MOLECULAR BIOLOGY OF INTRACELLULAR PROTEIN SORTING AND ORGANELLE ASSEMBLY

R.A. Bradshaw, M. Douglas and L. McAlister-Henn, Organizers
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Poster Sessions

Cytoplasmic Sorting - I: Contranslational Amino

SORTING IT OUT IN SECRETORY CELLS, Regis B. Kelly, Teresa Burgess and Linda
E 001 Matsuuchi. Dept of Biochemistry & Biophysics, University of California, San Francisco, CA 94143

As a protein moves through the cell it must select between alternative routes and know when it reaches its destination. The information that specifies choice of route and recognition of destination presumably resides in sorting domains on the protein surface. How proteins go from cytoplasm to endoplasmic reticulum, nucleus, mitochondria and how lysosomal enzymes are correctly routed from the Golgi are now reasonably well-understood, but in other cases, we are less clear how intracellular trafficking is determined. In our lab we have been exploring the sorting of secretory proteins. We find that failure to exit from the rough endoplamsic reticulum is associated with binding to a 75 kd protein, probably identical to heavy chain binding protein (BiP). We have also explored targetting at the Golgi level. In regulated secretory cells such as pituitary cells, hormone to be secreted is stored in dense secretory vesicles until the cell is triggered to secrete. In ACTH-secreting pituitary tumor cells some of the regulated pathway exists, but perhaps because of the rapid growth of the cells, much of the hormone exits by a second pathway, the constitutive pathway. Secretory proteins partition between the two pathways since the extracellular matrix protein, laminin, leaves exclusively by the constitutive pathway. To try to identify putative sorting domains that determine the pathway we have transfected the cells with cDNAs encoding secretory proteins that take either the regulated or the constitutive pathway. A hybrid protein made by fusing a regulated to a constitutive secreted protein exited by the regulated pathway, suggesting that the regulated pathway had a dominant sorting domain. To look for a sorting domain we are studying secretion of variants of trypsinogen constructed by oligonucleotide directed mutagenesis. Correct sorting does not require either the trypsinogen signal peptide, or the first 13 amino acids of the amino-terminus. When secreted proteins are segregated from each other in the Golgi region they are presumably packaged into separate organelles that migrate to the cell surface. To identify the vesicles carrying constitutively secreted proteins we have generated a cell line that hyperexpresses the immunoglobulin kappa light chain.

Terminal Processing

E 002 **THE AMINO TERMINAL PROCESSING OF PROTEINS: METHIONINE AMINOPEPTIDASE AND N^{α}-ACETYLTRANSFERASE.**

Ralph A. Bradshaw, Ryo Yamada and Richard L. Kendall, Dept. Biol. Chem., Univ. of California, Irvine, CA 92717

Methionine aminopeptidase and N^{α}-acetyltransferase, two enzymes that are associated with ribosomes, are widely distributed in eukaryotic organisms. Each acts on a selection of nascent polypeptide substrates, sometimes in concert and sometimes independently, to produce a number of variously modified N-terminal derivatives. Both enzymes are strongly effected by the penultimate residue but additional specifying influences in the sequence or structure of the substrate are likely. Interestingly the biological importance of these reactions is unclear; the majority of cytoplasmic proteins are N^{α}-acetylated (as the result of modification of either the initiating methionine or the adjacent residue) but significant numbers of unblocked molecules are also found. No correlation between stability and/or turnover and either state has been demonstrated. Furthermore, it is unclear how acetylation (or lack of same) is related to other, less prevalent, modifications such as N^{α}-myristylation.

As an approach to this problem, we have initiated isolation and characterization studies of both enzymes. Using rat liver as a source and synthetic substrates to assay, partial purification of each has been achieved from polysome preparations. The N^{α}-acetyltransferase shows an unusually lability that is not apparently related to the stability of protein. Activity is largely lost upon dialysis, gel filtration and DEAE chromatography but not on CM chromatography. The methionine aminopeptidase, in contrast, does not show such sensitivities. The properties and specificities of these preparations, particularly in relation to those measured or deduced from other sources, will be described. Supported by USPHS research grant USPHS DK32465 (formerly AM32465) and the Hitachi Chemical Co. Ltd., Tokyo, Japan.

