of secA, secB, and the suppressor mutants should help to define the role of the individual components in protein secretion.

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2. Oliver, D.B. and Beckwith, J.(1982). Cell 30, 311-319.
3. Emr, S.D., Hanley-Way, S., and Silhavy, T.J.(1981). Cell 23, 79-88.

1380 ORF EXPRESSION VECTORS: A GOOD METHOD FOR ANTIGEN PRODUCTION IN ESCHERICHIA COLI. Thomas J. Silhavy, Michael L. Berman, and George M. Weinstock. Basic Research Program-LBI, Frederick Cancer Research Facility, Frederick, Maryland 21701.

We have developed a new system for expressing foreign DNA in Escherichia coli. The system utilizes the ompF gene of E. coli, which encodes an abundant outer membrane protein of the cell. The 5' end of the ompF gene provides a regulated, high efficiency promoter, a ribosome-binding site and ATG initiation codon, and a signal sequence for export to the outer membrane. These sequences are coupled to the lacZ gene of E. coli such that expression of β-galactosidase from lacZ requires the ompF transcription and translation signals. However, the lacZ sequence is out of frame with respect to ompF, thus the hybrid gene is Lac⁻. Several restriction enzyme recognition sites that allow insertion of DNA fragments exist between ompF and lacZ. Insertion of a DNA fragment can realign ompF and lacZ so that β-galactosidase is produced provided that the inserted DNA is an open reading frame frame of the correct length for realignment. A tribrid protein is then produced with its amino terminus from OmpF, the translation product of the insert as its midsection, and functional β-galactosidase at its carboxy terminius.

We have inserted sequences from the herpes simples virus (HSV) thymidine kinase gene into this vector and used the resulting hybrid protein to raise antibodies that precipitate thymidine kinase from HSV-infected cells. We have also inserted sequences from the lexA gene of E. coli. The resulting tribrid protein can be precipitated by antiserum raised by immunization with intact LexA protein. This method of antigen production thus can be used for producing or detecting antibodies.

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1381 DIFFERENTIATION WITHIN THE BACTERIAL CYTOPLASMIC MEMBRANE, Bernard D. Davis, Daniele Marty-Mazars, Seikoh Horiuchi, Michael Caulfield, and Phang C. Tai, Bacterial Physiology Unit, Harvard Medical School, Boston, Ma. 02115.

The membrane of B. subtilis lysates can be separated in a sucrose gradient into a complexed fraction (CM), carrying ribosomes, and a ribosome-free fraction (FM). These differ strikingly in composition, with six major protein bands much more concentrated in the CM and two in the FM; 2-dimensional gel electrophoresis shows even larger differences. We have begun to correlate these differences with function. Thus the 64 Kd CM-protein is evidently a component of the apparatus of secretion, located on the cytoplasmic surface, since in vesicles it becomes accessible to proteolysis and to binding of antibody only after the ribosomes are released. It is also present in the cytoplasm (unlike certain other unique CM proteins); and antibody precipitates it in association with several other proteins, suggesting that it may serve in initiating secretion. Several other CM proteins also seem to be involved in secretion, since they are retained by the polysomes after extraction of membrane by mild detergents, and in vesicles some can be cross-linked to ribosomes. Moreover, vesicles carrying 70S ribosomes and those carrying longer polysomes yield different sets of retained proteins, suggesting a change in the organization of the secretory apparatus after the initial attachment of the signal peptide.

The membrane fractions also differ in their penicillin-binding proteins (PBPs). FM contains the bulk of the known PBPs, while CM contains several minor bands, some previously unrecognized. It thus appears that membrane domains involved in various aspects of protein secretion and wall synthesis can be separated. Such fractionation is clearly useful in studying the mechanism of protein secretion, and it may make bacteria useful for studying differentiation in membrane morphogenesis.

1382 GENETIC STUDIES ON THE MECHANISM OF SECRETION OF MALTOSE BINDING PROTEIN IN E. COLI, Philip J. Bassford, Jr., Vytas A. Bankaitis, Beth Ann Rasmussen, James P. Ryan, Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC 27514

The maltose binding protein (MBP) of the gram-negative bacterium Escherichia coli is secreted through the cytoplasmic membrane to an external compartment termed the periplasm. The MBP is similar to most other secreted proteins in both prokaryotic and eukaryotic systems in that it is initially synthesized with an amino-terminal "signal sequence" that is thought to be primarily responsible for initiating the secretion of this protein while it is still a nascent chain attached to the ribosome. We have several procedures that enable us to isolate mutations in the malE gene encoding the MBP that specifically alter the MBP signal sequence (1,2). Some of these alterations almost totally block MBP export, with the result that unprocessed precursor MBP accumulates in the cytoplasm (2,3). Other alterations only partially prevent export, and still others have no apparent effect at all. Phenotypic revertants of MBP signal sequence mutants have been obtained that restore MBP export with varying efficiency; the responsible mutations can be either linked or unlinked to the malE gene. The unlinked mutations allow us to identify genes that presumably encode components of the cell's secretion machinery. Several new classes of such suppressor mutations have recently been obtained in loci we have designated prlE and prlF. We have also initiated a detailed kinetic analysis of MBP synthesis, translocation and processing in various wild-type and mutant strains. We have found that, whereas wild-type MBP is exported very rapidly following its synthesis, in some instances suppressor-mediated export of mutant MBP is significantly slower, exhibiting a half-time of five min or more at 30 C. Finally, we are attempting to define the intracellular site of synthesis of export-defective MBP. Our preliminary results strongly suggest that MBP with a defective signal sequence is synthesized exclusively on free, cytoplasmic polysomes. This latter experiment, if confirmed, should provide strong support for our current concepts of the role of the signal sequence in initiating protein secretion.

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Intracellular Sorting and Import of Proteins into Organelles

1383 FACTORS RELATED TO THE CLATHRIN-COATED VESICLE CYCLE, David M. Schlossman, Sandra L. Schmid, William A. Braell and James E. Rothman, Department of Biochemistry, Stanford University Medical Center, Stanford, CA 94305.

We have found that coated vesicles and empty clathrin cages are rapidly and efficiently uncoated (disassembled) in vitro provided both ATP and a high speed supernatant (cytosol) fraction are supplied (1). An enzyme capable of uncoating has been purified to homogeneity from calf brain cytosol using ion exchange, hydroxylapatite, and ATP-Sepharose column chromatography. It consists of a polypeptide of apparent molecular weight 70,000 daltons by SDS PAGE, and it appears to be a dimer. The purified protein can bind ATP in the absence of clathrin as indicated by photo-crosslinking studies and by isolation of protein-[^{3}H]ATP complexes using gel filtration. Preliminary data indicate up to one mole of nucleotide can be bound per protein dimer with a dissociation constant of less than 1 μM. The purified protein also displays a strong clathrin-dependent ATPase activity, with ADP and Pi as the products. The ATPase is elicited by properly assembled cages and not by free triskelions. Finally, this purified uncoating ATPase (unc ATPase) binds noncovalently to clathrin during the act of uncoating. Released clathrin trimers (isolated by gel filtration) contain up to three moles of tightly bound unc ATPase (M_r = 70,000) in addition to the expected three moles of clathrin heavy chains (M_r = 180,000), and three moles of clathrin light chains (M_r = 33 and 36,000). Additional factors from the cytosol or membranes may be required for the recycling of the unc ATPase from this complex. This complex is unable to assemble into proper cages under standard conditions. Whether this "modification" in assembly properties is due to the bound unc ATPase itself or to some additional change in the clathrin is not yet clear.

1. Patzer, E. J., Schlossman, D. M. and Rothman, J. E. (1982). Release of Clathrin from Coated Vesicles Dependent upon a Nucleoside Triphosphate and a Cytosol Fraction. J. Cell Biol. 93:230-236.

1384 TRANSPORT OF PROTEINS INTO THE VARIOUS MITOCHONDRIAL COMPARTMENTS, Walter Neupert, Institute of Biochemistry, University of Göttingen, 3400 Göttingen, GFR

The majority of mitochondrial proteins is coded by nuclear genes, translated on cytoplasmic ribosomes and transported into the organelle by a posttranslational process. The rather complex transport process can be resolved into several distinct steps.

1) Mitochondrial proteins are synthesized as precursor proteins predominantly on free polysomes. The completed polypeptide chains are released into the cytosolic space. Most precursors differ from their mature counterpart with respect to size as they possess additional sequences with M_r's of 400-8000. Among the proteins without an extension are: the ADP/ATP carrier of the inner membrane, cytochrome c of the intermembrane space, and porin of the outer membrane. A number of findings indicate different conformations of precursor and mature forms. Even the hydrophobic membrane proteins appear to have soluble precursor forms.

2) Mitochondria bind precursor proteins via specific sites on their surface. Transport in vitro can be halted at the level of this binding by different procedures (e.g. ADP/ATP carrier, ATPase subunit 9, subunits of cytochrome bc_1 complex by dissipating the mitochondrial membrane potential; cytochrome c by inhibiting the addition of the heme group to the precursor apocytochrome c; and porin by performing transfer at 0°C. Number and affinity of binding sites could be determined for apocytochrome c and porin. Uptake of precursors into mitochondria appears to occur directly via these binding sites; i.e. they are receptors on the transport pathway. Not all precursors use the same receptor as demonstrated by the inability of apocytochrome c to compete for any other precursor tested so far.

3) Translocation of receptor-bound precursors into the mitochondria requires in most cases an electrical potential across the inner membrane (exceptions are cytochrome c and porin). It appears that translocation of receptor-bound precursors across the outer membrane into the inner membrane occurs by a single step.

4) Proteolytic processing of precursors at the correct cleavage site can be reproduced by a soluble protease activity extracted from mitochondria. Different lines of evidence indicate that processing of precursors occurs in the matrix space. Some precursors, such as cytochrome c_1, are processed in two steps, the second being catalysed by a different protease. The processing events may trigger the rearrangement of the polypeptide chain in the membrane.

The signals involved in recognition and the components involved in the translocation of precursors are apparently not specific for each individual precursor but are rather common to groups of different proteins. This view is strongly supported by the observation that isolated mitochondria import precursors which are not present in the cells from which these mitochondria are derived; e.g. yeast mitochondria import and process correctly the precursor to subunit 9 of the Neurospora ATP synthase. In yeast subunit 9 is synthesized within the mitochondria without an additional sequence.

1385 MECHANISMS FOR THE INCORPORATION OF PROTEINS INTO THE PLASMA MEMBRANE, David D. Sabatini, Michael J. Rindler, Ivan Emanuilov Ivanov, Department of Cell Biology, New York University Medical Center, 550 First Avenue, New York, N.Y. 10016, and Enrique Rodriguez-Boulan, Department of Pathology, Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, N.Y. 11203

Polarized monolayers of cultured epithelial cells, such as the kidney-derived MDCK cell line, when infected with enveloped viruses, provide a convenient model system to study the intracellular routes followed by newly synthesized glycoproteins to reach specific domains of the plasma membrane. The polarized nature of the monolayers is reflected in the asymmetric assembly of enveloped viruses, some of which, such as influenza and SV5, bud from the apical surfaces of the cells, while others, such as vesicular stomatitis (VSV), emerge from the basolateral surfaces. MDCK cells can sustain double infection with viruses of different budding polarity and within such cells the envelope glycoproteins of the two viruses are synthesized simultaneously and assembled into virions at different sites. Immunoelectron microscopic observations of doubly infected cells showed that glycoproteins of influenza and VSV traverse the same Golgi apparatus. This indicates that critical sorting steps must take place during or after passage of the glycoproteins through the organelle. Following passage through the Golgi, the HA glycoprotein accumulated almost exclusively at the apical surface, where the influenza virions assemble. Significant amounts of the G protein, however were detected on both plasma membranes in singly and doubly infected cells, although VSV virion assembly was limited to basolateral domains. These observations indicate that the site of VSV budding is not exclusively determined by the presence of G polypeptides on a given cell surface domain. It is possible that other cellular or viral components are responsible for the selection of the appropriate budding domain or that the G protein found on the apical surface must be transferred to the basolateral domain before it becomes competent for assembly.

Intracellular Sorting and Transport of Proteins

1386 THE SORTING AND TRANSPORT OF LYSOSOMAL ENZYMES, William S. Sly, Departments of Pediatrics, Genetics and Medicine, Washington Univ., St. Louis, MO 63110

Acid hydrolases are the glycoprotein products of 40-50 different unlinked nuclear genes. The mRNAs for these genes are transcribed on membrane bound ribosomes. High-mannose-type oligosaccharides are transferred from lipid-linked intermediates to asparagine residues of the enzymes as they enter the ER. To this point, the acid hydrolases share the route of biosynthesis and transport with all other secretory glycoproteins. However, each acid hydrolase contains information which allows it to be recognized by an addressing enzyme which transfers GlcNAc 1-P residues from UDPGlcNAc to mannose residues on the acid hydrolases. A second enzyme, which is greatly enriched in smooth membranes, removes the covering GlcNAc residues to expose Man 6-P recognition markers. This phosphodiester glycosyl hydrolase converts the enzyme into a high affinity ligand for the Man 6-P receptor. The newly synthesized enzymes bind to Man 6-P receptors which collect into vesicles for delivery to lysosomes. Apparently, the receptor-bound enzymes enter an acidic compartment where the low pH allows enzymes to dissociate from receptors, so the Man 6-P receptors can recycle and be reutilized. Lysosomotropic amines raise the pH of this compartment, prevent ligand release from receptor, and thus block receptor reutilization. By this means, they divert newly synthesized enzymes from lysosomes to the exterior and enhance secretion.

The Man 6-P receptor not only directs the localization of acid hydrolases, it also affects the processing of their oligosaccharides. The phosphorylated mannoses cannot be trimmed by Golgi mannosidases, and the Man 6-P-blocked oligosaccharides escape processing to complex-type, even though they are exposed to terminal transferases in the Golgi apparatus. Any enzymes which fail to be phosphorylated, and phosphorylated enzymes which fail to bind receptors are secreted. Some cell types express the Man 6-P receptor or other pinocytosis receptors on their cell surfaces that allow them to bind secreted enzymes and "recapture" them by receptor-mediated endocytosis. These cell surface receptors provide an alternate route to lysosomes. Alternate intracellular routes have also been suggested, but these remain to be demonstrated.

We will summarize new studies on the role of the endosome in sorting acid hydrolases, and on the ATP-dependent acidification of this compartment which appears to be important in the sorting and transport of acid hydrolases.

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1387 LYSOSOMAL ENZYME MEMBRANE RECEPTORS, George W. Jourdian, Departments of Biological Chemistry and Internal Medicine, University of Michigan, Ann Arbor, MI 48109

Adsorptive pinocytosis of lysosomal enzymes and the intracellular translocation of newly synthesized lysosomal enzymes in mammalian cells is believed to be mediated by specific interaction between phosphomannosyl recognition markers contained on the enzymes and a membrane-associated receptor. Glycoproteins exhibiting the binding specificity attributed to the receptor have now been isolated from the livers of several animal species (hamster, rat, and human) and from rat spleen and Swarm rat chondrosarcoma cells. In addition, phosphomannosyl receptors have been demonstrated in a large number of tissues and cells in culture. Antisera prepared against the receptors isolated from mammalian liver cross-react suggesting the presence of similar antigenic determinants on each receptor. Each receptor preparation exhibits a M_r approximately 215,000.

In this laboratory emphasis has been placed on the characterization of the phosphomannosyl receptor from bovine liver and its property of binding lysosomal enzymes. The receptor, a glycoprotein containing 4% carbohydrate, has been solubilized and isolated in homogeneous form. The binding properties of the receptor have been studied utilizing testicular β-galactosidase as a ligand. The binding constant of enzyme for receptor is 24.9 nM. The effect of pH on binding of enzyme to receptor resembles closely that for binding of enzyme to whole cells; maximum binding occurs between pH 6 and 8. In contrast to other carbohydrate-mediated recognition systems, the phosphomannosyl receptor does not require Ca^{++} for binding. The demonstration by chemical and enzymatic means of the presence of covalently-bound mannose 6-phosphate residues on the oligosaccharide chains of lysosomal enzymes, loss of enzyme binding to receptor on treatment with alkaline phosphatase, and the results of competition studies with monosaccharides, monosaccharide phosphates, and phosphorylated oligosaccharides indicate strongly that covalently-bound mannose 6-phosphate residues are a major determinant of the binding of lysosomal enzymes to receptor. Further, lysosomal enzymes known to carry the phosphomannosyl receptor including α-L-iduronidase, β-glucuronidase, and α-N-acetylglucosaminidase inhibit strongly the binding of β-galactosidase to receptor (K_i < 41.7 nM). The specificity of binding is further emphasized by the results of recent studies which show that a portion of the mannose 6-phosphate residues of β-galactosidase are blocked by a phosphodiester linkage with N-acetyl-glucosamine; the blocked residues do not bind to phosphomannosyl receptor.

The properties of the purified receptor suggest that it is involved in (1) the assimilation of exogenous lysosomal enzymes by cells, and (2) the intracellular translocation of newly synthesized enzymes.

1388 MUTATIONAL ANALYSIS OF THE FUNCTIONS OF THE HYDROPHOBIC DOMAINS OF INFLUENZA HAEMAGGLUTININ, Mary-Jane Gething and Joe Sambrook, Cold Spring Harbor Laboratory, P.O. Box 100, Cold Spring Harbor, N.Y. 11724

The complete RNA gene coding for the haemagglutinin (HA) from the A/Japan/305/57 strain of influenza virus has been converted to double-stranded DNA[1] and inserted into prokaryotic or eukaryotic expression vectors[2,3]. When the cloned HA gene is introduced into eukaryotic cells using vectors derived from SV40, it is efficiently expressed into a protein whose structure and biological properties are indistinguishable from those of authentic HA[3,4]. These expressing copies of the HA gene provide the material to analyze the effects of mutations on the structure and function of the molecule.

Two hydrophobic regions in HA determine its destination in the cell. The N-terminal signal sequence is required for the vectorial transfer of the nascent polypeptide across the membrane of the endoplasmic reticulum; the C-terminal hydrophobic sequence anchors the completed protein in the lipid bilayer. As the first step towards a detailed analysis of the role of these hydrophobic regions we have constructed mutants of HA by deleting from the cloned gene, DNA sequences that encode either the N- or C-terminal amino acid sequences of the protein. When these signal-minus or anchor-minus mutants are expressed from SV40-HA recombinant viral vectors the altered HA molecules are found in new locations. The signal-minus variant codes for a non-glycosylated, intracellular protein while the anchor-minus variant specifies a protein that is glycosylated and efficiently secreted from the cell[5]. Further experiments using mutants having smaller deletions and single amino acid changes in the hydrophobic regions will also be discussed.

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Protein Conformation and the Role of Membrane Potential in Protein Transport and Secretion

1389 ROLE OF MEMBRANE POTENTIAL IN SECRETION OF BACTERIAL PROTEINS Dale L. Oxender, Department of Biological Chemistry, The University of Michigan, Ann Arbor, MI 48109

The periplasmic proteins of the gram negative organism, E. coli are synthesized in precursor form on membrane bound ribosomes. The N-terminal signal sequence of these proteins is cleaved during their secretion across the inner membrane into the periplasmic space. The leucine-specific binding protein (LS-BP) protein of E. coli is a periplasmic protein involved in high affinity transport of leucine. The protein is synthesized on membrane bound ribosomes as a precursor with a 23 amino acid signal sequence. Pulse-labeling experiments with intact cells have shown that the completed precursor can be initially detected in the inner membrane and chased into the processed protein which is in the periplasmic space. The processing and secretion of the LS-BP can be inhibited by a variety of reagents including phenethyl alcohol and the proton ionophore, carbonylcyanide m-chlorophenylhydrazone (CCCP). Valinomycin, a potassium ionophore, also inhibits processing in spheroplasts. Processing can be restored in the CCCP and valinomycin treated cells by dilution into fresh medium. The concentration of CCCP inhibitory to processing also reduce the membrane potential of the inner membrane. When processing is inhibited, the precursor form of the LS-BP can be isolated with the inner membrane fraction. Only the mature form of the binding proteins is sensitive to osmotic shock treatment or spheroplast formation. These results suggest that the membrane potential plays an important role in the orientation of the precusor form of certain secretory proteins within the membrane to allow proper processing and secretion. We are currently studying the structural requirements for sensitivity to the membrane potential. In one approach we have examined internal deletions of the LS-BP missing up to 78 amino acid residues near the C-terminus. These altered binding proteins exhibit normal processing and secretion but they are rapidly degraded in the periplasm. The mature binding proteins are resistant to the periplasmic proteases. Attempts are being made to obtain strains devoid of periplasmic protease activity.

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1390 TRANSLOCATION OF NASCENT CHAINS ACROSS THE MEMBRANE OF ESCHERICHIA COLI IS INDEPENDENT OF ELONGATION OF THE POLYPEPTIDE, Linda L. Randall, Biochemistry/Biophysics Program, Washington State University, Pullman, WA 99164-4630

Although the export of protein by Escherichia coli has been studied extensively, the mode of passage of nascent polypeptides through the cytoplasmic membranes is unknown. Two distinct possibilities exist: 1) the polypeptide chain might be transferred in an extended form, one amino acid after the other, coupled to elongation of the protein, or 2) domains of the growing polypeptides might be translocated after their synthesis. The experiments reported here show that the latter mechanism applies to the transfer of at least two periplasmic proteins, the maltose-binding protein and the ribose-binding protein. This conclusion is based on studies of the accessibility of nascent chains to a protease, proteinase K, that was added to the outside of intact spheroplasts. If nascent chains were extruded simultaneously with their elongation they would be exposed on the outside of the membrane at very early times of synthesis. Alternatively if a domain were transferred, the polypeptide would remain on the inside of the membrane, inaccessible to the external protease, until the entire domain were transferred.

We have previously reported (1) that removal of the signal sequence is a late event relative to completion of the nascent polypeptides. No nascent chains are proteolytically processed until they have reached 75% of their entire length. In the experiments reported here, all those chains that had been proteolytically matured were susceptible to digestion by the externally added protease. On the other hand, all nascent chains that carried the signal sequence were inaccessible to the external protease even though they had been elongated to greater than 75% of their full length. If cells were disrupted prior to treatment with proteinase K, all nascent chains were digested. These observations imply that nascent polypeptides are not translocated one amino acid after the other in an extended form, but rather that nascent chains are on the cytoplasmic side of the membrane until time of maturation.

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1391 CONFORMATIONAL STUDIES OF MODEL POLYPEPTIDES IN MEMBRANES, Steven C. Quay, Claudia C. Condie, Angelo N. Heropoulos, Morton J. Cohn, Kevin Clifford, Michael Kravetz, and Kenneth W. Minton, Department of Pathology, Stanford University School of Medicine, Stanford CA 94305.

Melittin, the major toxin of the honeybee (Apis meliffera), is ideally suited for studying the relationship of structure to function in lipid-protein interactions and for examining the kinetic and thermodynamic implications of the coupling of electrostatic, hydrophobic, and trans-membrane electrochemical gradients to particular membrane functions. We are systematically studying the four major conformational states of melittin: the aqueous melittin monomer; the aqueous melittin tetramer; the electrically-silent membrane-bound form; and the voltage-dependent, anion channel conformation.

The melittin monomer-tetramer self-association reaction was studied by fluorescence spectroscopy. At 23°C, pH 7.15, ionic strength 0.50, the dissociation constant, K_d, is 3.20×10^{-6} M. Melittin has an amino acyl group with a proton ionization constant at about 10^{-6} M which must be unionized for tetramer formation to occur. The change in K_d with temperature indicates the forward reaction (tetramer formation) proceeds primarily by entropic changes. The observed enthalpic and entropic values for the tetramerization reaction are consistent with the expected contributions of both nascent hydrogen bonds and hydrophobic stabilization to the reaction.

If self-association of melittin to form tetramers occurs by hydrophobic interaction of the apolar surface of monomers, as suggested by our thermodynamic studies of tetramerization, one would expect less efficient collisional quenching of the fluorescence of Trp-19 in the tetramer, since this residue is located on the hydrophobic face of melittin. Formation of a soluble tetramer does in fact decrease the accessibility of Trp-19 to quenching by acrylamide, Cs^+, and I^-, by 30 to 60%. These studies support the model of melittin tetramerization proposed above.

The reaction of monomeric and tetrameric melittin with TNBS was studied as a function of pH to determine the ionization constants of Lys-21 and -23. The location of TNBS substitution was determined by cleavage of TNBS-melittin adducts with trypsin (at Arg and unsubstituted Lys residues) or with o-iodosobenzoic acid (at the single Trp residue), purification by Sephadex LH-20 column chromatography, acid hydrolysis, and TLC to identify amino acids. The results indicate that Lys-21 has a pK of 6.5 while Lys-23 has a pK of 8.6 in monomeric melittin. These pK values are ca 7.4 in the tetramer.

These studies on aqueous melittin provide the quantitative framework with which to examine the membrane binding of melittin and the nature of the voltage-dependent channel conformation. These studies represent the current focus of our laboratory.

1392 VOLTAGE-DEPENDENT ORIENTATION OF MEMBRANE PROTEINS, Robert Blumenthal, Christoph Kempf, Joseph van Renswoude, John N. Weinstein, and Richard D. Klausner, NIH, Bethesda, Md. 20205

All membrane proteins thus far studied are oriented asymmetrically with respect to the plane of the membrane. A central problem in the study of biological membranes is to identify how the asymmetry is established and maintained. Does the biosynthetic process determine once and for all the orientation of the protein with respect to the bilayer, or can other forces affect the protein's disposition? We have approached this question by studying the topology of the hepatic asialoglycoprotein receptor and of melittin in lipid bilayers. In both cases the membrane potential affects position and orientation in the membrane.