E 003 AMINO TERMINAL PROCESSING OF ACTIN, Peter A. Rubenstein, Larry Solomon, and Debra J. Martin, Department of Biochemistry, University of Iowa College of Medicine, Iowa City, IA 52242.
Actin NH_2- terminal processing is characterized by the post-translational removal of an Acetyl-amino acid from the newly translated actin and reacetylation of the newly exposed NH_2-terminus. Class II actins, those whose genes code for a protein with a Met-Cys-Asp(Glu) NH_2-terminus but which actually begin Ac-Asp(Glu), probably lose the initiator Met as a free amino acid early in translation since the second amino acid in the chain is small and neutral. The Cys is then quickly acetylated and is removed as Ac-Cys followed by reacetylation of the new NH_2-terminus. The acetylated intermediate has a half-life of about 15 minutes. Under unusual circumstances, the Met can be removed in an acetyl dependent fashion as well. The enzyme that carries out removal of the Ac-Cys (processing enzyme) is highly specific for actin and requires a native actin structure for activity. We have used site directed mutagenesis of actin to study the specificity requirements of the processing enzyme. It has been shown that some kind of a SAM dependent step may be involved in the processing. Since actin has a 3-MeHis which derives its methyl residue from SAM, we wondered whether His methylation was required for efficient processing. We replaced the MeHis with either Arg or Tyr. In neither case was processing affected indicating that if SAM was involved, it was not due to His methylation. Actins have a conserved Asp-Asn-Gly-Ser-Gly near the NH_2-terminus beginning at pos. 11. When we converted Asp_{11} to Glu saving the negative charge, no effect on processing was seen. However, when the Asp was replaced with Asn, processing was blocked indicating that a negative charge at that position is a binding site for the enzyme or that without it, the NH_2-terminal region assumes a conformation no longer recognized by the processing enzyme. The Asn actin also loses its ability to bind to DNAse I which normally binds to all actins. This is the first time that the NH_2 terminal region of actin has been implicated as being involved in DNAse binding. Others had shown that at least part of this binding site spanned a region that included the MeHis. Our His mutants retained the ability to bind DNAse I.

PROTEIN MYRISTYLATION. Bartholomew M. Sefton and Janice E. Buss. Molecular Biology and Virology Laboratory, The Salk Institute, San Diego, California.

E 004 A number of well-characterized proteins in eukaryotic cells are covalently modified by addition of the rare 14 carbon saturated fatty acid, myristic acid. These include $p60^{src}$ (the transforming protein of Rous sarcoma virus), the catalytic subunit of the cAMP-dependent protein kinase, and proteins containing the amino terminus of the mammalian retroviral *gag* protein precursor.

In every case, myristic acid is linked through an amide bond to the α-amino group of amino-terminal glycine. Comparison of the sequences of the amino-termini of myristylated proteins reveals no homology, save for the glycine to which the myristyl moiety is bound. The identity of the sequences pecifying myristylation therefore remains unknown. Only amino-terminal glycine can however undergo myristylation. Any mutation which leads to replacement of the myristylated glycine or prevents removal of the initiating methionine residue abolishs myristylation.

Unlike the palmitylation of proteins, which is a post-translational event occurring in or on cellular membranes, myristylation occurs very early in the life of a protein in the cytosol. It may be that myristylation occurs on nascent polypeptide chains.

In $p60^{src}$, the myristyl moiety plays an essential role in binding the protein to the cytoplasmic face of cellular membranes. All mutations preventing myristylation also prevent membrane binding. The fatty acid appears to play a similar role in retroviral nucleocapsid proteins. This may not be the sole function of this form of protein modification. A number of as yet unidentified myristylated cellular proteins are found exclusively in the cytosol.

E 005 SPECIFICITY DETERMINANTS FOR N-TERMINAL ACETYLATION AND REMOVAL OF N-TERMINAL ACETYLMETHIONINE, Finn Wold, Department of Biochemistry and Molecular Biology, University of Texas Medical School, Houston, TX 77225.

Attempts to identify short N-terminal sequence patterns that unequivocally distinguish acetylated and nonacetylated proteins have been largely unsuccessful. Since the limited available information suggests that both the removal of the initiator methionine and the acetylation of the N-terminus may take place cotranslationally at the stage when 25-50 residues have been assembled in the nascent polypeptide chain, it seems reasonable to consider that the complete set of specificity signals that determines whether a protein is to be acetylated or not may require an N-terminal sequence of this length to be expressed. Scoring the individual residues for their statistical appearance in proteins as secondary structure (helix, sheet, turn) formers (+), indifferent (0) or breakers (-) (1) and then comparing the first 40 residues of a large number of acetylated and nonacetylated eukaryotic proteins from the data bank, we found that an Apple IIc computer could separate the acetylated from the nonacetylated ones using the "perceptron algorithm" developed by artificial intelligence researchers some 30 years ago (2). The major areas of distinguishing structural features appear to be in the sequences 1-10, 16-24, 34-40, and on the basis that the separation of the two groups is unsuccessful if less than 40 residues are used for the comparison it appears that all three regions of information are involved in the process. It has not yet been possible to determine the nature of the secondary structure specificity signals; it should nevertheless be reasonable at this time to explore the possibility that both the removal of the initiator methionine and the subsequent acetylation of the newly exposed residue are regulated by the secondary structure features encoded into the first 40-50 residues. Regarding the removal of the initiator methionine, we have found an enzyme in rabbit muscle that catalyzes the specific removal of acetylmethionine from both short (3 residues) and long (11 residues) acetylated peptides. We have not found it to remove any other acetylamino acids from peptides, and this enzyme could thus play a role in the specific removal of the initiator methionine after an obligatory acetylation step. The relative importance of this acetylmethionine-cleaving enzyme and the more generally considered putative methionine aminopeptidase cannot be assessed at this time.

1. Levitt. M. (1978) Biochemistry 17, 4277-4285.
2. Sampson, J.R. (1976) "Adaptive Information Processing", Springer Verlag, NY, pp 131-135.

Cytoplasmic Sorting - II: Karyophillic Transport

E 006 THE IDENTIFICATION AND CHARACTERISATION OF NUCLEAR TARGETING SIGNALS USING XENOPUS OOCYTES Alan Colman, Nigel Dimmock, Ian Jones and John Davey, Department of Biological Sciences, University of Warwick, Coventry CV4 7AL UK

The Xenopus oocyte provides an attractive system to investigate protein migration into the nucleus. Nuclear proteins or their genes can be microinjected into the oocyte and the intranuclear movement of the protein can be accurately assessed since manual removal of the nucleus is both rapid and clean. We have used the oocyte to investigate sequences present in the influenza nucleoprotein (NP) which facilitate its entry into the nucleus. By deletion and fusion mutagenesis we provide strong evidence for a discrete amino acid sequence (between residues 327 and 345) which is essential for the nuclear accumulation of NP. However it is not clear whether this region actually facilitates nuclear entry. Rather we believe it to be a binding site to some intra-nuclear component and we are currently searching for an entry signal. Our results and the problems involved in distinguishing putative "binding" signals from "entry" signals will be discussed in relation to other known nuclear targeting signals.

NUCLEAR PROTEIN LOCALIZATION IN YEAST

E 007 Michael N. Hall, Dept. of Biochemistry and Biophysics, University of California, S.F., San Francisco, California, 94143.

We are studying the molecular mechanism by which the yeast α2 protein accumulates in the nucleus. Evidence for the following will be presented. (1) The α2 protein has two distinct nuclear localization signals. (2) Both signals are required for efficient or proper localization; either signal alone can mediate an "impaired" form of localization. (3) The mechanism by which α2 is delivered to the nucleus is an active process. The issue of whether the two signals act independently at different steps in a localization pathway or whether they act synergistically at the same step(s) will be discussed.

A NUCLEAR LOCATION SIGNAL, Alan Smith, Seng Cheng, Robert Harvey,

E 008 William Markland and Bruce Roberts, Integrated Genetics, Inc., 31 New York Avenue, Framingham, Massachusetts.

A basic sequence of amino acids Pro Lys Lys 128 Lys Arg Lys Val Glu found in SV40 Large-T antigen appears to be a prototype nuclear location signal. Extensive deletion and single amino acid mutation analysis of the signal sequence shows that Lys 128 is crucially important, whereas, other amino acids within the tract are less critical. Transposition of the wild type sequence to large cytoplasmic proteins results in their transport to the nucleus. This observation appears to be largely but not exclusively independent of the location of the sequence within the protein. Analysis of other eukaryotic proteins indicates similar sequences may act to locate other proteins to the nucleus. Some of these sequences appear near the terminii of proteins and in some cases, notably polyoma virus Large-T, there appear to be multiple nuclear location signals.

Analysis of DNA replication using Large-T variants with different amino acids substitutions at position 128 shows that some cytoplasmic Large-T species are able to replicate SV40 origin-containing plasmid DNA and implies a small amount of the protein is able to enter the nucleus and stimulate DNA synthesis. This result highlights the danger of attributing specific activities of Large-T to different subcellular fractions based on the studies of Large-T encoded by the various cytoplasmic mutants. A cell-free system able to translocate Large-T into isolated nuclei has been devised. The system is unable to translocate the Thr 128 mutant of Large-T and appears to be independent of exogenous energy or cytoplasmic proteins.

Cytoplasmic Sorting - III: Mitochondrial Transport

E 009 GENETIC AND BIOCHEMICAL ANALYSIS OF PROTEIN STRUCTURE FOR MITOCHONDRIAL IMPORT, Michael G. Douglas, Alessio Vassarotti, and Wen-ji Chen, Dept. of Biochemistry, University of Texas Health Science Center, Dallas, Texas, 75235.