In the first study we show that the hepatic receptor is inserted from the aqueous medium into lipid bilayers. When ligand is added, the receptor changes its conformation or disposition in the membrane. Under the influence of a trans-positive membrane potential, the receptor, a negatively charged protein, appears to "electrophorese" across the membrane, exposing binding sites on the other side. These studies provide the first demonstration of an intrinsic membrane protein crossing, or partially crossing, a lipid bilayer in response to an electrical potential.

In the second study we show that melittin will assume a transbilayer orientation under the influence of an applied potential. Melittin is an amphipathic peptide; its sequence contains a hydrophobic stretch of 19 amino acids followed by a cluster of 4 positively charged residues at the carboxy terminus. The hydrophobic region contains two positively charged residues. In response to trans-negative electrical potential, melittin appears to assume a transbilayer orientation.

These findings have led us to postulate that electrostatic forces can influence the disposition, and perhaps the orientation, of membrane proteins. Given the inside-negative potential of most or all cells, we would expect transmembrane proteins to have clusters of positively charged residues adjacent to the cytoplasmic ends of their hydrophobic transmembrane segments, and clusters of negatively charged residues just to the extra-cytoplasmic side. This expectation is born out by examination of the few transmembrane proteins (glycophorin, M13 coat protein, $H\text{-}2K^b$, HLA-A2, HLA-B7, VSV G protein and mouse $Ig\mu$ heavy chain) for which we have sufficient information on both sequence and orientation. Surface and dipole potentials may similarly affect the orientation of membrane proteins.

Our analysis of the membrane potential as a bias for correct protein orientation does not depend on a model for protein insertion ; i.e. whether it occurs by a process of self-assembly, or whether it is mediated by a complex translocation machinery.

Protein Transport and Secretion in Yeast and Bacillis Species

1393 PROTEIN SECRETION AND ORGANELLE ASSEMBLY IN YEAST, Randy Schekman, Department of Biochemistry, University of California, Berkeley, CA 94720

Protein secretion is a major aspect of cell metabolism and provides a mechanism for assembly of internal organelles and the cell surface. The cellular functions which execute the secretory process in yeast have been identified genetically by the isolation of temperature-sensitive lethal mutants that block the secretory pathway at one of four stages. Three of these stages are defined by class A sec mutants which accumulate secretory glycoproteins inside one of three distinct organelles: endoplasmic reticulum (ER), Golgi bodies, or secretory vesicles.[1] Glycoproteins and secretory organelles accumulate at a nonpermissive temperature (37°C) and proceed to a succeeding stage in the pathway when cells are returned to a permissive temperature (25°C) even in the absence of new protein synthesis.[2,3] Another type of sec mutant (class B) fails to produce active secretory enzymes even though secretory polypeptides are synthesized.[4] Some of the mutants in this class are blocked in the translocation of secretory polypeptides across the ER membrane.

The secretory pathway is responsible for localization of major yeast plasma membrane surface proteins. The export of at least four permease activities and six externally labeled plasma membrane proteins is blocked thermoreversibly in the sec mutants.[5] The transport organelles, secretory vesicles in particular, may carry secreted enzymes with only a subset of plasma membrane proteins. Purified secretory vesicles do not contain chitin synthetase and vanadate-sensitive ATPase, two bonafide integral plasma membrane proteins. Another vesicle may be responsible for transport of these and other membrane proteins.

Part of the secretory pathway is also responsible for localization of vacuolar glycoproteins. Carboxypeptidase Y (CPY) is synthesized as an inactive proenzyme which is matured in the vacuole by cleavage of an 8 Kd amino-terminal propeptide. Proenzyme forms of CPY accumulate thermoreversibly in sec mutants that are blocked in movement from the ER or from the Golgi body, but not in mutants that block transport of secretory vesicles.[6] Vacuoles isolated from sec mutant cells do not contain the proCPY produced at 37°C. These results suggest that vacuolar and secretory glycoproteins require the same cellular functions for transport from the ER and from the Golgi body, where the Golgi body represents the branch point in the pathway.

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1394 GLYCOSYLATION AND PROTEOLYTIC PROCESSING OF α-FACTOR PRECURSOR DURING SECRETORY TRANSPORT. J. Thorner[1], D. Julius[1,2], A. Brake[1,3] and L. Blair[1], Departments of [1]Microbiology and Immunology and [2]Biochemistry, University of California, Berkeley, CA 94720 and [3]Chiron Corporation, Emeryville, CA 94608.

Haploid cells of the α mating type of Saccharomyces yeasts secrete a linear, 13-residue-long peptide, called α-factor (1). This molecule acts as a mating pheromone to prime haploids of the opposite mating type, a cells, for conjugation with α cell partners (2). The α pheromone is initially synthesized as a larger poly-protein precursor, prepro-α-factor, which contains multiple copies of the mature α-factor sequence (3,4). The α-factor segments are excised from the precursor by both endoproteolytic and exoproteolytic cleavages. Final maturation requires the removal of extra N-terminal residues having a repeating $(-Glu-Ala-)_n$ or (-Asp-Ala-) structure and is catalyzed by a heat-stable membrane-bound dipeptidyl aminopeptidase (4). This step and several other post-translational modifications of prepro-α-factor have been studied in α cells carrying temperature-sensitive mutations (sec) that block secretion (5). Mutants that are blocked at very early stages in the secretory process, prior to or during translocation into the endoplasmic reticulum (ER), accumulate a form of prepro-α-factor that is indistinguishable in molecular weight (MW) from the protein synthesized in vitro with polyA+ RNA from α cells. Mutants that overaccumulate ER-like structures, and apparently block further secretory movement, accumulate a single higher MW form of prepro-α-factor. The increase in MW is consistent with the addition of three N-linked mannose-rich oligosaccharide chains. If glycosylation is prevented in vivo by the presence of tunicamycin, or if the glycosyl chains are removed from the high MW form by treatment with endoglycosidase H in vitro, the MW is reduced to that of the in vitro translation product, suggesting that during translocation of prepro-α-factor into the lumen of the ER the signal sequence is not removed. The fully glycosylated form of the precursor persists in mutants that are blocked in protein transport at some stage within the Golgi apparatus and even in certain mutants which accumulate secretory vesicles. However, in most of

the mutants that are blocked at the stage of secretory vesicles, the precursor has undergone extensive proteolytic scission and apparently mature α-factor molecules are the predominant species present. (Supported by NIH grant GM21841 to JT.)

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1395 SECRETION VECTORS FOR *BACILLUS* SPECIES, Ilkka Palva, Recombinant DNA Laboratory, University of Helsinki, Haartmaninkatu 3, SF-00290 Helsinki 29, Finland

A secretion vector has been constructed to promote secretion of foreign gene products in *Bacillus*. The α-amylase gene from *B. amyloliquefaciens* was cloned in *B. subtilis* using plasmid pUB110 as a cloning vector[1]. The promoter signal sequence region of the α-amylase gene was characterized[2] and the secreted α-amylase was found to be correctly processed in the new host[3]. A series of secretion vectors was constructed by removing the structural part of the α-amylase gene using BAL31 nuclease, and HindIII linkers were added after the α-amylase signal sequence or at different lengths downstream of the signal sequence cleavage site.

The promoter signal sequence region of β-lactamase gene from pBR322 was removed, and HindIII linkers were added to the 5' and 3' ends of the structural gene. The modified β-lactamase gene was joined to a series of secretion vectors, transformed into *B. subtilis* and the β-lactamase activity produced/secreted by the transformants was measured. All constructions where the β-lactamase gene was fused after the complete α-amylase signal sequence, resulted in an efficient secretion of β-lactamase to the culture medium, whereas in a construction with β-lactamase gene joined within the α-amylase signal sequence, most of the β-lactamase activity was cell-bound[4].

The human interferon α-2 cDNA devoid of its own signal sequence was inserted into two secretion vectors. In these constructions the full signal sequence of α-amylase is followed by either 0 or 4 amino acid residues of the mature α-amylase before the mature IFN α-2. When *B. subtilis* was transformed with these constructions, the interferon activity was efficiently secreted to the growth medium in all growth phases, and in both cases the secreted interferon was correctly cleaved after the last amino acid residue of the α-amylase signal sequence. Expression of other genes joined to the secretion vectors is discussed.

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Specialized Secretion of Polypeptides and Toxins

1396 POST-TRANSLATIONAL MODIFICATION AND PROCESSING OF ESCHERICHIA COLI PROLIPOPROTEIN IN VITRO. Henry C. Wu, Masao Tokunaga, Hiroko Tokunaga and Judith M. Loranger, Department of Microbiology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814.

The biosynthesis of outer membrane Braun's lipoprotein in Escherichia coli involves successive modification and processing reactions, namely glyceryl transferase, O-acyl transferase, signal peptidase and N-acyl transferase (1). Similar reactions are presumably involved in the biogenesis of other inner and outer membrane lipoproteins including Bacillus licheniformis penicillinase synthesized in E. coli (2). In vivo studies using a cyclic antibiotic, globomycin, suggest that modification of prolipoprotein to form glyceride-containing prolipoprotein may precede processing. Furthermore, the specific inhibition of processing of precursor forms of Braun's lipoprotein as well as other distinct lipoproteins by globomycin strongly suggests that the prolipoprotein signal peptidase is distinct from the M13 procoat protein signal peptidase which also appears to correctly process the precursor proteins of a number of periplasmic and outer membrane proteins.

We have developed an in vitro system which carries out all the modification and processing reactions using unmodified prolipoprotein, phospholipids and detergent-solubilized cell envelope as the enzyme source. Using this in vitro system, we have been able to show that the processing of prolipoprotein by signal peptidase requires prior modification of prolipoprotein with glyceryl transferase (3). Furthermore, the prolipoprotein signal peptidase is distinct from the M13 procoat protein signal peptidase (4). These results provide evidence for the existence of a unique modification and processing system for prolipoproteins in both Gram-negative and Gram-positive bacteria.

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1397 SYNTHESIS, PROCESSING, AND SECRETION OF VERY LOW DENSITY LIPOPROTEIN, David R. Janero, Patricia Siuta-Mangano, Kurt W. Miller, and M. Daniel Lane, Department of Physiological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Estrogen-induced chick liver cells synthesize and secrete large amounts of very low density lipoprotein (VLDL). These cells, in a primary monolayer culture system developed in this laboratory, are being employed to study operative and regulatory aspects of VLDL biogenesis and secretion. Pulse-chase experiments with [^{3}H]leucine showed that ~10 min are required for the synthesis of the principal VLDL protein constituent, apoprotein B (apo-B), and 30 min are required for newly-translated apoproteins to be secreted. Apo-B is synthesized on membrane-bound polysomes as a contiguous polypeptide chain of 350K rather than by post-translational assembly from small peptide precursors. Translocation of puromycin-discharged apo-B nascent chains into the endoplasmic reticulum lumen and their subsequent secretion are independent of both ongoing protein synthesis and the attachment of the polypeptides to the ribosomes. Core oligosaccharides are added to the nascent apo-B chain co-translationally in at least two stages, i.e., at molecular weights of ~120K and 280K. Inhibition of N-linked glycosylation of apo-B with tunicamycin affects neither the incorporation of the normal glycerolipids and "C" apoproteins into VLDL nor the secretion of VLDL, indicating that aglyco-apo-B can serve as a functional component for VLDL assembly and secretion. Active synthesis of VLDL apoproteins, however, is required for glycerolipid secretion. Pulse-chase experiments with [^{3}H]glycerol revealed that VLDL [^{3}H]triglyceride is secreted in parallel with VLDL [^{3}H]apopeptides pulse-labeled from [^{3}H]leucine. However, a significant fraction of VLDL [^{3}H]phospholipid is secreted prior to both the [^{3}H]triglyceride and the [^{3}H]apopeptides. The differential kinetics of hepatic glycerolipid secretion as VLDL indicate that triglyceride assembly is an early event in VLDL biogenesis proximal to the site of apoprotein synthesis, whereas a fraction of the secreted phospholipids associate with the maturing VLDL particle late in the secretory pathway. Within the context of current models for VLDL structure, the late assembly of phospholipids into VLDL is taken to reflect a surface maturation of nascent, triglyceride-rich lipid-protein complexes just prior to their secretion as VLDL.

1398 PRODUCTS OF PROENKEPHALIN PROCESSING AND THEIR SECRETION FROM THE ADRENAL MEDULLA, Daniel L. Kilpatrick and Sidney Udenfriend, Roche Institute of Molecular Biology, Nutley, NJ 07110

The adrenal medulla is rich in enkephalins and enkephalin-containing peptides (EC-peptides) ranging in size from M_r 1000 to M_r >20,000 (1). Together, these EC-peptides represent precursors, intermediates and free enkephalins. Sixteen different EC-peptides have been isolated and characterized (2). Through a combination of protein and cDNA sequencing data obtained from cloning experiments, the entire sequence of bovine adrenal proenkephalin has been determined (3,4). Proenkephalin contains 7 enkephalin sequences: 4 of [Met]enkephalin, 1 of [Leu]enkephalin and 2 [Met]enkephalin extended sequences, [Met]enkephalin-Arg^6-Phe^7 and [Met]enkephalin-Arg^6-Gly^7-Leu^8. Within proenkephalin each of these enkephalin sequences is bracketed by paired basic amino acid residues (Arg or Lys), the normal recognition sites for proteolytic processing. Free enkephalins represent less than 10 percent of the total enkephalin sequences present in bovine adrenal medulla and several of the larger EC-peptides have been shown to possess greater biological activity than free enkephalins in certain opiate assay systems (2). From all this it appears that EC-peptides are not merely intermediates in the biosynthesis of enkephalins, but are physiologically important in their own right. The adrenal EC-peptides are localized in the medullary chromaffin cells in the same secretory granules that also store catecholamines (5). It is also known that on cholinergic stimulation of the adrenal gland the contents of the granules are released by a process of exocytosis. By assaying perfusates from stimulated adrenal glands it was found that all the EC-peptides are released along with catecholamines in the same proportions as are found in the intact granules (6,7). Since the EC-peptides are released into the circulation they most likely act at peripheral sites. Their exact role remains to be determined.

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1399 MUTATIONS THAT AFFECT THE PROCESSING OF THE LOW DENSITY LIPOPROTEIN RECEPTOR, Wolfgang J. Schneider, Helge Tolleshaug, Joseph L. Goldstein, and Michael S. Brown, Department of Molecular Genetics, University of Texas Health Science Center, Dallas, Texas 75235

The cell surface receptor for low density lipoprotein (LDL) is an acidic glycoprotein of 160 kd. Mutations in the structural gene for this molecule occur in the disease familial hypercholesterolemia (FH). Cultured fibroblasts from FH heterozygotes express on their surface half the normal number of functional LDL receptors; those from homozygotes express either no functional receptors or a greatly reduced number. Since the LDL receptor normally mediates the cellular uptake and degradation of LDL, its deficiency in subjects with FH leads to accumulation of LDL-cholesterol in plasma, resulting in premature atherosclerosis.

We have studied the biosynthesis of the LDL receptor in human fibroblasts by incorporation of [^{35}S]methionine followed by immunoprecipitation with a monoclonal anti-receptor antibody. The receptor is synthesized as a precursor with an apparent M_r of 120 kd that is converted to the mature form of 160 kd within 15 to 30 min. This increase in M_r of 40 kd is much greater than the increase observed when other types of cell surface proteins are processed. This novel form of processing does not appear to be simple addition of oligosaccharide. The mechanism underlying the apparent 40 kd increase is not known; however, its significance became apparent from the study of a unique patient with the clinical phenotype of homozygous FH. Genotypically, this patient is a compound: he has two different mutant alleles at the LDL receptor locus, both producing receptors immunoprecipitable with the monoclonal antibody. The genotype of this patient was established by study of his parents' cells: the heterozygous mother and father each possess one normal and one mutant allele, and the child has inherited both defective genes. Thus, from the mother, the patient inherited a mutant allele which produces a 120 kd protein that cannot be processed; the mutant allele inherited from the father produces a receptor that is synthesized initially as a 170 kd protein. Like the normal receptor, this abnormal receptor undergoes a 40 kd increase to a mature form of 210 kd. - We have now identified several different mutant alleles at the LDL receptor locus. Most of the mutant gene products undergo processing, others are slowly or not at all converted to a mature form. However, if processing occurs, the increase in apparent M_r is 40 kd in all cases. Thus, the increase appears to be due to a specific reaction for which a certain domain on the precursor molecule(s) is the substrate. Mutations affecting this domain appear to affect processing.

Studies in normal cells as well as in cells from FH patients with identified structural mutations in the LDL receptor protein are now underway to define the biochemical basis for the genetic disruption of posttranslational processing of the LDL receptor in FH. (W.J.S. is an Established Investigator of the American Heart Association.)

Role of Proteases in Protein Processing, Turnover and Degradation
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1400 "THE ATP-DEPENDENT PATHWAY FOR PROTEIN DEGRADATION IN E. COLI, MITOCHONDRIA, AND MAMMALIAN CELLS," by Alfred L. Goldberg, Lloyd Waxman, Michel Desautels, Chin Ha Chung, and Keiji Tanaka. Harvard Medical School, Boston, Ma. 02115. In mammalian and bacterial cells, proteins with highly abnormal conformations are rapidly hydrolyzed. This process involves complete digestion by a soluble pathway that requires ATP. Liver mitochondria can also rapidly degrade abnormal proteins generated within the organelle. This process also requires ATP and is localized to the mitochondrial matrix. The energy requirement for protein breakdown in E. coli results from the ATP requirement of protease La. This enzyme which is the product of the lon gene catalyzes the rate limiting step in protein breakdown, although ATP-independent proteases are involved in later steps. Protease La cleaves proteins and ATP in a coupled reaction. Protein substrates stimulate ATP hydrolysis, and rates of ATP cleavage and peptide bond hydrolysis are proportional. Protease La has a molecular weight of 420,000 and contains four identical subunits. The phenotypic consequences of lon mutations probably result from their decreased capacity for proteolysis. One unusual mutant, R9 encodes a labile protease, which when inactive can inhibit the wild type protease by formation of mixed tetramers. This mechanism can account for its dominant phenotype in vivo. An enzyme resembling protease La has been purified from liver mitochondria, where it also seems to catalyze the rate-limiting steps in ATP-dependent proteolysis.

Fluorogenic tripeptides have been found which are substrates for these proteases. The hydrolysis requires ATP and Mg++ but differs in several respects from that of proteins. Use of these model substrates has allowed us to dissociate peptide and ATP hydrolysis. These reactions appear to occur sequentially in the normal proteolytic mechanism. Protease La is also activated 2-4 fold by DNA. The enzyme binds with high affinity to double stranded or single stranded DNAs and this process enhances proteolytic and ATPase activities. Interaction of protease La with substrates releases it from the DNA. The biological significance of this potential DNA-binding cycle is unclear.

In reticulocytes, ATP has been suggested to enhance proteolysis, by allowing conjugation of substrate proteins to ubiquitin. However, proteins whose amino groups are blocked so that they can't be linked to ubiquitin still undergo ATP-stimulated proteolysis. These experiments suggest that ATP serves distinct roles at different steps in the degradative process. The ATP-activated process, which is independent of ubiquitin, involves ATP cleavage and is inhibited by hemin. It probably involves direct effects of ATP on a protease. In fact, vanadate, which inhibits ATP-dependent proteases in mitochondria and E. coli also affects this process in reticulocyte extracts. An alkaline protease that is activated two-fold by ATP has been purified from red cells. This endoprotease is very large (450,000 daltons) and has multiple active sites. Although this enzyme does not hydrolyze ATP, a variety of evidence indicates that it plays a key role in this degradative pathway.

1401 MULTIPLE PROCESSING STEPS IN THE GENERATION OF PEPTIDES FROM HONEY BEE VENOM AND FROG SKIN, G. Kreil, W. Hoffmann, A. Hutticher and C. Mollay, Institute of Molecular Biology, Austrian Academy of Sciences, Billrothstrasse 11, A-5020 Salzburg, Austria.

Melittin, the main constituent of bee venom, is derived from a precursor composed of 70 amino acids. Liberation of the lytic peptide requires three distinct processing steps: a) cleavage of the signal peptide; b) formation of the COOH-terminal amide with concomitant loss of a glycine; c) cleavage of the pro-region. Partially purified signal peptidase from rat liver has been shown to cleave prepromelittin at a single bond, the pre-pro junction. Both reaction products, promelittin and intact signal peptide, have been detected (1). In collaboration with W. Wickner, it has moreover been shown that signal peptidase from E. coli also hydrolyzes prepromelittin at the same site. Hydrolysis of the pro-part proceeds via stepwise cleavage of dipeptides by a dipeptidylpeptidase (2). The enzyme from queen bee venom sacs has been isolated and studied in some detail.
Caerulein, a decapeptide related to cholecystokinin and gastrin, is one of the main components in the skin secretion of Xenopus laevis. A cDNA bank was constructed from skin mRNA and screened with cDNA primed with a deca-deoxynucleotide complementary to the mRNA sequence of the Trp-Met-Asp-Phe - region of caerulein. Three different clones containing information for caerulein precursors have been sequenced. In each one, pairs of arginine residues flank a 15 amino acid peptide which includes the caerulein sequence. After cleavage

at, and excision of, these arginines, two more processing steps must take place to yield the decapeptide: a) formation of the terminal amide again involving a glycine residue; b) cleavage of the tetrapeptide Phe-Ala-Asp-Gly, which precedes the caerulein sequence in these precursors. The skin secretion of Xenopus laevis contains dipeptidylpeptidase activity which can liberate dipeptides terminating with alanine or glycine.
We propose that stepwise cleavage of dipeptides represents a new type of precursor processing operating in the generation of peptides from bees, frogs as well as yeast (3).

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1402 MICROINJECTION STUDIES ON PROTEIN DEGRADATION IN CULTURED MAMMALIAN CELLS

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For the past three years my laboratory has used red cell-mediated microinjection to study protein degradation in cultured mammalian cells. Proteins are introduced into red blood cells during hypotonic hemolysis, and these loaded red cells are fused with culture cells using Sendai virus (1). This approach has certain advantages for the study of selective proteolysis within cells. Proteins can be modified and then introduced into cells to discover those features of protein structure significant in determining degradation rates. Moreover, injection of a specific, radioiodinated protein allows one to determine the half-life, location and size of the injected protein without confusion from other labeled proteins. By use of this procedure, we have shown that specific proteins are degraded at different rates after injection into cultured cells and that degradation occurs largely in cytosol rather than within lysosomes (2). Our present efforts focus on the role of ubiquitin marking in selective proteolysis (3). Ubiquitin was radioiodinated and injected into HeLa cells. Fractionation and subsequent NaDodSo$_4$-polyacrylamide gel electrophoresis showed that HeLa nuclei contained two major labeled proteins: ubiquitin and the histone H2A-ubiquitin conjugate, protein A24. HeLa cytosol contained ubiquitin and a series of ubiquitin-protein conjugates of diverse molecular weights. When injected HeLa cells were treated with phenylhydrazine to denature the cotransferred hemoglobin, a series of prominent ubiquitin-globin conjugates appeared. The identity of these conjugates was established by microinjection experiments in which both proteins were labeled. At low doses of phenylhydrazine, the intracellular concentration of globin-ubiquitin conjugates was proportional to the rate of hemoglobin degradation. This result together with the observation that ubiquitin conjugation to globin is markedly enhanced by phenylhydrazine induced denaturation of globin provides support for the hypothesis that the covalent attachment of ubiquitin to proteins signals proteolysis. Ubiquitin marking does not, however, appear to be an obligatory step in the degradation of all proteins since we have found that guanidinated proteins are not conjugated to ubiquitin, and yet they are degraded after injection into cultured cells and they are also degraded by an ATP-dependent, hemin-sensitive pathway in reticulocyte lysates. Under hemin inhibition in this latter system, ^{125}I-guanidinated lysozyme is converted to sulfhydryl-sensitive higher M.W. forms. The smallest of these new species has a MW of 26,000 daltons raising the possibility that the 12,000 dalton protein, thioredoxin, may also serve as a marker for proteolysis.

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Engineering Protein Secretion in Microorganisms
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1403 THE USE OF GRAM-POSITIVE ORGANISMS FOR PROTEIN SECRETION, Shing Chang, Sheng-Yung Chang, Jane McLaughlin and David Mark, Cetus Corporation, Berkeley, CA 94710

The leader (signal) peptide at the amino-terminus of the prepenicillinase is essential for the translocation of this protein across cell membrane. The cloned penP gene originated from Bacillus licheniformis has been shown to be expressed in both E. coli and B. subtilis (1) and the secretory precursor penicillinase is processed into a soluble form (the exo-enzyme) and a membrane-bound (lipoprotein) form in these host cells (2,3). Mutations causing alterations in the leader peptide have been constructed which affect the secretion and the modification steps. These include a small deletion, which removes the leu-ala-gly-cys-ala pentapeptide sequence at positions 24 to 28 in the leader sequence, and a point mutation which alters the process leading to the formation of the lipoprotein form. We fused the wild type as well as the mutated leader peptides to foreign proteins devoid of their natural leader sequences and constructed hybrid "pre"-proteins. Depending on the specific sequences used to create these fusions, the expressed "pre"-proteins can be transported across the B. subtilis membrane with different efficiencies, and the eukaryotic proteins detected in the growth medium.