The β-subunit of the mitochondrial F_1ATPase is synthesized as a cytoplasmic precursor containing a transient presequence of 19 residues which is required for its import into the organelle. In order to address the function of this presequence in mitochondrial import and the role of protein structure in this process, genetic and biochemical analyses of this region have been performed. Four experiments will be discussed. First, by CD measurements a synthetic 19 residue F_1 β presequence peptide will form an amphipathic α-helix in solvents of reduced polarity. In neutral aqueous buffer the peptide is essentially a random coil. This presequence peptide will inhibit *in vitro* import of the F_1-β precursor at a concentration which surface tension experiments indicate will insert into and disrupt a monolayer. However at lower concentrations of presequence peptide (estimated molar ratio of 30 peptides/F_1-β precursor) a reproducible stimulation of *in vitro* import is observed. This observation suggests a possible cooperative effect of the presequence peptides for post translational insertion into the membrane. Second, deletion of the first major α-helical region of the pre F_1-β protein including the presequence prevents import into mitochondria. In the appropriate selection screen, surrogate import signals will form at the amino terminal end of the presequence deletion construct. Analysis of these surrogate import signals reveals that 6 independent mutations to either reduce the content of acidic residues within or to extend the first available α-helix in the presequence deletion construct will yield a functional import signal. This experiment indicates that the formation of an amphipathic structure at the amino terminus containing at least a single basic residue will direct inefficient *in vivo* mitochondrial import. The efficiency of import is directly related to the number of basic residues in the amphipathic structure. Third, mitochondrial import of a gene fusion product of ATP2, encoding the amino terminus of pre F_1-β and CUP1 encoding the yeast Cu^{2+}-metallothionine (CuMT) at the carboxy terminus is blocked both *in vitro* and *in vivo* by chelation of the hybrid protein with Cu^{2+}. The divalent cation does not inhibit import of preF_1-β under conditions which inhibit that of preF_1-βCuMT. This analysis shows that protein unfolding is required for the efficient delivery of a protein into mitochondria *in vivo*. Fourth, genetic and biochemical studies to further define the requirements for protein import indicate that a membrane potential is required to initiate translocation of the F_1-β precursor, however, the completion of import requires additional soluble factors.

E 010 MULTIPLE PATHWAYS OF PROTEIN TRANSPORT INTO MITOCHONDRIA, W. Neupert, Institut für Physiologische Chemie der Universität, Goethestr. 33, 8000 München 2, FRG

We have investigated import pathways of different proteins which are targeted to the various subcompartments of the mitochondrion: porin, a channel forming protein of the outer membrane (OM); ATPase subunit ß, a matrix protein; ADP/ATP carrier and ATPase subunit 9 of the inner membrane (IM); cytochrome c_1 and Fe/S protein as examples of proteins facing the intermembrane space (IMS) while being anchored to the IM; and cytochrome c, a soluble IMS component. All these proteins are imported by pathways that can be dissected into a number of steps.

1) Proteinaceous import receptors appear to be required for recognition of precursors by mitochondria and initiation of translocation. Receptor binding did not require a membrane potential. With porin, the ADP/ATP carrier and cytochrome c, binding sites were titrated and affinity and number of sites were determined. The cytochrome c receptor is different from the porin receptor, but the latter seems to also recognize precursors of IM proteins. The porin receptor was reconstituted into liposomes. Binding to receptors leads to a tight interaction with the OM.

2) Proteins of the IM and the matrix enter the mitochondria via translocation contact sites. At these sites OM and IM come sufficiently close together to be spanned by a precursor polypeptide chain. Precursors can be trapped in such an intermediate position by importing them at low temperature. A membrane potential is required to transport precursors into this intermediate position, but not for completing import. Both, precursors with aminoterminal extensions, and precursors with internal targeting signals, such as the ADP/ATP carrier, are imported via translocation contact sites. These sites appear to be identical to the sites of close contact generally observed in electronmicrographs of mitochondria.

3) Intramitochondrial sorting was analysed for proteins targeted to the IMS or the outer surface of the IM. In particular, the pathway of the Fe/S protein of complex III was analysed. It was found that the precursor (32 aa extension) was first translocated completely into the matrix where it could be accumulated in the presence of chelators when the processing peptidase was inhibited. When the enzyme was reactivated, the precursor was cleaved to an intermediate (8 aa extension). This intermediate was then translocated back across the IM which was accompanied by cleavage to the mature size and was assembled with other subunits of complex III on the outer surface of the IM. We propose that this complex pathway reflects a mechanism which exists in prokaryotes and has been conserved as a prokaryotic symbiont has evolved into the mitochondrion. Transfer of the gene to the nucleus of the host cell, according to this "conservative sorting hypothesis", was accompanied by the addition of a mitochondrial targeting sequence and the formation of translocation contact sites to route precursors back on their "ancestral pathways".