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1404 HUMAN INTERFERON SECRETION BY YEAST, David V. Goeddel*, Ronald A. Hitzeman*, David W. Leung*, L. Jeanne Perry†, William J. Kohr†, and Arjun Singh*, Departments of Molecular Biology* and Protein Biochemistry†, Genentech, Inc., South San Francisco, CA 94080

Human Interferon (IFN) genes have been expressed in the Yeast Saccharomyces cerevisiae under the control of yeast promoter sequences. IFN activity is secreted into the growth media when the IFN genes contain signal peptide coding regions. The signal peptides are correctly processed yielding mature IFN molecules.

Genetic Approaches to Protein Transport in Procaryotes

1405 MUTATIONS WHICH ALTER THE SIGNAL SEQUENCE OF THE PERIPLASMIC PROTEIN ALKALINE PHOSPHATASE IN E. COLI, Susan Michaelis, John Hunt, and Jon Beckwith, Harvard Medical School, Boston, Mass., 02115

To study the secretion of the periplasmic enzyme alkaline phosphatase we have developed a system for isolating mutations which render its signal sequence non-functional. A phoA-lacZ gene fusion was constructed using recombinant DNA techniques. The fusion encodes a hybrid protein that contains approximately half of alkaline phosphatase joined to β-galactosidase. The hybrid protein is localized to the membrane where it exhibits abnormally low β-galactosidase activity. Lac^+ mutant derivatives of the fusion-bearing strain were isolated. Among these are mutations that lie within the phoA portion of the fused gene and cause internalization of the hybrid protein. When these mutations are genetically recombined onto an otherwise wild-type phoA gene they interfere with export of alkaline phosphatase to the periplasm and cause accumulation of cytoplasmic precursor as well as reduced enzymatic activity. The degree of inhibition of export as detected by SDS-polyacrylamide gel analysis correlates with the extent of loss of alkaline phosphatase enzymatic activity in these mutants. DNA sequence analysis for two mutants demonstrates that both contain lesions in the signal sequence-coding region of phoA. DNA sequencing is currently underway for a collection of 60 mutants exhibiting a broad range of enzymatic activity from 1% to 85% that of wild-type.

1406 GENETIC APPROACH TO THE STRUCTURE, LOCALIZATION AND ASSEMBLY OF THE λ RECEPTOR IN E.coli K12, Maurice HOFNUNG, Alain CHARBIT, Jean Marie CLEMENT, Christian MARCHAL, Henri VILLARROYA, Unité de Programmation Moléculaire et Toxicologie Génétique, INSTITUT PASTEUR, Paris, France.

The λ receptor in E.coli K12 is an outer membrane protein needed for the adsorption of various phages and for the entry of maltose (at low external concentration) and maltodextrins. By analysing the sequence changes and the dominance properties in mutants resistant to phages using the λ receptor we obtain information on the functional topology of the protein. The ability to find its location and to assemble can be also considered as a function and is thus within the reach of this approach.

1407 PROTEIN K EXPRESSION AND LOCALIZATION IN ESCHERICHIA COLI K-12 STRAINS WITH OMPR AND ENVZ MUTATIONS, Joyce Sutcliffe and John Foulds, National Institutes of Health, Bethesda, MD 20205

Using a technique that was designed in the laboratory of Dr. C. Schnaitman for cloning of porin proteins on phage lambda, the structural gene for protein K, a major outer membrane protein in E.coli K1 strains, has been cloned. To confirm that the cloned fragment is producing protein K, two-dimensional peptide maps of the cloned protein and authentic protein K were compared. Expression of protein K in a porin-deficient strain restores the sensitivity of this strain to colicins E2 and E3 as well as increases its rate of uptake of nutrients to allow growth on minimal media. These results indicate that protein K functions as a porin. Previously, protein K was found to be structurally similar to OmpF and OmpC porins. Regulation of protein K by ompR and envZ genes was examined by constructing lysogens of isogenic K-12 strains that contain ompR101, ompR4 or envZ473. The strains were grown on nutrient broth (NB) or trypticase soy broth (TSB) and proteins in the cytoplasmic, inner and outer membrane fractions were examined by SDS-polyacrylamide gel electrophoresis. The expression of protein K is influenced by the osmolarity of the medium; the production of this protein is favored in TSB. Protein K's expression and proper translocation occurs in the absence of a totally functional ompR gene product. However, protein K does not appear in any fraction when strains containing the envZ473 mutation are grown in NB or TSB. This suggests that protein K may be under transcriptional regulation of envZ without ompR involvement.

1408 Fusions of Secreted Proteins to E.coli Alkaline Phosphatase. Andrew Wright, Charles Hoffman and Yolanta Fishman, Tufts Medical School,Boston,Mass. 02111.

We have constructed a series of plasmid vectors carrying the E.coli alkaline phosphatase gene (phoA) for use in studies of protein secretion. We used the enzyme Bal31 to remove the promoter and signal sequence of the phoA gene so that expression of the gene and secretion of its product are absolutely dependent on fusion to other genes which specify secreted products. The modified phoA genes are flanked by PstI sites and exist in all three reading frames relative to these sites. The carboxyl termini of the modified genes are intact. phoA genes of this type lacking more than 100 nucleotides of the amino terminal coding sequence can still specify enzymatic activity. We have fused portions of genes specifying both periplasmic (β-lactamase) and outer membrane proteins (lamB)to these modified phoA genes and from both classes of fusions obtain proteins with phosphatase activity. β -lactamase - phoA fusions give rise to fusion proteins all of which are secreted to the periplasm and show phosphatase activity. Fusions ranged from those containing only the β -lactamase promoter and signal sequence region to those containing 547 nucleotides of the β -lactamase coding sequence. Most of the secreted proteins were processed,however in some cases efficient secretion without processing occurred giving active enzyme. A limited number of lamB-phoA fusions have been examined. One of these containing the signal sequence plus 643 nucleotides of lamB is found distributed between the periplasm and membrane fraction. The effect of shorter and longer lamB sequences on phosphatase localization is currently under study and will be discussed.

1409 GENETIC ANALYSIS INTRAGENIC INFORMATION REQUIRED FOR LOCALIZATION OF THE OUTER MEMBRANE PROTEIN LAMB, Spencer A. Benson, Erhard Bremer, and Thomas J. Silhavy, Basic Research Program-LBI, Frederick Cancer Research Facility, Frederick, MD 21701

Gene fusions between the lamB gene which encodes a major outer membrane protein and the lacZ gene which encodes the cytoplasmic protein β-galactosidase have been used to study protein localization. A lamB-lacZ fusion which encodes a hybrid protein containing a complete LamB signal sequence plus 241 amino acids of the mature protein is processed and localized efficiently to the outer membrane. The β-galactosidase moiety appears to be inactive when present in the outer membrane. This property has been exploited for selection of mutations that alter the localization of the hybrid protein. The majority of 200 independent mutations appear to be spontaneous deletions that reduce the amount of LamB material present on the hybrid protein. Sequence analysis of these deletions shows that a signal sequence plus 45 amino acids of the mature protein is sufficient for export of the hybrid protein from the cytoplasm. On the other hand, a protein containing a complete LamB signal sequence plus 15 amino acids of the mature protein is found only in the cytoplasm. Therefore, at least a portion of the information specifying export must lie in the protein segment between amino acid 15 and 45 of the mature LamB protein. Research sponsored by the National Cancer Institute under Contract No. NO1-CO-23909 with Litton Bionetics, and a NRS Award to S.B. (#1F32CA067-01). E. Bremer is a recipient of a Deutsche Forschungsgemeinschaft fellowship.

1410 ANALYSIS OF A MEMBRANE ANCHOR SEQUENCE IN A BACTERIOPHAGE f1 PROTEIN, Jef D. Boeke, Dept. of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, Nicholas Davis, and Peter Model, The Rockefeller University, New York, NY 10021

Bacteriophage f1 encodes an adsorption protein (gene III protein), which is inserted into the host bacterial inner membrane. Plasmids containing gene III have been constructed. These direct the synthesis of gene III protein (gIIIp) in Escherichia coli cells. The plasmid-encoded protein appears to be inserted into the membrane in the same way as gIIIp produced in phage infections. However, when truncated forms of the protein (produced by in vitro deletion mutagenesis of the plasmid or by infecting cells with gene III amber mutants) are specified, these mutant gIIIp's pass through the inner membrane and into the periplasm. A highly hydrophobic sequence near to the COOH-terminus of gIIIp, termed membrane anchor sequence, appears to be responsible for retention of the protein in the membrane; mutants lacking all or part of this sequence produce abnormal proteins which pass through the membrane. Membrane anchoring of such truncated gIIIp's can be restored by fusion of a similar membrane anchor sequence from another inner membrane protein to the truncated gIIIp COOH-terminus. Analysis of features of membrane anchor sequences and their importance will be discussed.

1411 IDENTIFICATION OF MUTANTS ALTERED IN SUBUNIT V OF CYTOCHROME c OXIDASE IN S. CEREVISIAE, Joan E. McEwen, Michael G. Cumsky, Scott D. Power, Christine Ko and Robert O. Poyton, MCD Biology, University of Colorado, Boulder. CO 80309

Cytochrome c oxidase from S. cerevisiae is an inner-mitochondrial membrane protein composed of three polypeptides (subunits I-III) encoded by the mitochondrial genes oxi-1, oxi-2 and oxi-3, and four polypeptides (subunits IV-VII) encoded by nuclear genes not yet identified. Our long-term goals are to: 1) identify mutants altered in nuclear cytochrome oxidase genes; 2) clone these genes; and 3) manipulate these genes in vitro in order to identify protein domains required for targeting of cytoplasmically-synthesized cytochrome oxidase polypeptides to the inner mitochondrial membrane and holoenzyme complex. So far, we have studied cytochrome oxidase-deficient mutants from eight nuclear complementation groups. By use of the Western immunoblot technique to detect each cytochrome oxidase subunit in whole cell extracts and mitochondria prepared from these mutants, we have identified two mutants in the same complementation group that lack the 12.5 kd subunit V of cytochrome oxidase. These mutants instead make variant forms of subunit V of an apparent molecular weight of 14.5 kd in one case and 9.5 kd, in the other. A revertant of the latter has regained 27% of the wild-type level of oxidase activity and makes a new variant subunit V of apparent 13.5 kd MW. We purified cytochrome oxidase from the revertant and showed that the variant subunit V is assembled in the cytochrome oxidase holoenzyme. These mutants therefore appear to be altered in the structural gene for subunit V. We have isolated the corresponding wild-type gene by transforming one of these mutants with yeast DNA cloned in the plasmid YEp13. This gene is currently being characterized.

1412 GENETIC ANALYSIS OF PROTEIN EXPORT. Thomas J. Silhavy, Basic Research Program-LBI, Frederick Cancer Research Facility, Frederick, Maryland 21701.

A significant fraction of the proteins produced by all cells is localized to a non-cytoplasmic location. We are studying the molecular mechanisms that determine such localization, in particular the mechanism(s) by which proteins are exported from the cytoplasm of Escherichia coli. The choice of E. coli as a model organism permits the application of sophisticated genetic approaches to the study of protein export. A particularly successful genetic approach uses gene fusions. Numerous strains have been constructed in which an NH_2-terminal portion of an exported protein is fused to a large functional COOH-terminal portion of the cytoplasmic enzyme, β galactosidase. By determining the cellular location of the hybrid protein produced by such fusion strains, we have gained insight into the location of intragenic information specifying protein export. More importantly, many such fusion strains exhibit unusual phenotypes which have been exploited to isolate export-defective mutants. Genetic and biochemical analysis of these mutants has revealed critical intragenic regions that specify a noncytoplasmic location. In addition, we have identified genetic loci whose products appear to be necessary for the export process. The availability of export-defective mutants has permitted further genetic studies to determine how the various informational and cellular components interact in the export process. Research sponsored by the National Cancer Institute, DHHS, under Contract No. NO1-CO-23909 with Litton Bionetics, Inc.

1413 ANALYSIS OF SITE-DIRECTED MUTATIONS WITHIN THE SIGNAL PEPTIDE OF E. COLI LIPOPROTEIN. G.P. Vlasuk[1], S. Inouye[1], K. Itakura[2], H. Hsuing[3], and M. Inouye[1]; [1]Department of Biochemistry, SUNY Stony Brook, Stony Brook, NY 11794; [2]Division of Biology, City of Hope Research Institute, 1450 E. Duarte Road, Duarte, CA 91010; [3]Lilly Research Laboratory, Eli Lilly Co., Indianapolis, IN 46285

The outer membrane lipoprotein (Lp) of E. coli is initially synthesized in the cytoplasm as a higher molecular weight precursor prolipoprotein (plp) which has an NH_2-terminal "signal sequence". The plp signal peptide shares several structural homologies with signal sequences from other bacterial secretory proteins which include: a) a positively charged NH_2-terminus; b) a sequence of 10-15 hydrophobic amino acids directly following the basic NH_2-terminus; c) serine and threonine residues following the hydrophobic core and located close to the carboxy-terminus; and d) glycine residues located within the hydrophobic domain. The functional significance of these regions with regard to protein secretion across the cytoplasmic membrane has been proposed in the loop model. To test the various proposals in this model, we have constructed a variety of specific mutations within the plp signal peptide using synthetic oligonucleotide directed mutagenisis. Examples of these Lp signal sequence mutations can be summarized as follows: 1) Alteration of the charge at the NH_2-terminus from +2 (wt) to +1, 0, and -2; 2) systematic replacement and/or deletion of the two glycine residues in the hydrophobic core; and 3) replacement of the serine and threonine residues located close to the cleavage site. Analysis of the effects these mutations had on Lp biosynthesis and secretion has allowed us to elucidate the function of the various regions of the plp signal peptide in the secretion of this protein across the cytoplasmic membrane.

1414 THIOL-β-LACTAMASE: REPLACEMENT OF THE ACTIVE-SITE SERINE OF RTEM β-LACTAMASE BY A CYSTEINE RESIDUE, Irving S. Sigal, Betty G. Harwood, and Rene Arentzen, E. I. du Pont de Nemours & Co., Experimental Station, Wilmington, Delaware 19898

By utilizing synthetic oligonucleotides in conjunction with recombinant-DNA methods the primary sequences of proteins can now be systematically altered, thus facilitating structure-function studies of enzymes. The role of particular amino acids can be determined by making specific substitutions at the nucleotide level in the structural genes of those enzymes. We have initiated a study to determine the catalytic role of particular functional groups at the active sites of hydrolytic enzymes. By employing a specifically designed molecular linker, the nucleotide sequence for pBR322 β-lactamase was modified in order to replace the active-site serine by a cysteine residue. The resulting mutant gene codes for a thiol-β-lactamase which remains active yet is chemically distinguishable from the wild-type serine enzyme. The thiol-β-lactamase is serving as a model system for future enzyme alterations.

Leader Peptidases and Processing

1415 A DISTINCT SIGNAL PEPTIDASE FOR LIPOPROTEINS. Masao Tokunaga, Judith M. Loranger and Henry C. Wu. Department of Microbiology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814.

The murein lipoprotein in Escherichia coli contains a unique structure, N-acyl diglyceride-cysteine, at its NH_2-terminus. Lipoprotein is first synthesized as a precursor protein, prolipoprotein. Biogenesis of the mature form of lipoprotein involves successive modification and processing reactions. In vitro studies have shown that the processing of prolipoprotein by signal peptidase requires prior modification of prolipoprotein with glycerol or glyceride. This novel substrate requirement for the prolipoprotein signal peptidase suggests that glyceride-modified cysteine and its neighboring amino acid sequence provides a unique recognition site for the prolipoprotein signal peptidase.

Using modified prolipoprotein purified from globomycin-treated cells as a substrate for in vitro assay, we have partially purified and characterized the prolipoprotein signal peptidase from E. coli. Prolipoprotein signal peptidase is localized in the cytoplasmic membrane of the cell envelope, and it is distinct from the M13 procoat protein leader peptidase. In the presence of non-ionic detergents, the enzyme does not require phospholipids for activity, and it is strongly inhibited by globomycin (I_{50} = 5 nM). In addition, the pH and temperature optima of the prolipoprotein signal peptidase are 8.0 and 37-45°C, respectively.

1416 STRUCTURAL REQUIREMENTS FOR SECRETION OF LS-BP IN E. COLI, Penelope M. Nazos, Robert Landick, T. Z. Su, Bruce R. Copeland and Dale L. Oxender, The University of Michigan, Ann Arbor, MI 48109

The leucine specific binding protein (LS-BP), a periplasmic component of the high affinity leucine transport system in E. coli is being used in our laboratory as a model to study the structural requirements for protein secretion. LS-BP is initially synthesized as a precursor form with a 23 amino acid residue signal sequence that is removed during its secretion into the periplasm. A variety of reagents which alter the membrane potential such as CCCP and valinomycin inhibit the processing and secretion of LS-BP but the inhibition can be reversed by dilution into fresh medium. These results suggest that the membrane potential plays a role in the secretion of the LS-BP probably by orienting the precursor molecule within the membrane. Using site directed mutagenesis internal deletion mutations resulting in the loss of up to 78 amino acid residues near the C terminus of the protein have been constructed. The secretion process and the sensitivity of secretion to CCCP of these altered LS-BP's appear to be normal. All of the processed mutants that we have isolated, however, are degraded in the periplasmic space. If processing is inhibited, the precursor accumulates in the membrane and is protected from degradation. As an additional approach in the study of secretion we are fusing LS-BP to cytoplasmic proteins such as tryptophan synthetase (trpA) and examining the secretion properties of the hydrid protein. We are also studying the interaction of LS-BP with the additional components of the secretory system such as secA protein which has been identified by Oliver and Beckwith.

(This research is supported by NIH Grant GM 11024)

1417 SPECIFIC PROCESSING OF THE BACTERIAL ß-LACTAMASE PRECURSOR IN SACCHAROMYCES CEREVISIAE, Rainer Roggenkamp, Jürgen Hoppe* and Cornelis P. Hollenberg, Institut für Mikrobiologie, Universität Düsseldorf, 4000 Düsseldorf, F.R.G. and *Gesellschaft für Biotechnologische Forschung mbH, 3300 Braunschweig, F.R.G.

Among the bacterial enzymes which can be expressed in yeast, ß-lactamase is of special interest, since the enzyme is synthesized as a preprotein in E. coli containing a signal sequence of 23 amino acids. Recently, we reported that the yeast cell is able to produce the active gene product by processing the bacterial precursor.

Since the expression of the ß-lactamase gene in yeast is low, the bacterial promoter was replaced by the yeast ADH1 promoter leading to an 100-fold increase in enzyme activity. This allowed the purification of the mature form of ß-lactamase from yeast crude extracts to apparent homogeneity. No difference with regard to molecular weight and specific activity could be detected when compared to the purified enzyme from E. coli. Furthermore, the amino terminal sequence was identical to that of the bacterial processed enzyme demonstrating that both bacteria and yeast recognize the same cleavage site. The amount of mature ß-lactamase was calculated to be 0.1-0.2 % of total yeast protein, demonstrating that substantial rates of processing of the precursor occurs in yeast. Despite this, the high rate of precursor synthesis led to its accumulation in the cell. Immunoassay and activity staining showed that the precursor protein is present in large excess over the processed form and has a very low specific activity compared to that of the mature enzyme. Current investigations concerning the location of ß-lactamase in the cell and the question whether ß-lactamase in yeast is cotranslationally processed at the yeast endoplasmic reticulum will be discussed.

1418 RECONSTITUTION OF ASYMMETRIC ASSEMBLY OF M13 PROCOAT PROTEIN INTO LIPOSOMES WITH BACTERIAL LEADER PEPTIDASE, Yoshiko Ohno-Iwashita and William T. Wickner, Molecular Biology Institute, University of California, Los Angeles, CA 90024

The leader peptidase of Escherichia coli cleaves a 23-residue leader sequence from M13 procoat to yield mature coat protein in virus-infected cells. We have reconstituted pure leader peptidase into vesicles of E. coli lipids and found that these liposomes are active in the conversion of procoat to coat. Trypsin removes all but 10% of the leader peptidase, yet the vesicles retain nearly full capacity to convert procoat to coat, suggesting that only procoat which inserts across the liposomal membrane is a substrate for leader peptidase. Over 70% of the coat protein produced by these liposomes span the membrane with correct orientation. On the other hand, leader peptide released from procoat is not retained in these liposomes but is recovered in supernatant fraction after centrifugation of the reaction mixture. The rate at which leader peptidase inside protease-treated liposomes cleaves externally-added procoat is comparable to the rate of procoat cleavage by the same amount of leader peptidase in detergent micelles, suggesting a rapid integration of procoat across a liposomal membrane.

1419 **LOCALIZATION OF THE ESCHERICHIA COLI THIOREDOXIN IN AN OSMOTICALLY SENSITIVE COMPARTMENT OF THE CYTOPLASM,** Vincent Pigiet nd Charles A. Lunn, The Johns Hopkins University, Baltimore, MD 21218

Thioredoxin is a ubiquitously distributed protein present in unusually high concentrations per cell, ~2 x 10^4 copies per cell in E. coli. Model studies in vitro have implicated thioredoxins in disulfide redox processes and in particular protein disulfide and reduction. Studies in E. coli are consistent with localization in an osmotically sensitive compartment, but bounded by the inner (cytosolic) membrane as thioredoxin is neither released or is accessible to external probes in spheroplasts. This osmotically sensitive compartment contains only a few proteins and is thought to correspond to the region of the membrane fusion zones (i.e., the Bayer's junctions) implicated in the processing of macromolecular transport outside the cell. The ability of thioredoxin to catalyze protein disulfide exchanges, together with the membrane localization, may suggest a role for thioredoxin in processing of disulfide proteins during export.

1420 POST-TRANSLATIONAL PROCESSING AND THE BIOGENESIS OF THE LAC PERMEASE. Victor A. Fried and Michael E. Ando, St. Jude Children's Research Hospital, Memphis, TN 38101

The lac permease is an integral cytoplasmic membrane protein encoded by the lacY gene in Escherichia coli. The DNA sequence of the lacY gene predicts a primary translation product of 46.5K daltons. We have been studying the biogenesis of the permease in strain T185 which has the lacY gene amplified on a plasmid. Two forms of the permease are induced in this strain and were shown to be related by 2D tryptic fingerprints. One of these forms, B, has an apparent M_r = 30K daltons on 10% SDS PAGE and is the mature permease observed in haploid strains, while the second form, A, is 31.5K daltons. Labeling experiments with N-ethylmaleimide demonstrated that the A form has a lower affinity for substrate than the B form. The ratio of the A to B forms was not constant, and A accumulated with time after induction. Pulse-chase experiments suggest that A may be a precursor of the B form. The accumulation of A could be explained by inhibition of the processing mechanisms by the overproduction of the lacY gene product. We propose that the primary translation product of the lacY gene is rapidly converted to the A polypeptide by a single endoproteolytic cleavage since a 15K dalton polypeptide appears concomitant with A. The post-translational processing of A to the mature B form is not yet clear. We have demonstrated that B migrates anomalously on SDS gels while A migrates with a normal charge-to-mass ratio. It is unlikely that such a change could be due to proteolytic modifications alone. In vivo labeling has demonstrated that B is not modified by acylation, phosphorylation or glycosylation. However, preliminary studies indicate that acetylation may be involved in this final maturation event.

1421 COMPLETE SEQUENCE OF THE ROUS SARCOMA VIRUS env GENE: IDENTIFICATION OF STRUCTURAL AND FUNCTIONAL REGIONS OF ITS PRODUCT, Eric Hunter, J. M. Hardwick, J. Wills, Univ. of Alabama in Birmingham, Birmingham, AL 35294

The nucleotide sequences coding for the envelope glycoproteins of Rous sarcoma virus have been identified by a combination of protein and nucleic acid sequencing. Regions responsible for initiating translocation of the nascent polypeptide across the endoplasmic reticulum (signal peptide) and for anchoring the glycoprotein complex in the viral membrane have been located.

Subcloning of these coding sequences in place of the late region of SV40 has allowed the expression of a normally glycosylated, functionally active glycoprotein complex on the surface of monkey cells (CV-1). Through the use of site directed mutagenesis the role of specific amino acids in the signal peptide, signal peptidase cleavage site and membrane anchor region have been investigated and will be discussed.

1422 GENETIC MAPPING OF THE E. COLI LEADER PEPTIDASE GENE (LEP): A NEW APPROACH FOR DETERMINING THE MAP POSITION OF A CLONED GENE, Pamela A. Silver and William Wickner, Molecular Biology Institute, University of California, Los Angeles, California 90403

The gene for leader peptidase, termed lep, has been mapped to between purI and nadB at minute 54 and 55 on the E. coli chromosome. Mapping involved: (i) cloning the gene into the plasmid pBR322, (ii) transforming the plasmid into a polA strain where it cannot replicate autonomously, (iii) selecting by ampicillin-resistance the rare cell in which the plasmid had recombined into the chromosome and (iv) mapping the chromosomal site of plasmid integration (and thus drug-resistance) by Hfr matings, P1 transduction, and 3-factor crosses. The map postion was confirmed by assay of the enzyme content of cells bearing an F-factor which covered that region of the chromosome.

1423 THE PRECURSOR FORM OF CYTOCHROME P-450scc IS PROCESSED BY ADRENOCORTICAL MITOCHONDRIA AND NOT BY HEART MITOCHONDRIA, Michael R. Waterman and Martha F. Matocha, University of Texas Health Science Center, Dallas, TX 75235

Cytochrome $P\text{-}450_{scc}$ is a mixed-function oxidase located in the inner mitochondrial membrane of adrenocortical mitochondria which catalyzes the side chain cleavage reaction of cholesterol, yielding pregnenolone. This hemoprotein is encoded by nuclear genes and its synthesis is regulated by ACTH. This laboratory has shown that cytochrome $P\text{-}450_{scc}$ is synthesized in vitro as a precursor protein which is 5,500 molecular weight larger than the mature enzyme (49,000 daltons). Addition of bovine adrenocortical mitochondria to total translation products programed by bovine adrenocortical poly A^+-RNA results in disappearance of immunoisolatable $P\text{-}450_{scc}$ presursor and appearance of immunoisolatable mature $P\text{-}450_{scc}$. The newly synthesized, mature form is found to be localized in the mitochondrial fraction and is trypsin-insensitive, while the precursor form is trypsin-sensitive. Addition of bovine heart mitochondria to translation products does not result in the appearance of immunodetectable mature $P\text{-}450_{scc}$ while immunodetectable precursor $P\text{-}450_{scc}$ remains after the processing incubation. However, under the same conditions, both adrenocortical and heart mitochondria can process the precursor form of citrate synthase, a protein common to both tissues. We conclude that cytochrome $P\text{-}450_{scc}$, a steroidogenic enzyme, is processed by mitochondria from steroidogenic tissues where it resides, but not by mitochondria from non-steroidogenic tissues. Whether this results from lack of recognition or lack of specific proteolytic activity or both in non-steroidogenic mitochondria is currently under investigation.

1424 IN VITRO SYNTHESIS AND PROPERTIES OF FUMARATE REDUCTASE OF ESCHERICHIA COLI. Gary Cecchini, Edna B. Kearney, and Robert P. Gunsalus. Univ. of Calif. at San Francisco and VA Medical Center, San Francisco, CA 94121 and Univ. of Calif. at Los Angeles, CA 90024.

Fumarate reductase (FRD) is a membrane-bound flavoenzyme found in anaerobically grown E. coli, where it participates in anaerobic respiration with fumarate as the terminal electron acceptor. In aerobically grown E.coli, this enzyme, which is also capable of catalyzing succinate dehydrogenation, is replaced by the enzyme succinate dehydrogenase (SDH), which is remarkably similar but apparently better adapted for succinate oxidation via the respiratory chain. We have constructed mutants of E.coli deficient in either FRD or SDH. Utilizing an E. coli Hind III plasmid bank and transformation of mutant strains of E.coli we obtained a clone which restored wild type SDH activity to a SDH mutant. Further characterization of the isolated plasmid, termed pGC1002, and measurement of FRD activity revealed that FRD was amplified 20-fold and that excess FRD encoded by the plasmid was able to overcome the aerobic repression of FRD and thus allow complementation of SDH mutants. Utilizing a coupled in vitro transcription/translation system, we found that 4 polypeptides are synthesized by the plasmid, one corresponding to the flavoprotein subunit (72K), one of 26K, and two small peptides of approximately 13K and 11K. Construction of a plasmid containing the genes for only the two large subunits demonstrated that the two small subunits are also required for enzyme activity in membrane vesicles as this plasmid did not amplify FRD activity in the membrane fraction of the organism but gave rise to a slight ($\sim$2-fold) increase in soluble FRD activity. These findings suggest a possible role for the two small peptides in the binding of FRD to the membrane.

1425 IN VIVO BIOSYNTHESIS, PROCESSING AND TRANSPORT OF OPIOMELANOCORTIN PEPTIDES IN RAT BRAIN, Jeffrey F. McKelvy, State University of New York at Stony Brook, New York 11794

Both the pituitary gland and the brain express the gene for opiomelanocortin (ACTH, MSH and opiate) peptides. Unlike the pituitary, however, which releases these peptides to the blood, the brain distributes the peptides throughout the CNS via diverse axonal projections from a single site of origin of cell bodies in the hypothalamus. A central question is whether the brain system processes the common precursor to ACTH and endorphin peptides (proopiomelanocortin, POMC) so as to deliver the same peptides to all target sites in the brain. We have approached this question by infusing ^{35}S-met and ^{3}H-lys in vivo, via osmotic minipumps, into the site of origin of POMC cell bodies, and analyzing the labeled peptides delivered to specific sites in the CNS (hippocampus, septum, paraventricular nucleus, preoptic area). Peptides were characterized by immunoprecipitation, HPLC, sulfoxidation and double-label tryptic mapping. Evidence was obtained for a differential distribution of opiate vs ACTH-related peptides to different brain sites. Moreover, the 31K common precursor (POMC) was transported intact to sites considerable distances from its site of synthesis. These results suggest that POMC processing and transport may be under several regulatory influences in the brain and that the transport of precursor polypeptide may be of functional significance.

1426 TRANSPORT OF PROTEINS ACROSS THE MITOCHONDRIAL OUTER MEMBRANE, Gordon C. Shore[1], Carole Argan[1], Carol J. Lusty[2], and Marco Colombini[3], McGill University[1], Montreal, Public Health Research Institute[2], N.Y., University of Maryland[3], MD.

We are investigating components of outer mitochondrial membrane which are required for recognition and/or uptake of incoming cytoplasmic precursor proteins destined for interior compartments of rat heart mitochondria. Experiments which were based on applying exogenous perturbations to intact mitochondria, e.g., washes with high concentrations of KCl, controlled proteolysis by phospholipase-free chymotrypsin or trypsin, reacting membrane components with difluorodinitrobenzene, and treating mitochondria with anti-sera directed against outer membrane proteins, showed that at least for the precursor (M_r 39,000) to the liver mitochondrial matrix enzyme, ornithine carbamyl transferase (M_r 36,000), recognition and/or uptake by heart mitochondria in vitro is mediated by intrinsic protein(s) which is partially exposed at the surface of the organelle. Although the total protein composition of mammalian outer mitochondrial membrane is complex, the complexity is greatly reduced when visualized on SDS-polyacrylamide gels following covalent incorporation of a fluorescing photoaffinity probe, azidopyrene, which labels only those regions of polypeptides embedded in the lipid bilayer: a protein, or perhaps family of proteins, of M_r 32,000 constitutes a major fraction of the intrinsic protein population. At least part of this fraction is made up of a polypeptide which forms a gated pore, 20-30A° in diameter, in outer mitochondrial membrane. When the complex (designated VDAC) was purified and inserted into a synthetic lipid bilayer, it was found to interact with apocytochrome c (the imported form of cytochrome c) but not with holoenzyme.

1427 SELECTIVE PREMATURE TERMINATION OF GROWTH HORMONE (GH) AND PROLACTIN (PRL) SYNTHESIS IN THE RAT PITUITARY, Neal H. Scherberg, Kimberly Barokas, Gabriel Tsuboyama and Samuel Refetoff, University of Chicago, Chicago, IL 60637.

The regulation of translocated protein maturation by endogenous processing was investigated by exploiting the rapid transfer of labeled amino acids from amino-acyl tRNA to nascent protein. 20% of ^{125}I-tyrosine was transferred from acylated tRNA into protein in 3 min at 28°C in homogenates of rat anterior pituitary. GH and PRL were principal labeled products accounting for 10% of protein-bound isotope respectively in tissue from male or females. When creatine phosphate (CP) was omitted, total transfer of isotope fell by two-thirds. Phosphoenolpyruvate could replace the CP requirement but nucleotide triphosphates could not: 1.5 mM ATP added in the absence of CP was more than 50% metabolized in 2 min and did not stimulate the incorporation of ^{125}I-tyrosine. The formation of GH and PRL was completely blocked in the absence of CP but the incorporation of isotope into higher weight proteins continued at a reduced rate. In the minus CP condition, 5 proteins in the male and 4 proteins in the thyroidectomized, GH-deficient female accumulated in the 9 - 22 x 10^3 daltons range. Incorporation into the same proteins preceded GH or PRL completion in the presence of CP. The kinetics, cross reactivity with antisera and absence of the male set from a GH-deficient pituitary imply a relation of the low weight proteins to the mature GH and PRL. GH messenger was stable in homogenates incubated with or without CP ruling out specific hydrolysis of the mRNA. The results suggest the existence of specific pauses in the synthesis of GH and PRL which become premature termination points when the ATP concentration is diminished.

1428 IMMUNOELECTRON MICROSCOPIC LOCALIZATION OF A SECRETORY PROTEIN IN BACTERIA, Bijan K. Ghosh, Guan Tinglu and Arati Ghosh. UMDNJ-Rutgers Medical School, Piscataway NJ 08854

We aim to visualize the location of alkaline phosphatase (APase), a secretory protein, in Bacillus licheniformis 749/C. Colloidal gold particles (150Å) were saturated with APase specific IgG. The frozen thin sections of mildly fixed cells (0.6% aldehyde at 4°C) were labelled with the above IgG-gold complex. This label was distributed in the membrane (Figs. 1-4), cytoplasm and wall (Fig. 5). The membrane contained 70% of the label. The label was distributed on the inside surface (Figs. 1,2), outside surface (Fig. 3) and within the membrane interior (Fig. 4). The gold label was mostly seen in clusters in association with the inside membrane surface. The labelling was quantified by stereological analysis of the labelled cryo-thin sections. The number of golds present per μ^2 section surfaces were as follows: derepressed cell, 11; derepressed cell pretreated with anti-APase IgG, 3; derepressed cell after 70% removal of the APase, 3; repressed cell, 1. The labelling was APase specific because, anti-APase IgG treatment blocked the binding, APase extraction diminished the binding and practically no binding was seen in cells lacking the APase (repressed cell). Binding of the gold in cluster to the inside membrane surface suggested the presence of specific APase binding sites which could be defined as membrane receptors (NSF PCM 8110613).

1429 ISOLATION AND PROPERTIES OF LEADER PEPTIDASE FROM E. coli, Paul B. Wolfe and William Wickner, University of California, Los Angeles, CA 90024

Leader peptidase from Escherichia coli has been purified to homogeneity from a plasmid-bearing strain which overproduces the enzyme up to 100-fold. It is a single 37000 dalton polypeptide found in both the inner and outer membranes. Leader peptidase is a transmembrane protein of the inner membrane of overproducing strains. The majority of the polypeptide chain is exposed on the outer surface of the inner membrane with a small segment exposed to the cytoplasm. This orientation agrees with the functional orientation of leader peptidase in reconstituted vesicles. The nucleotide sequence of the leader peptidase gene has been determined. It codes for a protein of 323 amino acids with a calculated molecular weight of 35,994 daltons. The deduced amino acid composition matches the composition of the purified enzyme and the size of purified leader peptidase on SDS-polyacrylamide gels is the same as leader peptidase synthesized in a cell-free translation system. These results suggest that leader peptidase assembles into the membrane without proteolytic processing.

Intracellular Sorting of Proteins

1430 MONENSIN INHIBITS RECYCLING OF MANNOSE RECEPTORS IN MACROPHAGES, Philip Stahl, Thomas Wileman, Rita Boshans and Paul Schlesinger, Washington University Medical School, St. Louis, MO 63110

Monensin, a carboxylic ionophore which mediates proton movement across membranes, blocks receptor-mediated uptake of ^{125}I-β-glucuronidase (^{125}I-β-gluc) by macrophages. Inhibition is concentration dependent- 50% inhibition ~1 μM. The results indicate that monensin inhibits receptor recycling. (i) Ligand binding to macrophage receptors is unaffected by monensin, (ii) inhibition of uptake of ^{125}I-β-gluc by monensin is time dependent, and (iii) internalization of receptor-bound ligand, following warming to 37°, is unaffected by monensin. The inhibition of ^{125}I-β-gluc uptake by monensin is partially dependent upon extracellular Na^+. Lysosomal pH, as measured by the fluorescein-dextran method, was increased by monensin and increased lysosomal pH produced by monensin correlated with inhibition of ^{125}I-β-gluc uptake. Similarly, removal of extracellular Na^+ reduced the lysosomal pH response to monensin. Binding studies with cells permeabilized with saponin, indicate that macrophages have an intracellular pool of binding sites which is approximately 5-fold greater than cell surface pool. Incubation of macrophages with monensin at 37° results in a substantial reduction in cell surface binding activity. However, total binding activity measured in the presence of saponin is only minimally affected by monensin treatment. These results suggest that monensin brings about a redistribution of binding sites from cell surface to some intracellular location and blocks receptor movement by neutralizing acid intracellular vesicles.

1431 EFFECTS OF MONENSIN ON THE INTRACELLULAR TRANSPORT OF HLA-DR ANTIGENS. Carolyn E. Machamer and Peter Cresswell, Duke Univ. Med. Ctr., Durham, NC 27710

HLA-DR antigens are comprised of two noncovalently associated glycoprotein chains (α and β) on the surface of B lymphocytes, monocytes, and a few other cell types. We have shown that a third chain, the invariant (I) chain, associates transiently with HLA-DR α and β chains during biosynthesis, presumably dissociating from the mature α-β complexes before or shortly after the complex reaches the cell surface. It is thought that the I chain may be important in α-β chain association or in transport of the complex to the cell surface. We have studied the biosynthesis and glycosylation of the I chain in human B lymphoblastoid cells utilizing pulse/chase labeling and two-dimensional gel electrophoresis. The I chain possesses two N-linked oligosaccharide side chains and probably two O-linked oligosaccharide cores as well, at least one of which has the structure Galβ1→3GalNAc-α-O-Ser/Thr, determined by sensitivity to endo-α-N-acetylgalactosaminidase after removal of sialic acid residues. Treatment of cells with the carboxylic ionophore monensin significantly alters the post-translational processing of the α-β-I complex. The rate of processing of the N-linked oligosaccharides on both the α and I chains from high mannose to the complex form is delayed as determined by acquisition of resistance to endoglycosidase H. At least one of the O-linked carbohydrate units on the I chain is never added, and the total number of sialic acid residues added to all three chains is reduced. In addition, the rate of dissociation of the I chain from the α-β chain complex is much reduced in monensin-treated cells. The alterations in glycosylation may be responsible for this decreased dissociation.

1432 AN ALTERNATIVE ROUTE FOR SECRETORY PROTEINS IN THE EXOCRINE PANCREAS, Adrien R. Beaudoin, André Vachereau and Pierre St-Jean, Centre de recherche sur les mécanismes de sécrétion, Département de biologie, Faculté des sciences, Université de Sherbrooke, Sherbrooke, P.Q., Canada, J1K 2R1

Two lines of evidence support the concept that secretory proteins are released from distinct cellular compartments under resting and stimulated conditions. In pigs, the specific activity of amylase released in pancreatic juice under resting condition is twice that found in the zymogen granule compartment from the same animal. Pulse and chase experiments also show marked disparities in the specific radioactivities of amylase (dpm/unit of activity) in resting secretion and in the zymogen granule fraction of the same pancreas. It is suggested that after synthesis and segregation in the rough endoplasmic reticulum, a fraction of the smooth vesicles are directed to the luminal membrane and thereby bypass the zymogen granule compartment. The residual fraction would follow the route described in Palade's model.

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1433 MEMBRANE AND SECRETORY μ CHAINS ARE SYNTHESIZED AND PROCESSED SEPARATELY IN A B LYMPHOBLASTOID CELL LINE, Linda Hendershot and Daniel Levitt, University of Alabama in Birmingham, Birmingham, AL 35294 and LaRabida-University of Chicago, Chicago, IL 60649.

Differential processing of the immunoglobulin μ heavy chain gene transcript results in two mRNA species that share the same 5' sequence but have distinct 3' sequences. These two messages code for the membrane-associated (μm) and secretory (μs) forms of IgM. Daudi, a B cell line derived from a Burkitt's lymphoma, produces both types of μ heavy chains. The μm is expressed on the surface membrane, but the μs remains in the cell cytoplasm (a situation also observed in normal B lymphocytes). We have found that the kappa light chains produced by these cells are preferentially associated with μm heavy chains. This preferential association is not due to the rapid degradation of μs ($t_{1/2}$=24 hr). While the μm heavy chains are completely glycosylated, the μs chains receive only core mannose sugars. When homogenized Daudi cells were treated with trypsin, the μs heavy chains were degraded but not the μm heavy chains. This finding suggests that only the μm proteins are transferred across the endoplasmic-reticulum even though both proteins should share the same N terminal sequence. Thus, two closely related μ chain proteins may be synthesized in different cellular compartments; the site of synthesis could therefore be an important regulatory mechanism during B cell differentiation.

1434 TWO TYPES OF GLYCOPROTEIN PRECURSORS ARE ENCODED BY THE SIMIAN ROTAVIRUS SA11, Brad L. Ericson, David Y. Graham, Bruce B. Mason, Henry H. Hanssen and Mary K. Estes, Baylor College of Medicine, Houston, TX 77030

The rotavirus genome codes for two glycoproteins: an outer capsid structural glycoprotein (VP7, apparent mol. wt. 38K) and a nonstructural glycoprotein (NS28K). The synthesis of these glycoproteins was analyzed in infected cells and in a cell-free system derived from rabbit reticulocyte lysates supplemented with dog pancreatic microsomes. The two rotavirus glycoproteins and their precursors were compared by immunoprecipitation, comparative peptide mapping, and digestion with beta-N-acetylglucosaminidase H (Endo H). The data showed that a 37K product synthesized in the cell-free system is the precursor to the 38K glycoprotein and that the 37K polypeptide contains a cleavable signal sequence (1.5K). The 37K polypeptide was glycosylated in vitro in the presence of microsomes to a polypeptide of 38K that was immunoprecipitated by monospecific antiserum to VP7. Endo H digestion of the 38K polypeptides from either infected cells or the cell-free system produced polypeptides of identical molecular weight, 35.5K. This 35.5K polypeptide represents the glycoprotein precursor lacking the signal sequence. These results were confirmed by comparative studies with a variant of SA11 that is defective in glycosylation of VP7. Similar experiments with the 20K precursor to the 29K nonstructural glycoprotein showed that the 20K polypeptide contains a noncleavable signal sequence. The synthesis of both the 38K and 29K glycoproteins required the presence of membranes, although glycosylation of the precursors was not necessary for membrane insertion. Both the 29K and the 38K glycoproteins were protected from trypsin digestion while in infected cell microsomal membranes. The 38K glycoprotein made in vitro in the presence of membranes was similarly protected, suggesting that the glycoproteins are extruded into membrane vesicles and glycosylated during synthesis.

1435 THE NATURE OF INTRACELLULAR μ CHAINS IN PRE-B CELLS, Yair Argon, Giovanni Galfre and Cesar Milstein, MRC Laboratory of Molecular Biology, Cambridge, U.K.

We have derived three fetal liver cell lines, transformed with Abelson murine Leukemia virus, which have properties analogous to pre-B cells: they produce immunoglobulin μ chains but not light chains, and the μ chains are not secreted nor are they expressed on the cell surface. The μ chains are synthesized on membrane-bound polysomes and are glycosylated to the high-mannose form. No terminally glycosylated μ chains can be detected by Endoglycosidase H resistance nor by labelling with ^{3}H-galactose. The μ chains are degraded with a half-life of 2-3 hours. They do not form covalent dimers nor larger aggregates, as do several myeloma heavy chains. Localization of μ chains by double-label immunofluorescence shows codistribution with endoplasmic reticulum (ER) markers but not with Golgi complex markers. These results suggest that in the absence of light chains, μ chains are not transported from the ER to the Golgi complex, and are degraded mostly in the ER. Since the cells express several B lineage surface markers and release viral particles to the medium, their intracellular transport machinary seems intact and the failure is apparently limited to Ig expression. The amount of μ protein or μ mRNA in all three cell lines is some 50-fold less than in an IgM secreting myeloma. mRNAs for both secreted μ and membrane μ are found and both can be translated in vitro. However, in vivo we can only detect one type of μ polypeptide, which by differential detergent extraction is membrane μ. A post-transcriptional control may, therefore, exist in pre-B cells to ensure that upon expression of light chain the cell will direct the resulting IgM to the surface, to act as antigen receptor.

1436 THE EFFECT OF MANNOSE 6-PHOSPHATE ON THE TURNOVER OF THE GLYCOSAMINOGLYCANS AND PROTEINS OF THE EXTRACELLULAR MATRIX, Calvin F. Roff and John L. Wang, Department of Biochemistry, Michigan State University, East Lansing, MI 48824

Human fibroblasts (SL66) were cultured in $^{35}SO_4^{-2}$ to label the glycosaminoglycans (GAGs). These labeled cells were then chased in the presence and absence of mannose 6-phosphate (M6P) and the distribution of radioactivity was analyzed in terms of three arbitrary fractions: (a)extracellular - radioactivity higher in cultures without M6P than in cultures with M6P; (b)pericellular - radioactivity lower in cultures without M6P than in cultures with M6P; and (c)intracellular - no difference between the two cultures. This effect was restricted to M6P, glucose 1-phosphate, and a M6P-containing phosphomannan. No difference in $^{35}SO_4^{-2}$ label distribution was observed when the experiment was performed with I cells, which lack lysosomal enzymes (LEs) carrying the M6P marker. However, the addition of LEs derived from normal cells to $^{35}SO_4^{-2}$-labeled I cells restored the M6P effect. Chromatographic analysis of the culture fractions after digestions with pronase and chondroitin ABC lyase revealed distinct molecular differences. The extracellular GAGs of M6P-treated cultures were of lower molecular weight than those from control cultures. The pericellular compartment of M6P-treated cultures showed material not found in control cultures. We have also observed differences in the SDS-PAGE profiles of ^{35}S-methionine labeled proteins derived from cultures treated with and without M6P. The medium of cultures chased with M6P does not contain the full complement of major polypeptides found in control cultures. These results suggest that one possible function of cell surface receptors recognizing the M6P moiety of LEs is to anchor certain of these enzymes proximate to their substrates at the cell surface.

1437 FATTY ACID ACYLATED PROTEINS IN SECRETORY (SEC) MUTANTS OF YEAST. Milton J. Schlesinger and Duanzhi Wen, Washington Univ. Sch. of Med. St. Louis, MO. 63110.

Four proteins can be labeled when ^{3}H-palmitic acid is added to sec mutants of yeast that accumulate endoplasmic reticulum (ER) membranes (Novick, P., Ferro, S. & Schekman, R. Cell 25: 461, 1981). The two higher mol. wt. proteins ($M_\gamma \cong 50$ kd and 150 kd) are asparagine-linked glycoproteins, based on their sensitivity to endo-glycosidase H (endo H) and to tunicamycin (TM). The lower mol. wt. protein ($M_\gamma \cong 20$ kd) does not label with ^{3}H-palmitic acid in TM-treated cells and the label is released by endo H. We tentatively postulate that fatty acid is attached to asparagine-linked oligosaccharide in this protein - possibly a new kind of glyco-lipid structure. The fourth protein ($M_\gamma \cong 30$ kd) is insensitive to endo H and TM, and is detected as a higher mol. wt. form (33 kd) in sec mutants accumulating golgi membranes and in those accumulating vesicles. None of these proteins are labeled to a significant extent with ^{3}H-palmitic acid in wild type yeast, in sec mutants grown at permissive temperatures, or in a sec mutant that blocks protein transport into the ER. Thus, three of these proteins probably reside at low levels in the normal yeast ER and one is localized to the vesicles. The nature of fatty acid linkage and the isolation of the protein components from intracellular membranes is under investigation.

1438 CHANGES IN THE CYTOPLASMIC SEQUENCE OF VSV G PROTEIN ALTER ITS RATE OF TRANSPORT TO THE PLASMA MEMBRANE, John K. Rose, John E. Bergmann+, Carol J. Gallione, and Robert Z. Florkiewicz, The Salk Institute, P.O. Box 85800, San Diego, Ca. 92138, and +Department of Biology, University of California, San Diego, La Jolla, Ca. 92093

The glycoprotein (G) of vesicular stomatitis virus (VSV) is inserted into the rough endoplasmic reticulum (RER) as a nascent chain and becomes anchored in the membrane at a hydrophobic sequence near the COOH-terminus. A highly charged (basic) COOH-terminal sequence of 29 amino acids protrudes on the cytoplasmic side. To determine if the cytoplasmic domain of G protein affects its transport to the plasma membrane we have introduced deletions removing the DNA sequences encoding this domain. These modified DNAs were expressed under control of the SV40 late promoter. All deletions which completely remove the cytoplasmic tail and most or all of the hydrophobic transmembrane domain generate proteins which are processed very slowly (relative to G protein) to an endoglycosidase H (endo H) resistant form. Once converted to an endo H resistant form the proteins are secreted rapidly. Two deletions removing over half of the cytoplasmic tail produce proteins with slow rates of processing. These proteins are transported to and remain anchored in the plasma membrane. Another deletion which removes only the two COOH-terminal amino acids and adds eight additional residues produces a protein which is not processed to an endo H resistant form. This protein is neither secreted nor transported to the cell surface. We conclude that the cytoplasmic domain of a transmembrane protein can influence its rate of transport from the RER to the plasma membrane and that the rate limiting step occurs at or prior to the time that endo H resistance is acquired.

1439 STABLE EXPRESSION OF VESICULAR STOMATITIS VIRUS GLYCOPROTEIN GENES IN EUCARYOTIC CELLS, Robert Z. Florkiewicz, Andrew Smith, John E. Bergmann+, and John K. Rose, The Salk Institute, P.O. Box 85800, San Diego, Ca. 92138, and +Department of Biology, University of California, San Diego, La Jolla, Ca. 92093

We have obtained stable expression of the vesicular stomatitis virus (VSV) glycoprotein (G) and a truncated form of G protein (TG) using a transforming fragment of the bovine papilloma virus (BPV) genome linked to the double-stranded cDNAs encoding the VSV G proteins. Transcription signals from the SV40 early region were included to direct expression of the cDNAs. Foci from mouse cells transformed with these DNAs were picked and cloned in soft agar. G and TG protein synthesis, processing, and transport were similar to what we observed previously during transient expression of these genes in COS-1 cells. G protein is processed rapidly to an endoglycosidase H resistant form and becomes anchored in the plasma membrane. However, in contrast to transient expression, a large fraction of the G protein appears to be cleaved (presumably near the COOH-terminus) and released from the cell surface. The TG protein (which lacks the COOH-terminal cytoplasmic and transmembrane domains) acquires endoglycosidase H resistance slowly (compared to G protein) and is also secreted slowly from the cells. Both biochemical and immunofluorescence data suggest that transfer from the endoplasmic reticulum to the Golgi apparatus is the rate limiting step for secretion of the truncated G protein.