E 011 SEVERAL ELEMENTS IN THE LEADERS PEPTIDE DIRECT THE ORNITHINE TRANSCABAMYLASE PRECURSOR TO MITOCHONDRIA, Leon E. Rosenberg, Arthur L. Horwich, Wayne Fenton, Frantisek Kalousek and Jan Kraus, Department of Human Genetics, Yale University School of Medicine New Haven, CT 06510.

The subunit precursor of the mammalian mitochondrial matrix enzyme, ornithine transcarbamylase (OTC), is directed from cytosol to mitochondria by its NH_2-terminal, cleavable leader peptide. We have carried out a detailed mutational analysis of the 32 amino acid long OTC leader from human and rat OTC to define the structural elements required for import of the precursor and processing of its leader peptide. In this analysis, SP6-derived plasmids containing wild-type or altered leader coding sequences were used to direct protein synthesis in vitro; subsequently, the respective OTC precursors were incubated with intact rat liver mitochondria or the matrix fractions therefrom, and studied by SDS-PAGE. Fusion constructs joining various portions of the leader with the mature subunit revealed that neither the NH_2-terminal 12 or 16 residues could direct import whereas a leader peptide comprising residues 8-25 could do so. Deletion analysis was corroboratory. Whereas import and cleavage were observed after removal of either the extreme NH_2-terminal or COOH-terminal portions of the leader, import was completely disrupted by removing residues 8-22. Remarkably, deletion of residues 30-37 containing the normal site of cleavage to mature OTC had no deleterious effect on import or processing indicating a lack of requirement for specific primary structure at this site of cleavage. Nearly 30 site-directed and random substitutions--some single, others double and even triple--have been produced. Single residue substitutions were found to impair import and/or cleavage only when either positively charged arg residues were replaced by charge-neutral ones or where an acidic residue (glu) was introduced in place of a charge-neutral one. Of the 4 arg residues in the leader (positions 6,15,23,26), arg 23 was most critical and appeared to be part of an α-helical region. Several single substitutions in the region between residues 23 and 26 led to appearance of cleaved products intermediate in size between pOTC and OTC. Double or triple substitutions supported the idea that net positive charge was important; they also showed that secondary structure (probably an α-helix) in the NH_2-terminus of the leader affects cleavage at its COOH-terminus. We conclude that the OTC leader directs import via several elements: net positive charge; a crucial midportion segment centered over arg 23; secondary structures--probably α-helices--at the NH_2-terminus and in the midportion; and, less certainly, by the presence of two successive cleavage sites--one near residue 23 and the second after residue 32--each of which participates in the normal pathway of OTC biogenesis.

E 012 WHAT GOES WHERE,WHY AND HOW IN MITOCHONDRIAL PROTEIN IMPORT, Adolphus P.G.M. van Loon, Dan Allison, Alison Baker, Martin Eilers, Eduard C. Hurt, Masajuki Ohba and David Roise, Biocenter of the University, Klingelbergstrasse 70, CH-4056 Basel, Switzerland.

The amino-terminal sequences of imported mitochondrial proteins contain information for specific transport to mitochondria and into the proper intramitochondrial space. Each mitochondrial transport sequence analyzed thus far contains a matrix-targeting signal, responsible for the specific transport of the attached protein to mitochondria and potentially into the matrix, and an intramitochondrial-sorting signal which is present if protein transport has to be stopped in the outer or inner mitochondrial membrane. Intramitochondrial sorting thus involves by the combination of various topogenic signals at the protein's amino terminus. The intramitochondrial-sorting signal in the cytochrome c_1 presequence contains a stretch of 19 uncharged amino acids followed by two acidic residues. Experimental evidence suggests that this sequence functions as a stop-transport sequence specific for the inner membrane. Matrix-targeting signals have common characteristics, although they differ in their primary amino acid sequences. Artificial matrix-targeting sequences can be constructed that consist of only three different amino acids (arginine, leucine and serine). The physico-chemical properties of artificial presequence peptides resemble those of authentic presequence peptides: they are highly surface active, can insert into and disrupt phospholipid bilayers in a membrane potential dependent fashion and probably form amphiphilic structures. The transmembrane transport most likely requires unfolding of the protein. Mitochondrial import of mouse DHFR fused to the presequence of subunit IV of cytochrome c oxidase can be specifically inhibited by prebinding an inhibitor to DHFR. What is the evolutionary origin of mitochondrial matrix-targeting sequences? Potential matrix-targeting sequences are encoded by many short eukaryotic or bacterial DNA sequences. Such cryptic sequences could only be used as mitochondrial transport sequences if placed at the amino terminus of an appropriate passenger protein. Comparing the transport efficiencies of many of these potential matrix-targeting sequences will reveal the requirements for a mitochondrial transport sequence.