1440 CELLULAR LOCALIZATION OF THE MAJOR EXCRETED PROTEIN OF TRANSFORMED MOUSE FIBROBLASTS, Susannah Gal, Mark C. Willingham and Michael M. Gottesman, Laboratory of Molecular Biology, National Cancer Institute, Bethesda, Md. 20205

Mouse fibroblasts transformed by a variety of agents, treated with phorbol ester tumor promoters or certain growth factors synthesize and secrete increased amounts of a phosphoglycoprotein of molecular weight 35,000 daltons designated MEP(Gottesman, M.M., Proc. Natl. Acad. Sci., 75: 2767, 1978). The phosphate in this protein has been found to be predominantly sugar-linked in the form of Man 6-phosphate(Sahagian, G.G. and Gottesman, M.M., J. Biol. Chem., 257: 11145, 1982). Since Man 6-phosphate has been implicated as the lysosomal recognition marker, we explored the subcellular localization of MEP using microscopic and biochemical analyses. In transformed cells, MEP is found predominantly in the Golgi apparatus and lysosomes as determined by indirect immunofluorescence and electron microscopic localization of affinity purified anti-MEP antibody. Immunoprecipitation of transformed cell extracts indicates the presence of at least two other lower molecular weight immuno-reactive forms of MEP. Pulse-chase experiments suggest that these proteins are related via a precursor-product relationship. Fractionation of subcellular organelles on a self-forming Percoll gradient indicates that the lower molecular weight forms of MEP predominate in the lysosomes while the secreted form is localized primarily in the Golgi region of the gradient. These results suggest that MEP might be a lysosomal protein which is overproduced in response to transformation and other growth stimuli.

1441 LEVELS OF SERUM RETINOL-BINDING PROTEIN (RBP) in the CIS- AND TRANS-COMPARTMENTS OF THE GOLGI APPARATUS FROM THE LIVERS OF CONTROL AND VITAMIN A-DEPLETED RATS, John Edgar Smith and Caroline E. Handler, Pennsylvania State University, University Park, PA 16802. Vitamin A circulates in blood bound to a specific transport protein, RBP, which is synthesized in the liver. This study was undertaken to explore the role of the cis- and trans-Golgi compartments in the marked reduction of RBP secretion that occurs in vitamin A-deficiency. Control and vitamin A-depleted rats were given an oral dose of ethanol. After 90 minutes their livers were fractionated by the method of Ehrenreich et al. (J. Cell Biol. 59:45-72, 1973). As expected the serum RBP levels had dropped in the depleted rats (control, 55 ± 8 ug/ml; depleted, 15 ± 1), while their liver RBP levels had increased (control, 124 ± 10 ng/mg protein; depleted, 1043 ± 6), The relative specific activity (specific activity of fraction/specific activity of homogenate) of RBP in the fractions enriched in the various compartments of the Golgi apparatus were: GF_3(cis-Golgi), control 5 ± 1, depleted 4 ± 1; GF_2 (trans-Golgi), control 10 ± 2, depleted 3 ± 1; GF_1 (secretory vesicles), control 20 ± 5, depleted 3 ± 1. From these data we conclude that the cis-Golgi can discriminate between apo-RBP (RBP without retinol) and holo-RBP (RBP with bound retinol) and that the delivery of RBP to the trans-Golgi compartment is greatly reduced if it does not contain bound retinol. By this mechanism apo-RBP would be retained within the liver cell, while holo-RBP was passed to the trans-Golgi compartment to be prepared for secretion from the cell. (Supported in part by BRG Grant 07082-16 awarded by the Biomedical Research Grant Program, Division of Research Resources, National Institutes of Health).

1442 RECEPTOR CONTROL OF NEUTROPHIL FUNCTION, P. M. Lad, M. M. Glovsky, P. A. Smiley and D. B. Learn, Kaiser-Permanente Regional Research Laboraotry, 4953 Sunset Blvd., Los Angeles, CA 90027

The neutrophil plasma membrane (PM) is a structural participant in protein internalization and lysosomal enzyme secretion. Receptors in the membrane inhibit (PGE_1, βadrenergic) and promote (f-met-leu-phe (FMLP) and C5a) these processes by mechanisms which involve cyclic nucleotides (cAMP, cGMP). We have isolated an hormonally sensitive PM, and have probed both its structural characteristics and the relationship of membrane receptors to enzymes of cyclic nucleotide metabolism. Cells were lysed by brief sonication with minimal lysosomal breakage and fractionated on sucrose and dextran gradients. Contamination of PM with granule (G) and nuclear (N) components, as assessed by enzymatic and SDS gel analysis, was minimal. Little PM was lost to G or N enriched fractions. Actin and myosin were associated with the membrane in amounts up to 30% membrane protein while ^{125}I Con-A overlay of the gels revealed glycoproteins at 162K, 90K, and 58K. The 162K protein comprised ~3% of the membrane. Study of the regulatory parameters showed that adenylate cyclase (AC) was activated by PGE_1, cholera toxin (A_1), and isoproterenol with the same order of potency as observed for inhibition of enzyme secretion. In contrast to the nucleotide dependent linkage of PGE_1 and β receptors to AC, FMLP and C5a gave no activation. However, a study of guanylate cyclase revealed activation by muscarinic cholinergic agonists and FMLP. Our results provide the basis for detailed study of membrane-cytoskeleton interactions, glycoprotein internalization and receptor related transduction mechanisms in the human neutrophil.

1443 THE PROTON AND CALCIUM MILIEU OF THE GOLGI AND SECRETORY VESICLE SYSTEMS OF MAMMARY ALVEOLAR CELLS. Margaret C. Neville and Patricia A. Staiert, University of Colorado School of Medicine, Denver, CO 80262.

Milk proteins are processed in the Golgi and secretory vesicle systems of the mammary alveolar cell. In addition lactose synthesis and the formation of the casein micelle take place within these vesicles. Many of the reactions involved require millimolar concentrations of calcium. We have studied lactose synthesis in intact Golgi and secretory vesicle systems obtained by gentle homogenization of diced mammary glands from lactating mice. Lactose synthesis was monitored by addition of [^{14}C]glucose and UDP-galactose to the vesicle preparation, incubation at 37°C for 2 to 10 minutes and separation of the vesicles on membrane filters. The reaction was linear for at least 10 minutes; similar results were obtained when [^{14}C]UDP-galactose was used as the radioactive species. Lactose synthesis was abolished by the addition of A23187, a calcium ionophore, and EGTA to the vesicle system and could be restored to 60% of the initial level by addition of millimolar concentrations of free calcium.

Mammary alveoli, dissociated with collagenase and incubated with 25 μM acridine orange for 10 min or longer, showed a bright orange reticular network when viewed by fluorescence microscopy with a K 510 suppression filter. Acridine orange, a weak base, is concentrated under the influence of the pH gradient in acidic vesicles and fluoresces orange. The orange fluorescence was abolished by 10 μg/ml monensin or 10 μM tetrachlorosalicylanilide.

These experiments suggest that the internal milieu of the Golgi apparatus and secretory vesicle systems of the mammary alveolar cell has both an acid pH and a calcium concentration in the millimole range. Supported by NIH grant HD 14013.

1444 SYNAPTIC VESICLES AND THE SYNAPTIC CLEFT CONTAIN AN IDENTICAL PROTEOGLYCAN. Steven Carlson, Kathleen Buckley & Regis B. Kelly. Dept of Biochem & Biophys, Univ of California, San Francisco, Ca 94143 USA

Although exocytosis involves the fusion of a secretory vesicle membrane with a plasma membrane, the protein composition of the two membranes is thought to be different. We have tested this hypothesis using nerve terminals from the electric organ of marine rays. Antibodies that bind to the external surface of the nerve terminal can be identified because they allow selective immunoadsorption of synaptosomal contents (Miljanich et al. (1982). J. Cell Biol. 94, 88). We have used immunoadsorption to screen a library of monoclonal antibodies raised to electric organ nerve terminals by Drs. Kushner and Reichardt (UCSF). Of the 16 positives, 15 show no detectable binding to synaptic vesicles. The 16th, mAb70, binds strongly to synaptic vesicle membranes. The antigen recognized by mAb70 is a proteoglycan-like molecule associated with the luminal surface of the vesicle membrane. Since it is the major unique antigen in synaptic vesicles, we had already purified the proteoglycan-like molecule. It contains a heparin-like glycosaminoglycan, has approximately equimolar amounts of uronic acid and N-acetylglucosamine and a total molecular weight of about 200 kd, about 30% of which is carbohydrate. The location of the antigen on the outside of nerve terminals was determined by immunoelectron microscopy using an HRP-labeled second antibody procedure. Binding of mAb70 was restricted to the synaptic cleft. An attractive interpretation of this result is that since synaptic vesicles only insert in the membrane at the synaptic cleft, they are vehicles for the insertion of cleft-specific extracellular matrix.

1445 SUBCELLULAR LOCALIZATION OF UDP-GLUCOSE:GLYCOPROTEIN GLUCOSE-1-PHOSPHOTRANSFERASE. Lillian A. Koro and Richard B. Marchase, Duke Univ. Medical Center, Durham, NC 27710

UDP-glucose:glycoprotein glucose-1-phosphotransferase (GlcPTase), an enzyme capable of transferring glucose-1-phosphate from UDP-glucose to endoglycosidase H-sensitive oligosaccharide chains, has been localized to a Golgi-enriched fraction of porcine liver cells. Our studies have utilized a doubly labelled β(^{32}P)UDP-(^{3}H)glucose and high mannose-type oligosaccharides derived from ovalbumin to quantitate the amount of GlcPTase present in the various subcellular fractions arising from sucrose gradient fractionation of liver homogenates. We have also characterized the electrophoretic profiles of the endogenouus acceptor glycoproteins of liver and other tissues and determined that acceptors for this enzyme are tissue-specific. In previous work (Koro and Marchase, Cell 30: In Press, 1982) we have demonstrated that the GlcPTase acceptor oligosaccharides derived from chick neural retina glycoproteins are similar to oligosaccharides from glycoproteins of neural retina bound to the cell surface through their interaction with the filamentous baseplate protein ligatin. This interaction of cell-surface glycoproteins with ligatin is mediated via terminal glucose-1-phosphates on their oligosaccharides. The GlcPTase we have described is similar to but distinct from the N-acetylglucosamine-1-phosphotransferase described by Reitman and Kornfeld (J. Biol. Chem. 256: 4275, 1981). We propose that GlcPTase also functions in the Golgi as a controlling enzyme in intracellular sorting of newly synthesized proteins, marking a particular subclass of glycoproteins for localization to the cell surface. (Supported by NIH grants NS 06233 and EY 04480).

1446 HVEM SHOWS A NETWORK LINKING STRUCTURES LIKELY INVOLVED IN THE TRANSPORT AND POSITIONING OF SYNAPTIC COMPONENTS, William L. Klein, and Tiffany Tsui, Northwestern University, Evanston, Illinois 60201. Cellular and molecular events underlying the assembly of synapses are poorly understood, especially for neurons of the central nervous system. A high degree of ultrastructural interaction seems necessary to orchestrate the proper transport and positioning of synaptic constituents. However, conventional EM analysis of differentiating neurites and their growth cones has shown little evidence for interactions between the abundant cytoskeletal and membranous components. In order to gain a better picture of differentiating neurites, we have examined the growth cones of cultured CNS neurons using high voltage electron microscopy (HVEM). HVEM gives a three-dimensional perspective of all cytoplasmic structures within a subcellular region, and examination of whole-mount specimens, rather than embedded and sectioned preparations, provides remarkable resolution. Our examination of growth cones has revealed a higher level of organization than previously seen, showing abundant linkages between smooth endoplasmic reticulum, microtubules, and filaments. Linkages between smaller vesicles via a filamentous network also are apparent throughout the growth cone but especially near the surface membrane. Prevalent structural interconnections between organelles may have important implications for the mechanisms of growth cone extension and retraction, for membrane assembly and pinocytosis, and for transmitter release.

1447 INTRACELLULAR PROCESSING OF HLA-DR OLIGOMERS, Vito Quaranta and Ralph A. Reisfeld, Research Institutes of Scripps Clinic, La Jolla, CA 92037

Biosynthesis of the human Class II histocompatibility antigens, HLA-DR, is an intriguing model for intracellular sorting processes. HLA-DR molecules, expressed at the surface of immune cells as two-chain (α,β) integral membrane heterodimers, apparently originate from transmembrane intracellular three-chain (α,β,γ) oligomers. A new monoclonal antibody, CY1, identifies a family of as many as three γ chains that are individually translated in a reticulocyte system driven by mRNA from the B lymphoid cell Raji, in the absence of microsomes. Pulse-chase experiments with Raji cells, followed by immunoprecipitation and two-dimensional electrophoretic analyses, show that these γ chains are all involved in HLA-DR biosynthesis due to their intracellular association with α and β chains. However, syntheses and association of α, β and γ chains are not synchronized. Pulse-labeled γ chains do not associate with α and β chains until 60 min after translation. During this time, pulsed α and β chains undergo glycosylation steps and transport in association with "cold" γ chains. These findings suggest that labeled γ chains are post-translationally placed on stand-by in an intracellular pool, and are utilized in an orderly fashion, only after pre-existing "cold" γ chains have exited the pool. An oriented pool is an unusual finding in glycoprotein oligomer assembly. In long-term chases, the γ chains immunoprecipitable by CY1 dissociate from HLA-DR oligomers and tend to disappear, while α-β dimers are inserted in the surface membrane. The lysosomotropic agents NH_4Cl and chloroquine cause a build-up of γ chains, suggesting that lysosomal degradation is involved in γ chain processing.

1448 SOMATOSTATIN DISCRIMINATES BETWEEN THE INTRACELLULAR PATHWAYS OF SECRETORY AND MEMBRANE PROTEINS, R.Green and D.Shields, Albert Einstein Coll.of Medicine,Bronx,N.Y.10461

Somatostatin (SRIF) is a 14-amino acid peptide that inhibits the secretion of a variety of polypeptide hormones. This suggests that SRIF should be a useful reagent to dissect late events in the secretory pathway. These experiments were designed to determine a)where in the secretory pathway SRIF acts, and b)if SRIF discriminates in its inhibition between secretory and plasma membrane proteins.

Growth hormone (rGH)-secreting cells (GH_3) were infected with VSV and pulse-chased with ^{35}S-met to follow the simultaneous intracellular transit of equivalent amounts of a secreted protein (rGH) and an integral membrane glycoprotein (VSV G). Secretion of rGH was monitored by immunoprecipitation, while appearance of G in the plasma membrane was detected by the TNBS/anti DNP cell surface labelling procedure, as well as by its recruitment into virions.

When SRIF (10ug/ml) was included in the chase, the secretion of pulse-labelled rGH was inhibited by 75%. G protein, however, appeared in the plasma membrane and in virions at control levels. In contrast, parallel exposure to the ionophore monensin (2×10^{-7}M) caused a dramatic inhibition, at all time points, of both secretion of rGH and incorporation of G into plasma membrane and virions.

The differential sensitivity of G and rGH to SRIF provides evidence for sorting of secretory and membrane proteins into physically or functionally distinct compartments. The data further suggest that this sorting occurs distal to the site of monensin arrest but proximal to the site of SRIF action. (Supported by ACS and NIH grants.)

1449 SUBCELLULAR LOCALIZATION OF THE SYNTHESIS AND GLYCOSYLATION OF CHONDROITIN SULFATE PROTEOGLYCAN CORE PROTEIN. N.B. Schwartz and G. Habib, Univ. of Chicago, Chicago, IL 60637.

The biosynthesis and processing of the protein core of chondroitin sulfate proteoglycan was studied in chick embryo sternal chondrocytes. When chondrocytes were pulsed for 20 min with [^{35}S]methionine, followed by subcellular fractionation, an immunoprecipitable core protein precursor of approximately 376,000 daltons was localized to the rough endoplasmic reticulum. Pulse-chase experiments showed the 376,000 dalton polypeptide as well as two additional higher molecular weight species in smooth membranes in the next two hours. This translocation did not occur in the presence of CCCP. Labeling studies with either [^{3}H]mannose or [^{3}H]glucosamine showed that N-linked oligosaccharide addition occurred co-translationally in the rough membranes. At this stage, the core protein had a high-mannose oligosaccharide which was sensitive to endo H, and inhibited by tunicamycin. After 2 hours, these were processed to resistant forms, presumably in the golgi complex. Continuous labeling with [^{3}H]glucosamine up to 6h indicated that the core protein in the smooth membranes became increasingly insensitive to endo H with a concomitant increase in sensitivity to keratanase. These results suggest that the core protein, containing N-linked oligosaccharides, was synthesized in the rough membranes and translocated intact to smooth membranes where further glycosylation modifications and addition of glycosaminoglycan chains occur.

1450 SORTING OF PEPTIDE HORMONES IN SECRETORY CELLS. Regis B. Kelly, Hsiao-Ping Moore, Barry Gumbiner. Dept of Biochem & Biophys., Univ of California, San Francisco 94143

Proteins take one of two routes from the Golgi to the cell surface in an ACTH and β-endorphin cell line AtT-20, derived from a mouse pituitary tumor. Mature ACTH and β-endorphin are synthesized as a large 30 K precursor proopiomelanocorticotropin (POMC). Some of the newly synthesized POMC enters the regulated route after leaving the Golgi. In this pathway it is cleaved to the mature forms of the hormone, stored in secretory granules for many hours, and released only on exposure of the cells to a secretagogue, in our case 8Br-cAMP. Four other proteins (68K, 60K, 37K and 15K) and some sulfated material also enter this pathway, are stored in apparently identical granules and are also released only on stimulation. Most of the proteins that are secreted do not use this pathway, however, but use a constitutive route (Gumbiner & Kelly, 1982. Cell 28, 51). Proteins taking this route reach the cell surface about 20 minutes after leaving the Golgi, do not enter storage granules and do not require a secretagogue. An example of a protein taking the constitutive pathway is the gp70 membrane-associated glycoprotein coded for by an endogenous murine leukemia-like virus. Some POMC also enters this pathway and is secreted rapidly and without proteolytic cleavage. Exposure of the cells to chloroquine diverts the POMC from the regulated route to the constitutive one, but does not impair exocytosis itself. The analogy between these results and those obtained in lysosomal enzyme targeting and receptor mediated endocytosis suggests the universality of a low pH step in protein sorting.

1451 BIOSYNTHESIS AND INTRACELLULAR TRANSPORT OF TWO CORONAVIRUS ENVELOPE GLYCOPROTEINS, Mark F. Frana, Elizabeth W. Doller, James N. Behnke, Constance Oliver, Susan Robbins, Lawrence S. Sturman, Kathryn V. Holmes, Univormed Services University of the Health Sciences, Bethesda, MD 20814, Center for Laboratories and Research, N.Y. State Dept. of Health, Albany, N.Y. 12201

Mouse hepatitis virus is a member of the coronavirus group of large, enveloped, positive-stranded RNA viruses. These viruses obtain their envelopes by budding from the RER and Golgi membranes. The viral envelope contains two glycoproteins: E1 (23K) and E2 (180K). Pulse chase and *in vitro* translation studies show that both glycoproteins are synthesized on membrane bound polysomes. E2 is an N-linked glycoprotein which is cotranslationally glycosylated, whereas E1 is an O-linked glycoprotein which is post-translationally glycosylated. E2 forms the viral peplomers or spikes. It migrates readily to the plasma membrane of infected cells. Proteolytic cleavage of the E2 glycoprotein from 180K to 90K is required for coronavirus-induced cell fusion. The E1 glycoprotein is a matrix glycoprotein deeply embedded in the lipid bilayer. The intracellular transport of the E1 glycoprotein is restricted. Newly synthesized E1 is transported from the RER to the Golgi apparatus where glycosylation occurs. It appears likely that cellular enzymes are utilized for glycosylation of this O-linked glycoprotein. Immunofluorescence and cytochemical experiments show that E1 accumulates in the Golgi apparatus and is not readily transported to the plasma membrane. We suggest that the restricted intracellular migration of the E1 glycoprotein may account for the intracellular budding site of coronaviruses. The coronavirus E1 glycoprotein represents an excellent model system for the study of the synthesis, transport and processing of O-linked glycoproteins.

1452 MONOCLONAL ANTIBODIES THAT RECOGNIZE DIFFERENT FORMS AND POST-TRANSLATIONAL MODIFICATIONS OF A LYSOSOMAL ENZYME, Robert C. Mierendorf, Jr., David A. Knecht and Randall L. Dimond, University of Wisconsin, Madison, WI 53706

Most of the lysosomal enzymes of the cellular slime mold, Dictyostelium discoideum, are developmentally regulated, highly modified proteins that are efficiently secreted under certain conditions. To further our studies on their synthesis, processing and secretion we have prepared a variety of monoclonal antibodies against several of these enzymes. Two separate screening methods were used to identify antibodies produced by spleen/myeloma hybrid cells in culture; one was an indirect (ELISA) assay based on the antibody binding to enzyme adsorbed on a 96-well plate, and the other was a direct assay of the antibodies' ability to precipitate enzyme activity with fixed S. aureus cells (Pansorbin). When purified alpha-mannosidase, an enzyme composed of 58,000 and 60,000 M_r subunits, was used as immunogen three general classes of antibodies specific for the enzyme were found. One class precipitates a 140,000 M_r precursor form in addition to the smaller subunits in labeled cellular and secreted fractions, whereas another recognizes only the two mature polypeptides under the same conditions. The third class fails to recognize any enzyme species in solution, but binds specifically to all three forms when they are either adsorbed on plastic or bound to nitrocellulose. Within these major classes, there are at least five subclasses of various specificities. In other work, monoclonal antibodies were prepared against purified N-acetylglucosaminidase and a crude mixture of lysosomal enzymes. With one exception, these antibodies recognize a post-translational modification, probably involving a sulfated sugar residue, shared by many or all of the lysosomal enzymes in this organism.

1453 ROLE OF THE GOLGI APPARATUS IN THE BIOSYNTHESIS AND TRANSPORT OF PHYTOHEMAGGLUTININ IN *PHASEOLUS VULGARIS* COTYLEDONS - Maarten J. Chrispeels, Department of Biology, University of California, San Diego, La Jolla, CA 92093

The developing seeds of *Phaseolus vulgaris* synthesize large quantities of proteins which accumulate in special membrane-bounded organelles called protein bodies. The two most abundant proteins in the protein bodies are phaseolin and phytohemagglutinin (PHA). Both proteins are made by membrane-bound polysomes and pass through the ER on their way to the protein bodies. Phaseolin is a high-mannose type glycoprotein, and glycosylation occurs cotranslationally in the ER. The carbohydrate moiety of PHA contains fucose and is not susceptible to digestion by endoglucosaminidase H, indicating that it is a complex oligosaccharide. The incorporation of fucose takes place in organelles which have a density of 1.13 $g.cm^{-3}$ and which were identified as vesiculated Golgi complex. Pulse-chase experiments indicate that fucosylated PHA moves out of the Golgi with a $t_{1/2}$ of 20-30 min to organelles with a density of 1.22 $g.cm^{-3}$. These organelles are newly formed protein bodies.

A pretreatment of 2h with 50μM monensin blocks the transport of the PHA out of the Golgi complex into the protein bodies. Monensin also blocks the transport of phaseolin out of the Golgi complex. These data provide the first biochemical evidence that the Golgi apparatus mediates the transport of storage proteins in developing seeds.

1454 ANALYSIS OF THE INTRACELLULAR LOCATION AND TRANSPORT OF HSV-1 GLYCOPROTEINS DURING INFECTION OF CELLS IN CULTURE. Bodil Norrild[1], Ismo Virtanen[2], V.P. Lehto[2] and Bente Pedersen[1]. Institute of Medical Microbiology, University of Copenhagen[1], Department of Pathology, University of Helsinki[2].

Herpes virus infection is known to have a profound influence on the cell-to-cell interaction and rounding and detachment of epithelial cells are observed. We have shown that the viral glycoproteins are synthesized in the endoplasmic reticulum of the cells, transported to the nuclear membrane and to the Golgi apparatus where the carbohydrate chains are partially processed. Our data also show that the terminal sugars of the carbohydrate chains are added beyond the Golgi apparatus, but before integration of the glycoproteins into the plasmamembrane.

The influence of the viral glycoproteins on the social behavior of the infected cells is illustrated for glycoprotein gD. This glycoprotein is transported to the tight junctions of the cells and also accumulates in the focal adhesion plaques where the cells are in close contact with the growth substratum.

1455 MISLOCALIZATION OF OVERPRODUCED VACUOLAR CARBOXYPEPTIDASE Y IN YEAST, Tom H. Stevens and Randy Schekman, University of California, Berkeley, CA 94720

Carboxypeptidase Y (CPY) is a glycoprotein which is localized in the lysosome-like vacuole of yeast. We have previously investigated the delivery of CPY to the vacuole using yeast mutants blocked at various stages in the secretory pathway. An endoplasmic reticulum-blocked and a Golgi-blocked secretory mutant prevent delivery of CPY to the vacuole at a restrictive temperature, but allow the correct localization of the accumulated CPY when the cells are returned to a permissive temperature.[1] Mutants that block secretion after the Golgi step (secretory vesicle-blocked mutants) have no effect on CPY localization to the vacuole. These results indicate that vacuolar and secretory proteins travel together from the ER to the Golgi body where sorting likely occurs.

The CPY gene has been cloned into the multiple copy plasmid, YEp13. The presence of this CPY plasmid in yeast results in the 3 fold overproduction of CPY. This overproduction causes the aberrant secretion of about 10% of the CPY synthesized. A secretory vesicle-blocked mutant that doesn't block vacuole delivery, does block the secretion of CPY. The aberrant secretion of CPY may result from the saturation of a vacuole protein receptor in the Golgi, with the unbound CPY being secreted.

1. Tom H. Stevens, Brent Esmon, and Randy Schekman (1982) Cell 30:439-448.

1456 CHARACTERIZATION OF MEMBRANE-ASSOCIATED ENZYMES IN ISOLATED SECRETORY VESICLES, Lynne L.W. Binari, Patricia A. Conrad and Richard H. Racusen, University of Maryland, College Park, MD 20742

For the past two years, we have been utilizing rapidly-secreting, cultured plant cells to study cellular exocytosis. The particular cells we are using secrete copious quantities of a pectinaceous polysaccharide into the suspending medium and, when fractionated, can be used to collect large numbers of intact (polysaccharide-containing) vesicles. Since membranes of secretory vesicles are expected to become components of the plasma membrane during fusion, analysis of this transitory membrane element may provide some insight into the process of membrane differentiation. To this end, we examined the activities of several membrane-associated enzymes in isolated secretory vesicles. We have characterized a cation-stimulated ATPase that is markedly similar to one that has been well-documented in the plasma membrane of plant cells. A reliable marker for membranes of plant Golgi apparati is also present in the vesicle membrane; however, an enzyme marker for endoplasmic reticulum exhibits no significant activity in the Golgi-derived vesicles. Although it is premature to decide how membrane-enzyme complements are altered during membrane flow, we are intrigued by the observation that both Golgi- and plasma membrane-related enzymes are apparently functional in secretory vesicle membranes.

1457 INTRACELLULAR TRANSPORT OF SPLEEN FOCUS FORMING VIRUS (SFFV) GLYCOPROTEINS. R. V. Srinivas and R. W. Compans, University of Alabama in Birmingham, Birmingham, Alabama 35294.

The SFFV gp52 glycoprotein has been identified as a recombinant molecule involving substitutions and deletions of the MuLV env gene. Unlike the MuLV structural glycoproteins, gp52 is defective in its transport to the cell surface. We have studied aspects of intracellular transport and membrane association of gp52 to investigate the possible mechanisms underlying the defective transport process. To determine whether a spontaneous denaturation occurs leading to aggregation of gp52, we studied the surface expression of gp52 in cells grown at different temperatures, as well as the solubility of gp52 in low concentrations of Triton X-100. No evidence of aggregation or of a temperature-dependent difference in transport was obtained. It was found that a panel of monoclonal antibodies to different epitopes of p15E, as well as an antiserum to a synthetic peptide corresponding to the carboxy terminus of MuLV envelope precursors, failed to react with gp52. Despite the possible absence of membrane anchoring regions of MuLV envelope proteins known to reside on p15E, gp52 was not found to be secreted into the culture fluids. Also, gp52 could be labelled with ^{3}H-palmitic acid, suggesting a membrane association. Proteolytic digestion of intact microsomal vesicles did not reveal a detectable cytoplasmic tail under conditions where this could be demonstrated on MuLV envelope precursors. We suggest that a loss of putative signals involved in mediating intracellular transport is a likely cause for the defective transport of the SFFV glycoproteins.

1458 STABILTY OF THE COATED VESICLE AND COAT STRUCTURE IN SOLUTION. P.K.NANDI,CLINICAL ENDOCRINOLOGY BRANCH,NATIONAL INSTITUTES OF HEALTH,BETHESDA,MD. 20205

Stability of the coated vesicles and clathrin coat (baskets) has been studied under a variety of solution conditions. Under appropriate conditions,both the coated vesicles and baskets could be stabilized up to pH 8 (and higher) without the presence of either calcium or magnesium ions. Of the two types of baskets viz. 150 S and 300 S species,the former is capable of retaining its structure in contrasts to the bigger baskets which dissociate under same experimental conditions. The process of the stability of the coat structure and coated vesicles can be described quantitatively. Other effects,in addition to the electrostatic effect,are found to be important in maintaining the coat structure alone and also when it is a part of the coated vesicles. The results on the relative stability of the coated vesicles and basket structure will be presented.

1459 DIFFERENT PROCESSING PATHWAYS FOR MEMBRANE AND SECRETORY IgM FORMS IN B-LYMPHOCYTE CELL LINES, Joseph Haimovich, Dept. of Human Microbiology, Sackler School of Medicine Tel-Aviv University, Tel-Aviv 69978, Israel

Cells of human and murine B-lymphocyte cell lines synthesize similar amounts of translatable mRNA's for membrane and secretory μ chains. However, only a minute amount of the produced secretory IgM is actually secreted while the rest is degraded. The intracellular precursor for the membrane form of IgM, on the other hand, is transported to the plasma membrane with no loss due to degradation. Secreted IgM, found in small amounts in the culture media of these cells, differs from membrane IgM, not only in its μ chain polypeptide backbone, but also in the size of the oligosaccharide groups attached to it. Because of their differences in both susceptibility to degradation and the extent of glycosylation it is suggested that membrane and secretory forms of IgM in B-cells are processed and transported via separate pathways.

1460 EVIDENCE THAT CARBOHYDRATE SPECIFIES INTRACELLULAR ROUTING OF GLYCOPROTEINS IN HEPATOCYTES, J. Brian Parent[1], Hans C. Bauer[1] and Kenneth Olden[1,2], [1]Howard Univ. Cancer Ctr., Wash., D.C. 20060 and [2]Lab. of Mol. Biol., N.C.I., N.I.H., Bethesda, Md. 20205.

We earlier proposed that the carbohydrates moiety of many glycoproteins functions as a "tag" which defines the route of intracellular transport (Biochim., Biophys. Acta. 650: 209, 1982). We used the liver cell line Hep G2 to test this model because it produces a variety of secretory proteins. Based on the carbohydrate structure, liver secretory proteins can be classified into three major categories: (1) no carbohydrate, e.g. albumin, thyroxine-binding prealbumin, (2) N-linked, complex biantennary oligosaccharides, e.g. transferrin (type 1), fibrinogen, and (3) at least one N-linked, complex triantennary oligosaccharide, e.g. ceruloplasmin, α_1-protease inhibitor , α_2-macroglobulin. A prediction of our model is that secretory proteins with similar carbohydrate will have the same intracellular retention time (RT) following synthesis; conversely, proteins with no or dissimilar carbohydrate may have different RTs. We measured RT using a pulse-chase protocol with ^{35}S-met and immunoisolation. In agreement with our model, we found that transferrin and fibrinogen with Class 2 oligosaccharide have similar RTs (130-150 min) and ceruloplasmin, α_1-protease inhibitor and α_2-macroglobulin with Class 3 oligosaccharide have similar RTs (55-60 min). In contrast, fibronectin and albumin have RTs of 8-10 min and 25-30 min respectively. A second prediction of the model is that treatment of Hep G2 with tunicamycin, an inhibitor of N-glycosylation, should alter the RTs of the above glycoproteins, but should have no effect on albumin which lacks carbohydrate and our results support this prediction. This work was partially supported by N.I.H. Grant #1-R01-GM-29804-01 from N.I.H.

1461 REACTIVITY OF THE CARBONYL FUNCTION OF THE KETOAMINE LINKAGES IN NONENZYMICALLY GLUCOSYLATED PROTEINS: PHYSIOLOGICAL CONSEQUENCES. A.S. Acharya and J.M. Manning
The Rockefeller University, New York.

Post-translational modification of proteins by nonenzymic glucosylation involves the formation of Schiff base adducts of the amino groups of proteins with glucose and subsequent Amadori rearrangement of the adduct to form stable ketoamine linkages. The carbonyl function of nonenzymically glucosylated RNase A reacts with dinitrophenylhydrazine in a manner analogous to that for the ketoamine linkage of the glyceraldehyde-hemoglobin adduct (Acharya & Manning, J. Biol. Chem. 235, 7318 (1980)). If the carbonyl function of the ketoamine linkages of the glucose adducts reacted with the amino groups of other proteins, covalent cross-linking of proteins would occur. Incubation of RNase A (0.5 mM) with 20 mM glucose for six weeks at 37°C resulted in the generation of fluorescent, cross-linked RNase. A product rich in di- and trimeric forms of RNase A was isolated by gel filtration; it exhibited a fluorescence 6 to 8 times that of the monomeric form. Upon affinity chromatography of the glucose RNase A adduct on Glyco Gel B, all the cross-linked, fluorescent products were bound to the affinity gel. The cross-linking as well as the development of fluorescence is prevented by the presence of $NaCNBH_3$ (reductive glucosylation). These results demonstrate that glucosylation, cross-linking and development of fluorescence are interrelated. The cross-linking found in nonenzymically glucosylated RNase can be considered as the reflection of reactivity of the carbonyl function of the ketoamine linkages of glucose with RNase A. This reaction could be of physiological consequence with proteins of low turnover such as collagen and lens crystallines. (Supported by NIH Grants HL-27183 and HL-18819.)

1462 THE ROLE OF CLATHRIN-COATED VESICLES IN THE INTRACELLULAR TRANSPORT OF NEWLY SYNTHESIZED LYMPHOID GLYCOPROTEINS, Christine Kinnon and Michael J. Owen,
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Clathrin-coated vesicles have been implicated in the shuttling of membranes and proteins between different intracellular compartments. We have investigated the role of coated vesicles in the intracellular transport of newly synthesized lymphocyte membrane-associated and secreted glycoproteins. Using coated vesicles prepared from the cultured human B lymphocyte cell line RPMI 1788, an association between HLA-A,-B and -DR antigens (membrane-bound glycoproteins) and IgM (a secreted glycoprotein) with coated vesicles was demonstrated by lentil lectin affinity chromatography and by immunoprecipitation using antisera directed against these glycoproteins. [^{35}S] Methionine pulse-chase experiments demonstrated that there was a kinetic correlation between the expression of newly synthesized glycoproteins and their presence in coated vesicles. Furthermore, these results suggested that the newly synthesized glycoproteins become associated with coated vesicles as they pass through the Golgi region of the cell, in agreement with the work of Rothman and co-workers using a viral glycoprotein (VSV G protein). One interpretation of these results is that coated vesicles may mediate the transfer of glycoproteins through the Golgi stacks.

Extragenic Components of the Protein Export Machinery

1463 BIOSYNTHESIS OF THE UBIQUINOL-CYTOCHROME c REDUCTASE IN YEAST: GENES FOR IMPORTED SUBUNITS, L.A.Grivell, A.P.G.M.Van Loon, A.C.Maarse and R.J.De Groot, Section for Molecular Biology, Laboratory of Biochemistry, University of Amsterdam, Kruislaan 318, 1098 SM Amsterdam, The Netherlands.

The ubiquinol-cytochrome c reductase in yeast consists of eight subunits, of which seven are encoded by nuclear genes, synthesized in the cytoplasm and imported by the mitochondrion. Several of these subunits are synthesized as precursors, with extensions ranging in length from 0.5-9 kD. Genes for five of the imported subunits (40K, FeS protein, 17K, 14K and 11K) have been cloned and partially characterized. Data so far available suggests that these genes are not closely linked and each hybridizes to a unique chromosomal map location, consistent with there being only a single copy per haploid genome.

Genes coding for 40K, FeS and 11K subunits have been re-introduced into wild-type cells on a multi-copy shuttle vector. This increase in gene copy number leads in several instances to over-production of the protein concerned without effect on the levels of other subunits; in the case of 11K and 40K subunits, the bulk of the excess subunit is found associated with the mitochondrial fraction. The implications of these findings for the regulation of the genes for imported subunits and of the import process itself will be discussed.

1464 THE ATP DIPHOSPHOHYDROLASE: PHOTOAFFINITY LABELING OF THE INTRINSIC MEMBRANE PROTEIN CHARACTERISTIC OF THE EXOCRINE PANCREAS SECRETORY GRANULE. **Denis LeBel, Research Center on the Mechanisms of Secretion, Faculty of Sciences, University of Sherbrooke,** Sherbrooke, Québec, Canada J1K 2R1. Studies on the ATP hydrolases of the exocrine pancreas have led to the identification of a very distinct activity of this tissue: the ATP diphosphohydrolase (J. Biol. Chem. 255: 1227, 1980). This enzyme hydrolyses ATP or ADP to AMP producing only orthophosphate (J. Biol. Chem. 257: 3869, 1982). This enzyme is an intrinsic protein of the zymogen granule membrane. In the studies reported here, we achieved photoaffinity labeling of a purified membrane preparation with [^{32}P]8-azido ATP. **When used at concentrations close to the** K_m of the enzyme (1-2 μM), [^{32}P]8-N_3ATP specifically labeled two proteins of the membrane. One of them was the 92,000 dalton glycoprotein, the major protein of the membrane which accounts for 25% of the coomassie blue staining on gels. A 58,000 dalton integral membrane protein was also labeled. The labeling intensity of the latter was much more important considering the smaller amount of this protein in the membrane. ATP and ADP were competitive in the photolabeling reaction for both proteins. **Reaction with [^{32}P]8-N_3ATP on a** purified ATP diphosphohydrolase preparation led to the labeling of the 58,000 dalton subunit of the enzyme (J. Biol. Chem. 257:3869, 1982). These results show that the catalytic subunit of the ATP diphosphohydrolase is the 58,000 dalton subunit which is also one of the two integral membrane protein reactive to [^{32}P]8-N_3ATP in the native zymogen granule membrane. **Labeling** of the 92,000 dalton glycoprotein of the zymogen granule membrane with [^{32}P]8-N_3ATP has not yet been associated with any known enzymatic activity involving ATP as substrate. (Supported by NSERC of Canada and by the Fonds FCAC du Québec).

1465 INTRACELLULAR PRECURSORS OF THE SECRETED ALKALINE PROTEASE OF SACCHAROMYCOPSIS LIPOLYTICA, David Ogrydziak, June Fukayama and Peter Mirabito, U.C. Davis, CA 95616

The yeast Saccharomycopsis lipolytica secretes a single alkaline extracellular protease (AEP) into the extracellular medium during exponential growth. Immunoprecipitation of labeled cell extracts revealed a major immunoprecipitate of the same mobility (Mr=35,000) on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) as the secreted AEP and one precursor (Mr=40,000) which is consistently found and another possible precursor (Mr=48,000). **Results of peptide mapping of intracellular forms of the protease and immuno**precipitation analyses of protease precursors synthesized in vitro will be presented. **The** amounts and mobilities on SDS-PAGE of intracellular forms of the protease in protease structural gene mutants, possible secretion mutants and other mutants which produce lower levels of AEP will be reported. Preliminary results indicate that one of the structural gene mutants (xpr2-34) accumulates a precursor with mobility different from the wild type precursor.

1466 λ S LYSIS PROTEIN: IDENTIFICATION AND MODE OF ACTION OF A LETHAL MEMBRANE PROTEIN, Ry Young, E. Altman, J. Garrett, and R. Schulz, Medical Biochemistry, Texas A&M University, College Station, TX 77843

The bacteriophage λ S protein regulates lysis of the infected cell. Lysis is caused by the secretion of bacteriolytic enzymes (pR, pRz) into the periplasm and the consequent destruction of the peptidoglycan. **The S protein allows penetration of the inner membrane (IM) by** the bacteriolytic enzymes, at a rigidly scheduled time long after the onset of expression of the lysis genes. **We have cloned the lysis genes of lambda and identified the S gene (108** codons) product as an 8.5 kd membrane protein, and also the product of the Sam7 non-functional allele (55 codons) as a ∿ **7 kd membrane protein. The pSam7 polypeptide appears** to accumulate in the outer membrane **(OM); this indicates that the amino-terminal sequence** of pS (and pSam7) contains signals which **destine the molecule for export to the OM, but that** the carboxy-terminal sequence (missing in **pSam7) contains export-inhibition sequences. We** suggest the pS molecule may exert **its lethal action at the zones of adhesion, after being** trapped there en route to the OM. **Using a novel envelope fractionation procedure, we have** found evidence for the association of pS in membrane material of intermediate density, consistent with a localization in the zones of adhesion. E. coli mutants have been isolated resistant to S action, as well as the action of the φX174 lysis gene E; these mutants define at least 5 genetic loci which we designate as sly genes (sensitivity to lysis) and which may be involved in adhesion zone structure and function.

1467 A GENETIC ANALYSIS OF THE ROLE OF 7S RNA IN PROTEIN SECRETION, Jo Ann Wise and Christine Guthrie, University of California, San Francisco, CA 94143

The targeting of ribosomes translating messenger RNAs encoding proteins destined for the endoplasmic reticulum is mediated by an 11S complex designated Signal Recognition Particle (SRP). My interest in this process was stimulated by the recent, quite unexpected, discovery that SRP contains, in addition to six polypeptides, a 7S RNA component which is essential for activity in vitro. I plan to undertake a genetic analysis of the relationship between 7S RNA structure and function in order to determine its precise role in the early steps of protein secretion. To achieve this goal, I will exploit the powerful genetic methods and versatile transformation techniques available in yeast. After cloning the gene for 7S RNA (which I have tentatively identified on the basis of size and possession of a 5' triphosphate residue), I will mutagenize it in vitro; secretion-defective mutants will be sought among the transformants. In addition to mutations which affect the assembly of the particle, the molecular events deduced from in vitro experiments using higher cells allow the prediction of several other classes of potential 7S phenotypes: (1) The SRP may fail to recognize ribosomes translating presecretory mRNAs; (2) the SRP may be defective for imposition or release of the translational block which is crucial for insuring proper compartmentalization; or (3) the SRP may be unable to interact with its receptor on the endoplasmic reticulum membrane. The availability of mutations in 7S RNA will make it possible to identify other cellular components which interact with this molecule, minimally the SRP proteins themselves, by selecting extragenic suppressors of the secretion defects.

1468 THE MOLECULAR SPECIES OF SECRETORY COMPONENT IN RAT BILE AND LIVER MEMBRANES: IDENTIFICATION BY WESTERN BLOT TECHNIQUES, Thomas M. Kloppel and William R. Brown, VA Med Cntr and Univ Colo Health Sci Cntr, Denver, CO 80220

Rat bile and rat liver membranes were examined by western blot analysis to detect the molecular species of secretory component (SC). Bile or liver membrane proteins were electrophoresed in polyacrylamide gels and electrophoretically transferred to nitrocellulose membranes (NCM). Protein profiles on NCM were probed with anti-SC antisera, and immunoreactive bands were visualized by indirect immunoperoxidase staining. Bile collected in the presence of proteolytic inhibitors showed 2 immunoreactive bands (Mr = 82 and 78 kd) representing free SC. SC purified from bile without proteolytic inhibitors migrated as a single protein with an Mr = 70 kd which agrees with previously reported values for free rat bile SC. SC reduced with 2-mercaptoethanol also migrated as a doublet but with a slightly larger apparent molecular weight. The free SC doublet bound dimeric IgA when NCM blots were probed with human dimeric IgA confirming the specificity of the anti-SC antibody. Crude liver membranes prepared with proteolytic inhibitors showed two immunoreactive SC-containing bands, Mr = 106 and 100 kd. Crude liver membranes prepared without protease inhibitors showed 2 smaller immunoreactive bands demonstrating the susceptibility of the membrane SC to limited proteolytic degradation. The results indicate that the molecular species of SC in liver membranes are larger than free SC proteins in the bile. These findings are consistent with the hypothesis that free SC in bile is a proteolytic product of liver membrane-associated SC. In addition, the previously reported Mr for free SC in bile probably is spuriously low because of in vitro proteolysis of SC.

1469 EXPORT-SPECIFIC MUTATIONS THAT RESTORE PROPER LOCALIZATION OF MALIOSE BINDING PROTEIN WITH A TRUNCATED SIGNAL PEPTIDE, Vytas A. Bankaitis and Philip J. Bassford, Jr., University of North Carolina, Chapel Hill, NC 27514

We have isolated a 21 bp deletion, designated ΔmalE308, whose endpoints lie within DNA sequences encoding the signal sequence of the periplasmic maltose binding protein (MBP) of Escherichia coli. This mutation results in the removal of residues 12 through 18 from the hydrophobic core of the MBP signal peptide. The missing region includes 3 of 5 residues previously shown, by analysis of point mutations, to be critical for proper localization of the MBP. Greater than 99% of the MBP synthesized in ΔmalE308 strains remains internalized in its precursor form. We have isolated 28 Mal^+ phenotypic revertants of a ΔmalE308 strain and have categorized these into three mutant classes: (i) 12 reversion mutations were very tightly linked to the malE gene and appeared to act only in cis. Many of these presumed intragenic suppressor mutations very efficiently restored export of mature MBP to the periplasm. However, 9 of the 12 suppressor strains exhibited some apparently aberrent processing of the MBP signal peptide. (ii) 15 ΔmalE308 suppressor strains mapped at the prlA locus. Of these, 3 alleles were more efficient suppressors of signal sequence mutations than any previously described. (iii) One ΔmalE308 suppressor mutation, designated prlE1, was located at 2.5 min on the E. coli linkage map (close to, but distinct from, the secA locus thought to encode a component of the cell's secretion machinery). The prlE1 mutation was recessive to $prlE^+$ and interacted with both malE signal sequence mutations and prlA mutations in an allele-specific manner. This implies a direct interaction of the prlE gene product with mutant MBP signal peptides and the prlA gene product.

1470 DEGRADATION OF EXTRACELLULAR FIBRONECTIN BY VIRUS-TRANSFORMED FIBROBLASTS. Wen-Tien Chen, Kenneth Olden, and Bruno A. Bernard, Howard University Cancer Center, Washington, D.C. 20060.

Virus-transformed fibroblasts show an increased production of proteases as well as a loss of extracellular proteins. The possibility arises that some of these proteases might be involved in degradation of cell surface proteins. To investigate this possibility, we have studied membrane-associated proteolytic activities of normal and transformed cells with a novel crosslinked protein-substratum: ^{14}C- or rhodamine-labeled fibronectin (FN) covalently linked to the surface of a fixed gelatin film. The coupled FN was stable, since 1) there was little release of radioactivity from ^{14}C-FN-coupled substrata during incubation with normal CEF and 3T3 cells, and 2) rhodamine-FN-substrata appeared as a uniform fluorescence carpet on which normal cells were grown. However, when RSV-transformed CEF and 3T3 cells were seeded on the ^{14}C-FN substratum, there was a time-dependent release of radioactivity; on the rhodamine-FN, discrete negative fluorescent spots were detected under transformed cells and along their migratory paths. Indirect double immunofluorescence analysis showed a correlation between vinculin localization and the FN-negative spot under transformed cells, indicating that degradation occurs at sites of cell-substratum contacts. These findings suggest that loss of cell surface proteins following transformation is due to a local membrane protease activity. Inhibitor studies and electrophoretic analysis of degraded products are under way to determine a potential class of proteases involved in this system.

1471 ISOLATION AND CHARACTERIZATION OF SECRETORY VESICLES FROM SACCHAROMYCES CEREVISIAE Tina Etcheverry, Genentech, Inc., South San Francisco, Ca. 94080 and Randy Schekman, University of California, Berkeley, Ca. 94720.

Recent evidence suggests that protein secretion and cell surface growth utilize the same cellular machinery, however, it is unknown if secretory vesicles are used to deliver membrane proteins to the plasmalemma. To approach this question, a purification scheme has been developed for obtaining intact secretory vesicles. The purification is aided by starting with a mutant strain (*secl*) which accumulates these vesicles. Major contaminating organelles are removed from the vesicle fraction by differential centrifugation under conditions of osmotic support, followed by density equilibrium on a Percoll gradient. This material is further purified by electrophoresis on a low percentage agarose gel. This last step optimizes purification by combining a charge-density separation with a sieving effect of the agarose. The secretory enzymes invertase and acid phosphatase are monitored during the purification process and used as a measure of specific activity. The activities of contaminating membrane markers are also monitored and found to be undetectable (<.1 percent) in the purified material. Two major plasmalemma proteins, chitin synthetase and the vanadate-sensitive ATPase, can be purified away from the vesicle fraction by this protocol, yet incorporation of these activities into a membrane form is occurring. This suggests that these plasma membrane proteins are incorporated into the plasmalemma by a mechanism unrelated to the secretory process.