Cytoplasmic Sorting - IV: ER Transport/Secretion

E 013 THE ROLE OF THE ENDOPLASMIC RETICULUM IN PROTEIN SECRETION, Jonathan A. Rothblatt, Michael Hortsch and David I. Meyer, Cell Biology Program, European Molecular Biology Laboratory, 6900 Heidelberg, West Germany.

The rough endoplasmic reticulum (ER) represents the site of entry into the secretory pathway and the site of assembly of most membrane proteins. Targeting of proteins to the ER is mediated by a pair of receptors, SRP and docking protein. The subsequent steps of translocation across the membrane are poorly characterized and the components mediating this process are unknown. We are approaching the identification of these components in two ways, biochemically and genetically. We have recently carried out further dissection of mammalian rough microsomes and found two activities required for translocation, beyond the SRP-docking protein targeting step. One is inhibitable by proteolysis and correlated with a loss in the ability of membranes to bind ribosomes, while the other can be inactivated by alkylation with NEM.

In order to take advantage of the ability to genetically inactivate translocation-relevant components by mutation, we have set up a cell-free system derived from yeast for studying protein translocation. One of the most interesting features of this system is the ability of the precursor of the yeast pheromone α-factor to be translocated across the membrane of the ER in the absence of ongoing protein synthesis, i.e., post-translationally. Studies are being carried out to biochemically characterize protein translocation in this system using such a post-translational assay. Both the cellular components that participate, as well as the structural features of the nascent secretory proteins being translocated are under investigation.

E 014 THE SIGNAL RECOGNITION PARTICLE RECEPTOR IS A COMPLEX THAT CONTAINS TWO DISTINCT POLYPEPTIDE CHAINS. Shoji Tajima, Leander Lauffer, Virginia L. Rath and Peter Walter. Dept of Biochemistry & Biophysics, University of California, San Francisco, CA 94143

Signal recognition particle (SRP) and SRP receptor are known to be essential components of the cellular machinery that targets nascent secretory proteins to the endoplasmic reticulum (ER) membrane. Here we report that the SRP receptor contains, in addition to the previously identified and sequenced 69 kd polypeptide (α-subunit, SRα), a 30 kd β-subunit (SRβ). When SRP receptor was purified by SRP-Sepharose affinity chromatography, we observed the co-purification of two other ER membrane proteins. Both proteins are ~30 kd in size and are immunologically distinct from each other, as well as from SRα and SRP proteins. One of the 30 kd proteins (SRβ) forms a tight complex with SRα in detergent solution that is stable to high salt and can be immunoprecipitated with antibodies to either SRα or SRβ. Both subunits are present in the ER membrane in equimolar amounts and co-fractionate in constant stoichiometry when rough and smooth liver microsomes are separated on sucrose gradients. We therefore conclude that SRβ is an integral component of SRP receptor. The presence of SRβ was previously masked by proteolytic breakdown products of SRα observed by others and by the presence of another 30 kd ER membrane protein (mp30) which copurifies with SRα. Mp30 binds to SRP-Sepharose directly and is present in the ER membrane in several-fold molar excess of SRα and SRβ. The affinity of mp30 for SRP suggests that it may serve a yet unknown function protein translocation.

E 015 MECHANISMS OF SECRETION AND MEMBRANE ASSEMBLY, Ross Dalbey and William Wickner, Molecular Biology Institute and Department of Biological Chemistry, University of California, Los Angeles, CA 90024.

We have performed extensive genetic analysis of two proteins of the plasma membrane of E. coli, the procoat protein of coliphage M13 and the E. coli leader peptidase, to determine which regions of each are essential for membrane assembly. Procoat protein has a typical cleavable leader peptide, yet also requires its hydrophobic, membrane anchor region of the mature protein and even its basic, carboxy terminal tail for its membrane assembly. These data rule out simple models of membrane assembly information beign contained in descrete packets which are decoded one at a time or in an amino to carboxy direction. Leader peptidase undergoes no cleavage during its membrane assembly. It has three hydrophobic segments, of which the second can be shown to be an "internal, uncleaved signal sequence." However, this signal cannot act in isolation to direct the polar carboxyterminus of leader peptidase across the plasma membrane. It needs either the first, or third, apolar domain to promote its membrane insertion. These studies, and those of other laboratories, indicate that different parts of a protein can act together in concert to promote membrane insertion.