1472 ACCUMULATION AND DELAYED SECRETION OF UNPROCESSED PROTEINS BY MONENSIN IN CULTURED RAT HEPATOCYTES, Yukio Ikehara, Yoshio Misumi and Kimimitsu Oda, Fukuoka University School of Medicine, Fukuoka 814-01, JAPAN

Previously we reported that monensin inhibits the conversion of proalbumin to serum albumin in cultured rat hepatocytes.[1] We further studied effects of monensin on the intracellular transport and processing of secretory proteins including α_1-protease inhibitor (α_1-PI) and albumin. Hepatocytes were pulse-labeled with [^{35}S]methionine and chased for various times in the presence or absence of the drug (1×10^{-6} M). Newly synthesized albumin and α_1-PI in the cell and medium prepared by immunoprecipitation with the respective specific antibodies, were analyzed by electrofocusing and SDS-gel electrophoresis followed by fluorography.
In addition, the processing of carbohydrate chains of α_1-PI was determined by its response to Endo H digestion. In control cells, α_1-PI was first synthesized as an Endo H-sensitive 51K-form, and processed to two Endo H-resistant forms having 51K and 56K, the latter of which was secreted into medium. In the monensin-treated cells, however, most of the newly synthesized α_1-PI was not processed to the final complex form (56K). The Endo H-resistant 51K form which was accumulated in the treated cells was finally secreted into the medium after prolonged times of chase. Proalbumin accumulated in the treated cells was also secreted into the medium. Similar results were obtained for transferrin and haptoglobin. Possible mechanism(s) for these alterations will be discussed in relation to the Golgi function.
1) Oda,K. & Ikehara, Y. (1982) Biochem. Biophys. Res. Commun. 105, 766-772.
(This work was supported in part by a grant from the Ministry of Education, Science and Culture of Japan)

Vectors for Directing Secretion of Foreign Proteins

1473 PROTEIN SYNTHESIS IN MEMBRANES OF E. COLI K-235 IS REQUIRED FOR REACTIVATION OF AN INACTIVE SIALYLTRANSFERASE COMPLEX. Frederic A. Troy and Christopher Whitfield, Univ. of Calif. Sch. Med., Davis, CA 95616

Capsular polymers containing polysialic acid in E. coli K-235 are not synthesized when cells are grown at 15°C. A defect in the synthesis or assembly of membrane-associated endogenous acceptor molecules appears responsible (Troy, F.A. and McCloskey, M.A. (1979) J. Biol. Chem. 254, 7377-7387). Unexpectedly, membranous sialyltransferase (ST) complexes isolated from cells grown at 15°C were shown to undergo a spontaneous reactivation of sialyl polymer (SP) synthesis when incubated at 33°C for 2-4 hr (Troy, F.A. et al. (1982) Biophys. J. 37, 85-86). Spontaneous reactivation has now been shown to possess two remarkable features. First, all of the ST activity associated with reactivation was localized in low buoyant density vesicles (LDV; ρ=1.1 g/cm^3), while a topologically distinct ST that catalyzed transfer of sialic acid to an exogenous acceptor was localized in hybrids of inner and outer membranes. Second, LDV catalyzed de novo protein synthesis and reactivation of SP synthesis was obligatorily coupled to protein synthesis. Temperature jump experiments identified four proteins to be correlated with in vitro reactivation. These results demonstrate that synthesis of specific membrane proteins are required for activation of the inactive ST complex. Five to 10% of the endogenous ST activity in 37°C membranes is localized in identical LDV suggesting the possibility that these vesicles are of physiological importance in membrane assembly processes and may represent functional adhesion sites between inner and outer membranes. Growth at 15°C leads to an enrichment in these vesicles presumably because of the altered thermotropic properties of the membrane phospholipids that result when cells are grown at low temperature.

1474 HUMAN LENS AMINOPEPTIDASE: PRESENT IN A STATE OF REDUCED ACTIVITY IN AGED NORMAL AND CATARACTOUS EYE LENSES, Allen Taylor and Mark Daims, Harvard University, Cambridge MA 02138

Immunodiffusion tests using rabbit antisera to bovine lens leucine aminopeptidase,blLAP, indicate that human lens homogenates contain a component which shares some, but not all, the antigenic determinants of blLAP. Microcomplement fixation analyses show that the concentration of LAP in human lenses is equal to that in bovine lenses (0.02% dry lens mass). This result is in contrast to all the previous literature which indicates that the activity of LAP in human lens is much lower, if extant, than that observed in lenses of other vertibrates. The LAP in human lens is present as several electrophoretically distinct but immunologically indistinguishable species, some of which may be associated with high molecular weight components. Thus, the discrepancy between activity levels and concentration levels appears to derive from the fact that LAP is present but damaged and inactive in aged human lens.

Active LAP hastens the rate of hydrolysis of lens alpha crystallin fragments. It is possible that disease and age related inactivation of LAP results in an inability of the aged lens to degrade and remove altered lens proteins or polypeptides. This malfunction may be related to the accumulation and aggregation of proteins which result in cataract.

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1475 USE OF SITE-DIRECTED MUTAGENESIS IN THE STUDY OF THE lon GENE PRODUCT IN Escherichia coli, Padmini Sampathkumar and Alfred L. Goldberg, Harvard Medical School, Boston, MA.

The product of the lon gene is an ATP-dependent protease (protease La) responsible for the degradation of abnormal proteins. In addition, it is a protein-stimulated ATPase and a DNA binding protein,and DNA stimulates both proteolytic and ATPase activities of this enzyme. In order to elucidate the interrelationship between these functions and to clarify their role in the various phenotypes of lon mutants,we have cloned a 3 kb fragment of lon DNA into a single copy R plasmid replicon and have undertaken a systematic mutagenesis of the lon DNA. Using a combination of several restriction enzymes, overlapping DNA fragments between 300-600 base pairs have been isolated that cover the length of the lon DNA. Single stranded DNA generated from these fragments have been used to form D-loops required for the D-loop mutagenesis of the cloned DNA with a single strand mutagen. The resulting mutants have been selected for their ability to stabilize an essential protein that is rapidly degraded by protease La and screened for their ability to induce transcription from an operon involved in the expression of the mucoid phenotype of lon$^-$ mutants. Each class of mutants have been further screened for their ability to confer resistance to UV or methymethanesulfonate in a lon$^-$ strain carrying the plasmid. Twenty-two mutants have been isolated which are localized to a 1 kb region of DNA corresponding to the C-terminal end of the protein. Six of these mutants stabilize the short-lived protein substrate of La, but differ in their ability to induce transcription from the operon involved in mucoidy and in their pattern of UV resistance. The properties of the proteins coded for by these mutant plasmids will be studied in vitro.

1476 ISOLATION OF THREE NEW PROTEASES AND A NOVEL PROTEASE INHIBITOR FROM ESCHERICHIA COLI
Chin Ha Chung and Alfred L. Goldberg, Harvard Medical School, Boston, MA 02115

To learn more about the pathways of intracellular protein breakdown in bacteria, cytoplasmic endoproteases, proteases Do, So and Ci, have been purified to near homogeneity using conventional procedures. Unlike protease La (the lon gene product), these enzymes are not activated by ATP. Proteases Do and So are inhibited by diisopropyl fluorophosphate and phenyl methylsulfonylfluoride, and therefore are serine proteases. Protease Do has an unusually high molecular weight of 520,000 and is composed of ten identical subunits (52,000 d). Protease So has a molecular weight of 140,000 and contains two identical subunits (78,000 d). Both enzymes degrade casein, globin, and denatured bovine serum albumin to acid-soluble peptides. Proteases Do and So differ in their sensitivities to various protease inhibitors. Protease Ci is a metalloprotease, sensitive to o-phenanthroline. This enzyme has a molecular weight of 110,000 and hydrolyzes small polypeptides (e.g., insulin, glucagon, and the auto α fragment of β-galactosidase), but not casein or globin. A periplasmic protein capable of inhibiting trypsin has been purified to homogeneity by conventional procedures and affinity chromatography. It is stable to heat and acid, but it is inactivated by pepsin. It has a molecular weight of 38,000 and is composed of two identical subunits (18,000 d). Each subunit can bind and completely inhibit one trypsin molecule. It also inhibits chymotrypsin, pancreatic elastase, and rat mast cell chymase, but does not inhibit the eight soluble endoproteases which we have recently isolated from E. coli. The physiological roles of these proteins remain to be elucidated.

1477 CARBOHYDRATE MOIETIES OF GLYCOPROTEINS PROTECT AGAINST PROTEOLYSIS.
Kenneth Olden[1,2] Bruno A. Bernard[1,2], and Sheila Newton[1], Howard University Cancer Center[1], Washington, DC 20060 and Laboratory of Molecular Biology[2], National Cancer Institute, Bethesda, MD 20205.
Protection against proteolysis by the oligosaccharide moieties of glycoproteins has been widely reported. To investigate the mechanism of the protective effect, we utilized fibronectin (FN) and bovine pancreatic ribonuclease (RNase). Nonglycosylated FN was degraded more rapidly than glycosylated FN by several proteases; this was due to the more rapidly degradation of the nonglycosylated fragment corresponding to the carbohydrate-rich, collagen-binding domain of the molecule. Other regions of the molecule were not more sensitive to proteases. Also, enzymatic removal of the charged terminal sialic acid did not significantly enhance the proteolytic susceptibility of the protein. When trypsin sensitivity of nonglycosylated RNase A was compared with three glycosylated species of RNase B which differed with respect to the size of the carbohydrate chain, RNase was digested 6-10 times more rapidly than fully glycosylated RNase B. RNase B forms with smaller carbohydrate chains were digested at intermediate rates, and chymotrypsin digestion was not as significantly influenced by the presence or size of the oligosaccharide chain. The clustering of carbohydrate chains in the collagen-binding domain of FN and the close proximity of the carbohydrate chain to the primary trypsin cleavage site of RNase suggest a direct steric hindrance of protease substrate interaction. The presence of carbohydrates may be biologically significant since i) intactness of the FN polypeptide chain is essential for its binding to collagen and ii) cleavage at the RNase tryptic sites results in loss of most of its enzymatic activity. Supported by NIH grant #R01-GM29804-01.

1478 EXPRESSION AND LOCALIZATION OF THE CLONED GENE FOR PHOSPHOLIPASE C FROM PSEUDOMONAS AERUGINOSA IN ESCHERICHIA COLI,
Stephen Lory, Jiaobin Ding, Phang C. Tai and Bernard D. Davis, Harvard Medical School, Boston, Ma. 02115.
It is not clear how gram-negative bacteria, with two membranes, secrete proteins outside the cell. To try to identify the genes involved in this process we have cloned into E. coli the gene for phospholipase C of P. aeruginosa, an enzyme that is secreted in large amounts. The gene was located in a 4.9 Kb fragment of Pseudomonas DNA, inserted into the BamHl site of the multicopy plasmid pBR322. It was expressed efficiently in E. coli, but with major differences from Pseudomonas: (i) In Pseudomonas the enzyme is repressed by inorganic phosphate, but in the E. coli recombinants either the plasmid or the host lacks some element in this regulatory system, and the enzyme is constitutive. (ii) Pseudomonas cells do not retain appreciable amounts of the enzyme, but in E. coli most of it is associated with the outer membrane and none is released to the medium. When the plasmid was modified by Tn5 insertion mutagenesis, or by insertion of a linker DNA at a unique StuI restriction site, studies in E. coli maxicells showed that the loss of phospholipase activity is associated with loss of a protein of M_r 80,000. Studies are in progress to see whether the enzyme held in the outer membrane is a precursor, to determine how it is held, and to try to identify a Pseudomonas gene(s), possibly absent in E. coli, that is required for completion of the process of secretion.

1479 SECRETION OF HUMAN INTERFERON-ALPHA FROM SACCHAROMYCES CEREVISIAE, Chung Nan Chang, L. Jeanne Perry, Ronald A. Hitzeman and Mark Matteucci, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080

The signal sequence of a yeast secretory protein was utilized to effect translocation of the human interferon-alpha (IFN-α2) through the Rough ER and the processes of the secretory pathway. Evidence to support these findings will be presented by analyses of both intracellular and extracellular products.

1480 EXPRESSION OF MATURE AND PRECURSOR HUMAN GROWTH HORMONE IN GRAM NEGATIVE BACTERIA, Gregory L. Gray and Herbert L. Heyneker, Genentech,Inc., South San Francisco, Calif.

Broad host range expression plasmids for mature human growth hormone and precursor human growth hormone were constructed for use in E. coli and other gram negative bacteria. The cellular location of the various products has been investigated.

1481 PROCESSING OF HUMAN PRE-β-INTERFERON IN E.COLI, Nina Irwin and Mark Ptashne, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138

We have expressed in E.coli two proteins, one with the primary sequence of mature human β-interferon and one with that of pre-β-interferon. In many strains of E.coli, pre-β-interferon is not processed and is instead rapidly degraded by E.coli proteases. In one strain of E.coli, however, this molecule is processed to a form slightly larger than the mature form of the protein. The "processed" interferon is found in E.coli membranes and is more stable than its precursor form.

1482 TRANSPORT OF HEMOLYSIN IN BACTERIA, Werner Goebel, Michael Härtlein, Sigrid Schießl and Jürgen Kreft, University of Würzburg, W-Germany

Hemolysin belongs to the very few proteins in Gram-negative bacteria, which are transported across the inner and the outer membrane. Transport of hemolysin in E. coli across the inner membrane occurs only after proteolytic processing of a large precursor protein, which is hemolytically inactive and is not secreted across the inner membrane. Transport of active hemolysin across the outer membrane requires a specific system consisting of two proteins which are located in the outer membrane. The genes required for synthesis of hemolysin and its transport are clustered on transmissible plasmids or the chromosome. Transfer of genes for hemolysin of a Gram-positive microorganism into E. coli leads to poor expression and partial secretion of the hemolysin across the inner but not the outer membrane, whereas in B. subtilis synthesis and transport of this hemolysin are as efficient as in the natural host.

1483 SYNTHESIS AND EXPRESSION OF AN **E.COLI** LIPOPROTEIN SIGNAL SEQUENCE - HUMAN INTERFERON HYBRID GENE. M D Edge and P A Meacock, ICI Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire SK10 4TG - ICI/University Joint Laboratory, University of Leicester, Leicester LE1 7RH. UK

A DNA fragment encoding Escherichia coli lipoprotein signal sequence (LPPss) has been chemically synthesised and joined to a synthetic human interferon - α1 (IFN-α1) gene. IFN-α1 is a secreted eukaryotic protein with cysteine as the first amino acid of its mature form; a property it has in common with lipoprotein. Since the potential cleavage point of the hybrid LPPss -α1 is identical to that of natural prolipoprotein, processing and export of mature IFN -α1 may be expected. The hybrid LPPss -α1 gene was placed under transcriptional control of a lac UV5 and a synthetic trp promoter on a plasmid vector. The trp promoter vector directs the formation in E.coli mini cells of the hybrid precursor form (MW$\sim$21,000). A protein corresponding in size to the processed product (MW$\sim$19,000) is also detected but in smaller amounts. E.coli cells producing the precursor peptide under conditions of trp promoter induction, either grow slowly or lyse readily. This suggests that the hybrid precursor protein is interfering with natural membrane biosynthesis. Experiments to analyse the distribution of the products of the gene between cytoplasm, periplasm and membrane fractions will be described.

1484 TWO DISTINCT ATP-DEPENDENT STEPS FOR PROTEIN BREAKDOWN IN RETICULOCYTES, Keiji Tanaka, Lloyd Waxman and Alfred L. Goldberg, Harvard Medical School, Boston, MA 02115

Protein degradation in rabbit reticulocytes is a nonlysosomal process requiring ATP. In this pathway, evidence has been presented that ATP is required for the covalent conjugation of ubiquitin to protein substrates. To test this hypothesis, we have blocked completely the amino groups of casein and albumin by methylation, acetylation, carbamylation and succinylation to prevent ubiquitin conjugation. These modifed proteins were still degraded by an ATP-stimulated process. The hydrolysis of proteins lacking amino groups and unmodified proteins was inhibited by hemin and by vanadate, an inhibitor of the ATP-dependent proteases in E. coli and mitochondria. These data suggest two dissociable actions of ATP, one which requires ubiquitin and one which does not. One possible candidate for the latter step is a cytoplasmic high molecular weight protease (550,000 daltons) that is directly stimulated twofold by ATP. We have purified this enzyme from red blood cells. The purified enzyme has three different endoproteolytic active sites as judged by their inhibitor sensitivities and substrate specificities. The three activities were inhibited by inhibitors of serine proteases and by sulfhydryl reagents. One site digests casein, globin and albumin and is activated by ATP; two others cleave different fluorogenic peptides in an ATP-independent fashion. Since all three activities are optimal around pH 8.0 and are inhibited by hemin, this novel enzyme complex appears to play an important role in the ATP-dependent pathway for degrading proteins.

1485 EXPRESSION AND STABILITY OF THE HSV I THYMIDINE KINASE GENE IN E. COLI, David L. Hare and John R. Sadler, University of Colorado Health Sciences Center, Denver, Colorado 80262.

The Herpes Simplex I Thymidine Kinase (HSV I TK) gene was expressed in E. coli by inserting a 203 bp lac UV5/L8 promoter-operator segment, in frame, 53 nucleotides 5' to the native TK translational start codon. The hybrid gene created by this fusion encodes a polypeptide which has 25 additional amino acids on the amino terminus of the HSV TK protein and phenotypically complements a tdk mutation of E. coli. This fusion polypeptide has been characterized by maxicell, immunoprecipitation, and native gel techniques and its activity is inhibited by anti-HSV antibody. In a TK expressor strain containing an episomal $lacI^q$ (overproduction of the lactose repressor) we get greater than 1000-fold coordinate regulation of the plasmid- encoded TK and chromosmal β-galactosidase activities with the inducer IPTG. Pulse-chase induction demonstrates the fused TK polypeptide to be as stable as β-galactosidase. HSV TK-specific RNA isolated from this strain has a short half-life, similar to many bacterial messages.

1486 CONSTRUCTION OF A PLASMID CONTAINING UNIQUE RESTRICTION SITES AT THE BEGINNING AND AT THE END OF THE SIGNAL CODONS OF THE β-LACTAMASE GENE BY SITE-SPECIFIC MUTAGENESIS: THE CONSEQUENCES OF THE PRECISE EXCISION OF THE SIGNAL SEQUENCE, James T. Kadonaga, Antoine E. Gautier, Andrew D. Charles, Michael D. Edge and Jeremy R. Knowles, Harvard University, Cambridge MA 02138

Using the 'piggyback' approach to oligonucleotide mediated site-specific mutagenesis, a derivative of pBR322 has been constructed which contains a unique EcoRI restriction site right at the beginning of the signal codons of the β-lactamase (bla) gene and a unique Bst EII site just at the end of the bla signal codons. Although the signal peptide encoded by the new plasmid (pTG2) differs from the wild type (pBR322) by two amino acid residues (Ser_2 to Arg_2 and Ala_{23} to Gly_{23}), the synthesis, transport, and processing of β-lactamase remain unchanged. The new plasmid has been designed to allow the study of the translocation and processing of the β-lactamase, by the construction of a number of point and deletion mutations in the signal codons of the bla gene. In addition, pTG2 might be used as a cloning vehicle for the export of proteins out of the cytoplasm. Two deletion mutants, in which the bla signal codons have been almost completely excised, have also been constructed. Bacteria containing either of these plasmids produce, but apparently cannot secrete, an active β-lactamase.

1487 EXPRESSION OF CALF CHYMOSIN (RENNIN) IN E. COLI, Peter A. Lowe, Spencer Emtage, Saroj Angal, Timothy J.R. Harris, Mike A.W. Eaton and Mike T. Doel, Celltech Limited, 250 Bath Road, Slough, SL1 4DY, England.

The gene for calf met-prochymosin obtained in this laboratory (Harris, T.J.R., Lowe, P.A., Lyons, A., Thomas, P.G., Eaton, M.A.W., Millican, T.A., Patel, T.P., Bose, C.C.,Carey, N.H. and Doel, M.T., Nucleic Acids Research (1982), 10, 2177-2187) has now been expressed in E. coli at high levels using a trp promoter/pAT153 construct. Like the natural enzyme the prochymosin produced in E. coli may be acid matured to a form exhibiting milk clotting activity and sensitivity to pepstatin the classical inhibitor of chymosin.

General Subjects

1488 THE EFFECT OF DENERVATION ON ENKEPHALIN-CONTAINING PEPTIDES AND CATECHOLAMINES IN RAT ADRENAL GLANDS. G. Fleminger, E. Ezra, R. Howells, D. L. Kilpatrick, and S. Udenfriend, Roche Institute of Molecular Biology, Nutley, N. J. 07110

We previously showed that enkephalin-containing peptides (EC-peptides) are markedly increased in rat adrenal glands after denervation (1). On reinvestigating this phenomenon, we found that in unilaterally denervated rats there was a lag of 12-24 hr before EC-peptides increased. Maximal increase (ca. 12-fold) was at 72 hr and mainly in the largest EC-peptides, representing either proenkephalin or large fragments of it. There were only small increases in free enkephalins and in intermediate-size EC-peptides even after 96 hr, indicating an extremely low rate of processing. As before, there were no increases in the contralateral innervated glands at any time, nor in adrenals of sham-operated rats. By contrast, catecholamines (CA) increased in both denervated and innervated glands within 30 min of transection, with maximal levels (6-9-fold) at 12 hr. There were similar increases in sham-operated rats, indicating that the effects were due to the surgery. By 24 hr CA levels in both denervated and innervated glands had returned to normal. This clear dissociation between the effect of denervation on enkephalins and on CA is interesting since both are present in the same chromaffin granules and are released concomitantly by nerve stimulation. The immediate increase in adrenal CA is probably due to activation of preformed CA synthesizing enzymes. The long lag period and the fact that the increase is mainly in what appears to be the gene product proenkephalin suggests that activation in this case is at the transcriptional level.

1. Lewis, R.V., et al., (1981) J. Neurosci. 1, 80-82.

1489 N-GLYCOSYLATION IS REQUIRED FOR MAINTENANCE OF FUNCTIONAL SODIUM CHANNELS IN NEUROBLASTOMA CELLS, Charles J. Waechter*, John W. Schmidt and William A. Catterall, *University of Maryland School of Medicine, Baltimore, Maryland 21201 and University of Washington School of Medicine, Seattle, Washington 98195

The addition of tunicamycin (TM) to the growth medium (1 μg/ml) inhibited N-glycosylation by 80-94% while protein synthesis was still 80-90% of control values. When cultured neuroblastoma cells (N18) were grown in the presence of TM, the number of sodium channels, as measured by high affinity saxitoxin (STX) binding, was reduced to 20-28% of control values over a 60 hour period. A Scatchard plot of the binding data revealed that growth in the presence of TM reduced B_{max} from 69 fmol/mg to 14 fmol/mg without a significant change in the K_D (2.7 nM). Neurotoxin-activated $^{22}Na^+$-influx mediated by the sodium channel was similarly reduced. TM also caused a reduction in cell surface STX-receptors in the presence of chloroquine (CQ) suggesting that the block in N-glycosylation did not reduce the number of sodium channels solely by accelerating sodium channel degradation. After the washout of TM, STX receptors returned at a rate of 1930/cell/hr. The reappearance of sodium channels required protein synthesis. When sodium channel biosynthesis is blocked by either cycloheximide or TM the number of STX receptors are lost at a rate of 1730/cell/hr. These results demonstrate that N-glycosylation is required for the maintenance of normal levels of the sodium channel in neuroblastoma cells.

1490 ISOLATION AND CHARACTERIZATION OF RECOMBINANT DNA CLONES PREFERENTIALLY EXPRESSED IN FEMALE FLIES OF DROSOPHILA MELANOGASTER, Uwe Walldorf, Bernd Hovemann and E.K.F. Bautz, Institut fur Molekulare Genetik, Heidelberg, F.R.G.

A collection of cDNA clones derived from RNA of two days old flies has been isolated. By screening with (A)+ RNA from male and female flies, respectively, we were able to isolate a small number of clones which showed a stronger hybridization signal for RNA from female flies than from male ones.

Two classes of positives have been identified: (a) clones coding for yolk protein precursors, whoseexpression is under hormonal control and female specific, (b) clones showing a five to ten fold higher RNA expression in female compared to male flies. Two different genomic clones have been found hybridizing to the second group of cDNA(B). They map to the chromosomal loci 48D and 100E, crosshybridize considerably and code for a protein of 50KD MW. The sex specific epxression of this gene family will be discussed in detail.

1491 PERIODIC BINDING PROTEIN SYNTHESIS IN E. COLI, James J. Anderson and Stephen Cooper, University of Michigan, Ann Arbor, MI 48109.

Tests for periodicity in the rates of protein synthesis during the E. coli division cycle have given negative results (Lutkenhaus et al., J. Bacteriol. 138: 352-360, 1979). However, other reports implicate involvement of cell division in the synthesis, or accumulation, of secretory and/or membrane associated proteins (cf. Shen & Boos, Proc. Nat. Acad. Sci. USA 70:1481-1485, 1973). We reexamined this question by quantitating rates of synthesis of the periplasmic leucine binding protein during the cell cycle of E. coli strain B/r. We avoided artifacts by pulse labelling random exponentially growing cells and secondarily separating "new born" cells as a function of time in the Cooper-Helmstetter "baby machine". Two-minute cell samples were gathered over 100 minutes, and a C^{14} labelled culture added as an internal standard. Periplasmic proteins were obtained from one portion of each sample and the leucine binding protein recovered by immune precipitation and SDS gel electrophoresis. Gel slices and total cell incorporation samples were oxidized to separate isotopes in a Packard sample oxidizer and the ratio of H^3/C^{14} for each sample determined. The results showed a two-fold periodic variation in binding protein synthesis rates over the cell cycle, with "new-born" cells having the highest rate. Kinetics of synthesis of the leucine binding protein in asynchronous cultures revealed a 3-minute lag in appearance of full-length binding protein in the periplasm (see also Ito et al., Cell 11, 551-559, 1977) and a curious 25% overshoot phenomenon of total binding protein synthesis. The cumulative data may be explained by either differential synthetic or turnover rates during the cell cycle.