Intracellular Targeting - I: ER/Golgi

E 016 VIRAL GLYCOPROTEINS: MEMBRANE TRANSPORT AND FUSION ACTIVITY, Ari Helenius, Connie Copeland, Robert Doms, Francois Boulay, Klaus-Peter Zimmer, William Balch, Ira Mellman, Departments of Cell Biology and Molecular Biophysics and Biochemistry, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510.

Most glycoproteins of enveloped animal viruses are synthesized in the endoplasmic reticulum (ER) and transported via the secretory pathway either to the plasma membrane or to other membrane organelles where virus budding occurs. As integral components of the viral membrane, the glycoproteins subsequently mediate the early interactions between virus and cell; adsorbtion to cell surface, endocytosis and penetration. While virus internalization by endocytosis usually depends on constitutive cellular functions, the fusion reaction is directly induced by the viral spikes glycoproteins. In most cases fusion is triggered by the acidic pH in endosomes.

We are studying the biosynthesis and functions of three viral fusion factors; Influenza hemagglutinin (HA), Semliki Forest virus E1,2,3 and the G-protein of Vesicular Stomatitis virus. Our present focus is on their conformation and state of oligomerization during various phases of their life cycle. All three proteins are homotrimers in their mature form. Our results for HA and G indicate that trimerization occurs post-translationally at the time when the proteins are to be transported from ER to the Golgi complex. In HA further changes in the quaternary structure occur as the protein passes through the Golgi to the plasma membrane. A final change is associated with its proteolytic activation which makes it fusion competent and modifies its sensitivity to acid pH. The formation of correctly folded trimers is crucial for transport of both HA and G.

When exposed to mildly acidic pH, the HA and the SFV spikes undergo irreversible conformational changes which lead to fusion activity. The ectodomains of HA are partially dissociated, and a previously hidden hydrophobic moeity is exposed for interaction with the target membrane. In SFV the E1 subunit interacts with cholesterol molecules present in the target membrane thus mediating the interaction between the membranes during fusion. In G a reversible conformational change takes place, the nature of which still remains unclear. It is evident that, while trimeric and similar in general properties, the three fusion proteins are different in their mode of action.

This work was supported by grants from NIH.

E 017 PLASMA MEMBRANE PROTEIN INTERMEDIATES ARE PRESENT IN THE SECRETORY VESICLES OF YEAST, Cherie L. Holcomb, William Hansen, Tina Etcheverry and Randy Schekman, Biochemistry Department, University of California, Berkeley CA 94720.

Secretory vesicles are an intermediate between the Golgi complex and the cell surface. A mammalian endocrine cell line has been shown to possess two types of secretory vesicles. One type of vesicle contains proteins that are constitutively secreted and the other contains proteins that are secreted in a regulated fashion (1). In the yeast Saccharomyces cerevisiae there is no evidence for a pathway of regulated secretion. However, yeast cells do export two major classes of proteins - plasma membrane proteins and those which are secreted. We have performed experiments to determine if these two classes of proteins are transported to the cell surface in the same vesicles. First, we have devised a procedure for partially purifying yeast secretory vesicles from a mutant strain (sec1) in which mature vesicles accumulate at a restrictive temperature (2). The purification includes differential and gradient centrifugations and an electrophoretic separation of membranes. The fractions obtained from this procedure are at least five fold enriched for secretory vesicles over any other cellular membrane. We have found that a newly synthesized plasma membrane protein and a newly synthesized secretory protein are both present in purified fractions. In addition, these proteins copurify through every step of the isolation. This suggests that the proteins occur in the same vesicles. To confirm this observation we have complexed secretory vesicles to Staphylococcus aureus cells via an antibody directed against the major plasma membrane ATPase. The secretory enzyme, acid phosphatase is recovered quantitatively in the complex. This result presents strong evidence that newly synthesized plasma membrane proteins and proteins destined for secretion reside in the same secretory vesicles. Physicla characteristics of the secretory vesicles and additional applications of the secretory vesicle purification procedure will also be discussed.

1. Gumbiner, B. and Kelly, R. B. 1982. Cell 28, 51-59.
2. Novick, P. and Schekman, R. 1979. Proc. Natl. Acad. Sci. USA 76, 1858-1862.

Intracellular Targeting - II: Genetic Analyses

E 018 PROTEIN SORTING IN YEAST: NATURE OF THE SIGNALS DIRECTING TARGETING AND MODIFICATION OF TWO VACUOLAR PROTEINASES. Scott D. Emr, Division of Biology, California Institute of Technology, Pasadena, CA 91125.