1492 STUDIES ON STRUCTURAL-FUNCTIONAL CORRELATIONS OF THE INFLUENZA HEMAGGLUTININ IN TERMS OF DIRECTIONAL TRANSPORT, Nancy L. McQueen, Alan Davis, Debi P. Nayak, UCLA School of Medicine, Department of Microbiology & Immunology, Los Angeles, CA. 90024

The influenza hemagglutinin (HA) has been expressed in a eukaryotic system using an SV40 cloning vector. This expressed HA has been shown to migrate to and insert into only the apical region of polarized epithelial monkey kidney cells. This is in agreement with previous findings that the HA is localized to only the apical surface of epithelial cells and the virus itself buds only from the apical region. It has been shown that glycosylation is not responsible for the directional transport of the HA. Therefore, since the cloned HA migrates to the appropriate region, it is likely that features of its polypeptide backbone are responsible for its directional transport. The G protein of vesicular stomatitis virus in contrast to the HA of influenza is localized only to the basolateral region of polarized epithelial cells. To determine regions on the molecules which are responsible for the directional transport, hybrid genes were constructed in which specific areas of the HA gene were replaced by those of the VSV-G gene and the site of membrane insertion of these hybrid proteins was determined.

1493 Functional Implications from the Crystal Structure of Alamethicin. Robert O. Fox, Jr. and Frederic M. Richards, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511

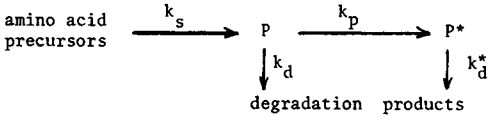
Alamethicin is a linear peptide antibiotic of 20 residues which spontaneously partitions into membranes and forms voltage-gated ion channels as an aggregate of 8 to 10 monomers. Thus, alamethicin serves as a useful model membrane protein, amenable to biophysical analysis. In an effort to characterize a molecular structure of the peptide similar to that found in lipid bilayers we have prepared crystals of alamethicin which diffract to 1.0 Å resolution from a methanol acetonitrile solvent system. The structure has been solved by isomorphous replacement and refined with geometric restraints at 1.5 Å resolution to a crystallographic residual of 16%. The three crystallographically independent alamethicin molecules are each in a predominantly α-helical conformation although they differ in the position of short 3_{10} helical regions and the extent of a bend induced by a central proline residue. Most of the solvent accessible polar groups lie on a narrow strip parallel to the helix axis resulting in a highly amphipathic molecular surface. A series of molecular models of the alamethicin ion channel have been constructed based on the monomer conformation seen in the crystal, which exhibit n-fold symmetry, highly complementary helix packing, and a polar channel interior. An annulus of Gln-7 residues provides the greatest restriction of the channel inner diameter. The Gln-7 annulus model can be hydrated to produce a clathrate-like structure. A molecular mechanism for voltage-gating of the alamethicin channel which has been inferred from the models will be presented. Supported by grant USPHS-GM-21714-08.

1494 ON THE CHARACTERIZATION OF MEMBRANE-ASSOCIATED PROTEINS BY PHASE PARTITION IN TRITON X-114, Pamela A. Maher and S.J. Singer, University of California at San Diego, La Jolla, CA 92093

The experimental discrimination of integral from peripheral membrane proteins, and the possibility of a finer discrimination among integral proteins of different structures, are the subjects of this study. The phase separation technique of Bordier (J.Biol.Chem.256,1604(1981)) was applied to the membranes of red blood cells, Torpedo californica electroplax, kidney microsomes, sarcoplasmic reticulum, rod outer segments, and vesicular stomatitis virus(VSV). This technique relies on the properties of Triton X-114, which forms a homogeneous solution at 0°C but separates into detergent-rich(DRP) and detergent-poor(DPP) phases above 20°C. As Bordier showed(loc.cit.), integral proteins such as glycophorin partition nearly completely into the DRP, whereas peripheral proteins such as spectrin go into the DPP. Similarly, the VSV G-protein and rhodopsin partition into the DRP. However, band 3 protein, the Ca^{++}-ATPase, and the Na^{+},K^{+}-ATPase show nearly equipartition between the DRP and DPP. Moreover, the acetylcholine receptor(AChR), clearly an integral protein, partitions completely into the DPP, in which it retained its multisubunit structure and binding capacity for α-bungarotoxin. The results of the phase separation technique must therefore be interpreted with caution, because we have shown that at least one integral protein(AChR) partitions indistinguishably from peripheral proteins. In addition, the technique may be useful in discriminating between integral proteins that exist either as monomers or as multimers(and/or pore-formers) in the membrane. (Supported by USPHS grant AI-0665).

1495 MATHEMATICAL MODEL FOR PROTEIN DEGRADATION AND POSTTRANSLATIONAL PROCESSING, Raymond D. Mosteller and Bruce E. Goldstein, USC School of Medicine, Los Angeles, CA 90033, and Jet Propulsion Laboratory, Pasadena, CA 91103.

A mathematical model has been developed that describes degradation and posttranslational processing of proteins in nongrowing and exponentially growing cells. The model is based on the following scheme:



where P* represents the modified form of protein P, which is synthesized according to the rate constant k_s, and k_d, k_d^* and k_p represent the first order rate constants of degradation and processing. Solutions to the general equations below have been derived and were used to calculate and plot theoretical results for pulse-chase and continuous labeling experiments using varying values for the rate constants and growth rate. The model can also be applied to any cellular component which is degraded or processed by first order kinetics.

$$dP/dt = k_s F(t) - (k_d + k_p)\,P \qquad dP^*/dt = k_p P - k_d^* P^*$$

$F(t)$ = growth rate function

1496 MOLECULAR CLONING AND DNA SEQUENCE ANALYSIS OF YEAST α-FACTOR STRUCTURAL GENES, Grant A. Bitter and Kenneth K. Chen, AMGen, Thousand Oaks, CA. 91320

Yeast α-factor is a thirteen amino acid peptide pheromone of known sequence which is secreted into the media by haploid cells of α mating type. We have used a 25 base[1] synthetic oligonucleotide as a hybridization probe to clone the α-factor structural genes from a Saccharomyces cerevisiae genome library. The α-factor genes have been subcloned on a 2.0kb EcoRI fragment, and the complete DNA sequence of the coding region has been determined. These results indicate that yeast α-factor is synthesized as a pre-polyprotein which contains four copies of α-factor peptide at the C-terminus. The α-factor peptides are separated by spacer peptides with the canonical sequence Lys-Arg-Glu-Ala-Asp-Ala-Glu-Ala. The first spacer peptide (amino terminal to first α-factor peptide) is missing an Asp-Ala dipeptide while the spacer between the first and second α-factor peptide contains a Glu instead of Asp at position 5 of the spacer. Although there is heterogeneity in the DNA sequence, the amino acid sequence of all four α-factor peptides is invariant. The pre-polyprotein contains an amino terminal extension, the sequence of which will be presented.

The utility of the α-factor system for secretion of foreign peptides from yeast is currently being investigated.

1. Kurjan, J. and Herskowitz, I. (1981) Cold Spring Harbor Meeting, The Molecular Biology of Yeast, Abstract No. 242.

1497 IDENTIFICATION AND CHARACTERIZATION OF A STRUCTURAL GENE FOR THE YEAST PEPTIDE MATING PHEROMONE, a-FACTOR. A. Brake[1], J. Merryweather[1], R. Najarian[1] and J. Thorner[2], [1]Chiron Corporation, Emeryville, CA 94608 and [2]Department of Microbiology and Immunology, University of California, Berkeley, CA 94720.

Haploid cells of the a mating type of the yeast Saccharomyces cerevisiae secrete a mating pheromone, called a-factor. Two peptides with a-factor activity have been reported by Duntze and his co-workers (1): H_2N-TyrIleIleLysGlyValPheTrpAlaAsxPro-COOH and H_2N-TyrIleIleLysGly-LeuPheTrpAlaAsxPro-COOH. Using a synthetic oligonucleotide probe complementary to a portion of the predicted nucleotide sequence of the a-factor coding region, two discrete chromosomal segments were isolated from a library of cloned yeast DNA fragments. DNA sequence analysis of one of these segments indicates that it could potentially code for a larger precursor polypeptide which, unlike the α-factor precursor, contains only a single copy of the Val-containing molecule at its carboxyl terminal end. Immediately preceding the a-factor sequence is a pair of basic residues which, as is the case for other peptide hormone precursors, provides a site for endoproteolytic cleavage. Again unlike the α-factor precursor, the a-factor precursor appears to lack any region with a repeating $(-Glu/Asp-Ala-)_n$ structure. While the a-factor sequence contained in the precursor is highly homologous to that of the peptides analyzed by Duntze et al, it is not completely identical. Nevertheless, it seems that the cloned segment is an expressed and functional a-factor gene because a complementary polyA+ RNA is found only in a cells and because presence of the cloned gene on a multi-copy plasmid causes a cells to overproduce a-factor activity at least 10-fold as determined by a bioassay.

1. Betz, R., Manney, T.R., and Dunzte, W. (1981) Gamete Res. 4: 571-584.

1498 MOLECULAR ORGANIZATION OF BACTERIORHODOPSIN AND RHODOPSIN, Najmutin G.Abdulaev, Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, Moscow, USSR.

Bacteriorhodopsin and rhodopsin are integral membrane proiens containing retinal as a light sensitive group. Light absorption by these proteins results in generation of a conciderable electric potential. It is known that the light energy is used by halophilic microorganisms for ATP synthesis and other vital functions. In the visual cells light absorption results in activation of ionic channels or enzymic systems.

Comparative study of the structural organization of these proteins was a necessary step in the elucidation of their functioning as light transducers. The complete amino acid sequences of bacteriorhodopsin and bovine rhodopsin were established and the arrangement of their polypeptide chains in the purple membrane and photoreceptor membrane, respectively, was determined. It is demonstrated that much more groups of the polypeptide chain are exposed into the aqueous phase in bovine rhodopsin than in bacteriorhodopsin. The polypeptide chain of rhodopsin traverses the photoreceptor membrane seven times; the N and C - terminal parts being located at the opposite sides of the membrane. A general feature for both bovine rhodopsin and bacteriorhodopsin is the location of the retinal-binding lysine residue at the C-terminal intramembrane segment (Lys-296 and Lys-216, respectively).

Modification of some functional groups in bacteriorhodopsin and rhodopsin and its influence on their biological functions were investigated. Particularly, different modifications of amino groups in bacteriorhodopsin do not affect the proton translocating function. Modification of carboxyl groups conciderable changes the photochemical cycle and proton transport function.

1499 CHEMICAL CHARACTERIZATION AND SPATIAL ORGANIZATION OF Na^+,K^+-ATPase. Nikolai N.Modyanov, Karine N.Dzhandzhugazyan, Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, Moscow, USSR.

Immunochemically homogeneous Na^+,K^+-ATPase has been isolated from outer medulla of pig kidneys. Two types of subunits, α and β, in an equimolar ratio compose the enzyme molecule. Their molecular weights are: 96 KD - for the α-subunit, 40 KD and 7 KD - for protein and carbohydrate moieties of the β-subunit, respectively. In membrane-bound Na^+,K^+-ATPase the subunits form oligomeric complex $\alpha_4\beta_4$, being apparently a functional unit of the enzyme. It follows from stoichiometry of alcylating ATP analog binding, sufficient for complete inhibition of the enzyme. The amino acid and carbohydrate compositions of the subunits have been determined and their N-terminal amino acid sequences have been found: H-Gly-Arg-Asx-Lys-Tyr- for α, and H-Ala-Asn(CHO) Gly-Thr-Ala- for β.

The N-terminal part of the β-subunit is exposed on the outer surface of the cell membrane, since one of its carbohydrate chains attaches to the Asn-2 residue. Spatial organization of Na^+, K^+-ATPase in the membrane has been studied using rabbit and pigeon antibodies against the enzyme and different preparations of the subunits. With intact cells and "inside-out" proteoliposomes as model systems and by diverse independent immunochemical methods antigenic determinants of the α-subunit have been found on both sides of the membrane. It allows us to suggest that the α-subunit participates in formation of a transmembrane cation-specific pathway in the enzyme molecule.

1500 EFFECT OF CHEMICAL AND PHYSICAL MUTATION REACTIONS ON HISTONES OF SNAILS , SPECIFIC INTERMEDIATE HOSTS FOR HUMAN SCHISTOSOMIASIS, Ibrahim M. Nabih and Maha S.Rizk , National Research Centre , Dokki, Cairo , EGYPT.

Studies on histones in both types of snails , Biomphalaria and Bulinus specificintermidiate hosts for the world wide human parasitic disease, Schistosomiasis , had shown that all sulpher containing amino-acids along with tryptophan are missing . Both of the aromatic amino-acids tyrosine and phenylalanine are poorely represented .
No change in the amino-acids pattern was shown in histones isolated from snails chemically pretreated with either of the mutagens , 5-bromouracil and the thiaxanthone analogue 1-(ß-diethylamino-ethylamino)-3,4-cyclohexeno - thiaxanthone .
On the other hand , histones isolated from snails pretreated with - radiation , 10.000 rad , showed destruction in the total protein amount accampanied with decrease in the amino-acids concentration .
This decrease in both of the total protein amount and the amino-acids concentration was more demonstrated in histones isolated from snails preradiated with 20.000 rad .

1501 PLASTIDS IN THE DEVELOPING ENDOSPERM OF RICINUS COMMUNIS. J.A. Miernyk, S.A. Boyle, P.S. MacDougall and D.T. Dennis, Biol. Dept., Queen's University, Kingston, Ontario, Canada. K7L 3N6

Leucoplasts in the developing endosperm of castor oil (R. communis) seeds are plastids specialized for the conversion of photosynthetically produced carbohydrate into fatty acids. We have previously detailed the occurrence of a complete set of glycolytic isozymes localized in the leucoplasts, as well as pentose-P isozymes (except glucose-6-P dehydrogenase), the pyruvate dehydrogenase complex, acetyl-CoA carboxylase, acyl carrier protein and fatty acid synthetase. Incubation of isolated, intact plastids with labeled glycolytic intermediates yields results completely consistent with the isozyme localization data. Preliminary examination of plastids from other sources supports the use of Ricinus leucoplasts as a model system. It is known from formal genetic analysis that spinach chloroplast aldolase and glucose-P isomerase, and Clarkia chloroplast glucose-P isomerase are coded for by nuclear DNA. No data is available for any other glycolytic or pentose-P enzyme from any plant source. We have begun to examine developing castor oil seeds for the site of transcription and translation of phosphofructokinase, enolase, pyruvate kinase and 6-phosphogluconate dehydrogenase. Initial results suggest that, in analogy to the small subunit of ribulose 1,5-P_2 carboxylase,plastid enolase is coded for by nuclear DNA, synthesized in the cytosol as a high M_r precursor, and transported post-translationally into the leucoplasts. Libraries of plastid and nuclear DNA are being assembled in order to probe unambiguously the coding location for the glycolytic and pentose-P enzymes.
Supported by NSERC of Canada.

1502 CONCOMITANT ALTERATION OF THE PEPTIDE COMPOSITION OF THE CYTOPLASMIC MEMBRANE AND $\Delta\Psi$ ENERGIZED AMINOACID TRANSPORTS AFTER METHOTREXATE TREATMENT IN S. PNEUMONIAE. COMPARISON OF WILD TYPE STRAIN AND METHOTREXATE RESISTANT MUTANTS. Michèle Gherardi and Marie-Claude Trombe, Centre de Recherche de Biochimie et de Génétique Cellulaires du CNRS Toulouse, France.

In S. pneumoniae isoleucine accumulation is monitored by a single $\Delta\Psi$ energized transporter. When wild type bacteria are submitted to sublethales concentrations of methotrexate (MTX), Vi of isoleucine transport increases from its standard valine 5.7 nmol./min/mg proteins to 19.8 nmol./min/mg proteins. No change is observed in Vi of glutamine uptake which is $\Delta\Psi$ independant. This effect occurs in the absence of protein synthesis. Analysis of the membrane composition of treated and non treated wild type bacteria in bidimension electrophoresis (O-Farrell gels) show four additional spots in the membrane of MTX treated bacteria. The results obtained suggest strongly that these spots correspond to proteins which are solubilized during the sonication of the non treated bacteria while they remain in the membrane in MTX treated bacteria.
A set of MTX resistant mutants : the amiA mutants do not exhibit change in their membrane properties either physiological of structural after similar MTX treatment. Therefore in S. pneumoniae the membrane appears as a primary larger for MTX. Interaction of MTX with the membrane leads to the alteration of dependent biological activity and protein arrangement in the membrane.

1503 TOPOGRAPHY OF NASCENT ALBUMIN EPITOPES, A.V. LeBouton, College of Medicine, University of Arizona, Tucson, AZ 85724

Previous immunocytochemical studies of nascent albumin have shown that: epitopes of albumin are apparently present on the cytosolic surface of bound ribosomes and the cisternae of the RER are devoid of free albumin. Neither of these findings agree with current dogma. The presence of albumin on ribosomes was tested by injecting rats with puromycin to abort nascent chains from their ribosome of conception. The ribosomes became invisible, but reappeared when the same section was stained non-specifically with uranyl acetate. The lack of free intracisternal albumin was then studied. To see if underfixed albumin was being leached from cisternae, the concentration of formaldehyde was doubled to 8%, or fixation was by perfusion rather than immersion. Lumina were still empty of albumin and bound ribosomes remained positive. To see if antibody was unable to enter cisternae in sufficient amounts, the length of contact with antisera was tripled to three hours or Triton X-100 (0.3%) was added. In both cases, the ribosomal reaction for albumin epitopes was increased but there was still no free albumin in cisternae. Finally, the possibility was explored that optimal, accurate, conditions existed only at the surface of specimens. There was a surface effect, but only in that the ribosomal reaction for albumin was more intense; there was still no albumin free within the cisternae. In some surface areas, however, unknown forces had greatly dilated the lumen of the RER. In these sites, a layer of membrane-associated albumin was found adjacent to the luminal side of the RER membrane, trans to the overlying positive bound ribosomes.

1504 MYO-INOSITOL BINDING PROTEIN FROM PSEUDOMONAS PUTIDA, Dominique Feiss, Joachim Frey and Jacques Deshusses, University of Geneva, Geneva, Switzerland.

We have recently isolated a periplasmic binding protein from a Pseudomonas putida specific for myo-inositol. In order to study its biosynthesis, pulse labelling and immuno precipitation were undertaken. A faint band appears in polyacrylamid gel electrophoresis being 1800 daltons heavier than the mature protein (30000 daltons). This band, suspected to be a precursor, disappears upon chase and is increased if the cells were preincubated with procaïne or TAME. Fingerprints would acertain this band as being the precursor.

The genetics of the myo-inositol transport system has been undertaken in this strain. Transport mutants, affected in the affinity and in the capacity, have been isolated.

1505 DOMAIN STRUCTURE AND IN VITRO ASSEMBLY OF PROCOLLAGEN I TRIMER, Kurt J. Doege and John H. Fessler, University of California, Los Angeles, CA 90024

A prerequisite for secretion of the procollagen I molecule is its intracellular assembly from two proα1 and one proα2 chains. Each chain has a distinct domain structure, a central collagen helix flanked by globular amino and carboxyl propeptides. Each propeptide contains internal disulfides, but the carboxyl propeptide is the site of interchain disulfides cross-linking the trimer. The following in vitro experiments strongly support the hypothesis that the carboxyl propeptides are instrumental in correct chain selection and assembly. The role of the various domains in the assembly of procollagen was studied in vitro by reducing the various disulfide bonds under dissociating conditions, and then monitoring by SDS-PAGE the glutathione-facilitated recovery of native structure. The collagenase-released C-propeptide undergoes sequential reduction, first of the interchain disulfides, then the intrachain disulfides, forming intermediates recognizable on SDS-PAGE. As denaturing agent is removed under disulfide-exchange promoting conditions, the folded monomeric structures are reformed first, followed by association to trimers of the correct chain composition. The disulfides formed in refolding have been confirmed as faithful to the originals by peptide mapping on HPLC. The effect of the other two domains on the refolding was also studied, as well as several of the refolding reaction conditions. The carboxyl propeptide folds in vitro to its native monomeric structure, and subsequently interacts specifically to assemble procollagen trimer.

1506 INTRACELLULAR ASSEMBLY, SECRETION AND SUPRAMOLECULAR COMPLEX FORMATION OF PROCOLLAGEN IV, John H. Fessler, Keith G. Duncan and Liselotte I. Fessler, Molecular Biology Institute and Biology Department, UCLA, Los Angeles, CA 90024.

The intracellular assembly of procollagen molecules prepares them for secretion and subsequent incorporation into extracellular, supramolecular complexes. Mechanisms must exist that assure sequential assembly. Each procollagen molecule consists of three proα chains, and folding of the carboxyl propeptide domain of each chain is instrumental in selection and juxtapositioning of three gene products for folding into a collagen triple helix. Interference in helix formation prevents export of the fiber-forming procollagens, but not of procollagen IV, which does not form fibers, and instead becomes incorporated into a molecular net that remains associated with cells as basement membrane. The amino and carboxyl ends of procollagen IV are specialized to form the junction points of these nets. We find that while the carboxyl propeptides of the fiber-forming procollagens are internally disulfide linked, which probably assists collagen helix formation, those of procollagen IV are not. This is associated with a slower assembly and secretion of these molecules. Extracellularly, the carboxyl propeptides of two adjacent procollagen IV molecules form a disulfide-linked junction. The amino ends of four procollagen IV molecules spontaneously associate in vitro to form the other network junction, and subsequently link covalently by disulfide exchange. We conclude that disulfide bridge formation is regulated during the intracellular sojourn of these molecules, and presumably some S-S links rearrange after secretion. We find that disulfide components of cell culture media can interfere in this process, and this has implications for the culture of cells that utilize secreted, disulfide-linked proteins.

1507 DIFFERENTIAL TURNOVER OF EXOGENOUS MEMBRANE POLYPEPTIDES IMPLANTED INTO THE HEPATOMA CELL MEMBRANE, James F. Hare, Michael Huston, and Phoebe Rich, Oregon Health Sciences University, Portland, Ore 97201.

In order to study turnover and recycling of cellular membrane polypeptides, we have developed systems to implant labeled membrane components into the cell membrane of hepatoma monolayer cultures. In one system we directly fuse iodinated red cell ghost membranes into hepatoma cell membranes with Sendai virus. In another system we inlay purified membrane polypeptides into liposomes derived from extracted Sendai envelopes (virosomes). When delivered to the cells in virosomes, rat liver mitochondrial cytochrome c oxidase polypeptides were degraded at about the same rate ($T_{1/2}$=16-18 h) as were endogenous, lactoperoxidase accessible hepatoma polypeptides. Human red cell membrane polypeptides, by contrast, when implanted into hepatoma cell membranes by viral mediated fusion became stably incorporated. These results indicate that cellular membrane degradation system(s) are selective but apparently do not specifically recognize implanted proteins as foreign. Cytological and biochemical studies will reveal the membrane disposition and ultimate cellular fate of implanted polypeptides.

1508 STRUCTURE-FUNCTION STUDIES WITH FUMARATE REDUCTASE OF ESCHERICHIA COLI, Joel H. Weiner, Bernard Lemire and John J. Robinson, Department of Biochemistry, University of Alberta, Edmonton, Canada T6G 2H7.

Fumarate reductase (FR) is the membrane-bound terminal electron transfer enzyme in E. coli grown anaerobically on glycerol-fumarate. The enzyme is made up of a 2-subunit catalytic head (69 Kd flavin containing and 27 Kd non-heme iron containing subunits) and a membrane anchor composed of two extremely hydrophobic subunits of 15 and 13 Kd. E. coli carrying a recombinant plasmid with the frd operon produces membranes in which FR is 75% of the membrane protein. The total protein content of the membrane doubles, totally due to the amplification of FR. Interestingly, other membrane-bound activities such as glycerol-3-P dehydrogenase D(-)lactate dehydrogenase and F_o/F_1 ATPase remain unaltered and thus are not displaced by FR amplification. The membranes are covered with 50 Å knobs on the cytoplasmic surface when negatively-stained and viewed in the electron microscope. These knobs are the catalytic 2-subunit head. This portion of the enzyme has been highly purified and is active on its own but requires anions for optimal activity and thermo- and alkaline stability. Addition of the anchor polypeptides removes the anion dependence and greatly stabilizes the enzyme. The knobs can be removed from the membrane by a urea wash leaving the anchor polypeptides intact. Purified catalytic heads can be specifically and functionally reconstituted with the membrane-bound anchors.

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1509 DO MITOCHONDRIA SHARE ENZYMES WITH THE REST OF THE CELL?, Anita K. Hopper[1] and Nancy C. Martin[2], [1]M.S. Hershey Medical Center, Hershey, PA 17033, and [2]University of Texas Health Science Center, Dallas, TX 75235

It has been a commonly held notion that proteins with analogous functions in the mitochondria and elsewhere in the cell are encoded by separate genes. However our genetic and biochemical studies have shown that mutations in single nuclear genes can affect enzymes in both the mitochondrial and the nuclear/cytoplasmic compartments. Specifically, S. cerevisiae nuclear mutations, trm1, trm2 and mod5-1, abolish the activity of tRNA modifying enzymes - (guanosine-N^2,N^2)-dimethyltransferase, (uridine-5)methytransferase and isopentenyl-pyrophosphate transferase activities, respectively and tRNA isolated from both the cytoplasm and mitochondria is affected. A likely explanation for these results is that single nuclear genes each encode both the nuclear and mitochondrial forms of the enzymes. We are currently attempting to understand the molecular basis for these observations by comparing the properties of the mitochondrial and nuclear/cytoplasmic forms of the proteins and characterizing recombinant DNAs containing the TRM1 and TRM2 sequences.