The secreted enzyme invertase (Inv) and the vacuolar hydrolases carboxypeptidase Y (CPY) and proteinase A (PrA) appear to transit together through early stages of the yeast secretory pathway. After passage through the Golgi complex, these proteins are sorted and targeted for delivery to their respective destinations. To ensure their proper delivery, these proteins must contain signals in their sequence or structure that allow them to be properly distinguished for routing to their correct cellular compartments. We have been interested in defining the nature of this protein sorting code and in identifying the cellular machinery that is responsible for deciphering the code and executing its delivery instructions.

We have employed a gene fusion approach to map the sequence determinants contained in the vacuolar glycoproteins CPY and PrA that direct accurate intracellular sorting and modification of these enzymes. Different sized amino-terminal segments of CPY and PrA have been fused to mature enzymatically-active invertase (Inv). We have found that relatively short amino-terminal domains of CPY (50 amino acids) and PrA (76 amino acids) contain sufficient information to quantitatively divert invertase to the yeast vacuole. Deletion of the putative vacuolar sorting signal from an otherwise wild-type CPY protein (deletion of amino acid residues 21-50) was found to result in the mislocalization of CPY protein to the yeast periplasm and extracellular medium. Furthermore, by examining the extent to which the oligosaccharides on various CPY-Inv hybrid proteins are modified, we have found that sequences in CPY mapping distal to the vacuolar protein sorting signal appear to regulate in a cis-dominant manner the degree to which the N-linked oligosaccharides on the enzyme invertase become elongated in the Golgi complex.

The efficient delivery of CPY-Inv and PrA-Inv hybrid proteins to the yeast vacuole by strains having no other source of active invertase results in the inability of such strains to grow on sucrose as their sole fermentable carbon source. This Suc^- phenotype has enabled us to select for mutants that "mislocalize" these hybrid proteins to the cell surface. Using this approach, we have defined a large number of trans-acting functions required for accurate protein targeting to the vacuole. Presently, these mutations have been assigned to some 34 different complementation groups. In addition, these vacuolar protein targeting (vpt) mutants have been shown to exhibit hybrid protein independent defects in the vacuolar sorting of wild-type CPY protein. Precursor CPY protein is secreted from these mutants into the periplasm and extracellular medium. Certain of the vpt mutants also exhibit other interesting phenotypes including defects in; assembly of a normal vacuole compartment, endocytosis of FITC-dextran, and growth at 37°C. We believe that the gene fusion approach and an analysis of such vpt mutants will aid in understanding how intracellular protein traffic is regulated in eukaryotic cells.

E 019 PROTEIN FOLDING AND INTRACELLULAR TRANSPORT, Mary-Jane Gething*, Karen McCammon*, Karl Normington, Chuck Chao and Joe Sambrook, Department of Biochemistry and *Howard Hughes Medical Institute, University of Texas Health Science Center, Dallas, TX 75235.

The hemagglutinin of influenza virus is synthesized as monomeric subunits that are co-translationally translocated across the membrane of the rough endoplasmic reticulum (ER). We have shown using chemical crosslinking, velocity sedimentation, protease sensitivity and antibodies specific for native or denatured epitopes that folding and assembly of HA monomers into trimeric structures takes approximately 7-10 minutes and is completed before the protein leaves the ER (1). Evidence will be presented to show that folding is the rate limiting step in transport of HA from the ER. Mutants of hemagglutinin that fail to be transported from the ER are blocked at different stages of the folding pathway. Unfolded molecules of hemagglutinin are associated with BiP, a cellular protein of 77kD that has been shown previously to bind to IgG heavy chain in the ER of certain myelomas (2). The reason why assembly of native structures is required for transport of proteins through the exocytotic pathway will be discussed.

1. Gething, M.J., McCammon, K. and Sambrook, J. (1986) Cell 46, 939-950.
2. Bole, D.G., Hendershot, L.M. and Kearney, J.F. (1986) J. Cell Biol. 102, 1558-1566.

E 020 TRANSPORT OF HYBRID GLYCOPROTEINS IN NON-POLARIZED AND POLARIZED CELLS. John K. Rose, Lynn Puddington, and Jun-Lin Guan. Molecular Biology and Virology Laboratory, The Salk Institute, P.O. Box 85800, San Diego, Ca. 92138. The requirements for efficient cell-surface transport of two membrane-anchored forms of soluble proteins have been investigated. These proteins, rat growth hormone (a non-glycosylated secretory protein) and the alpha subunit of human chorionic gonadotropin (a glycosylated secretory protein), can both be expressed and secreted as monomeric, soluble proteins. Membrane-anchored forms of both proteins were generated by fusing the appropriate cDNA sequences with the sequence encoding the transmembrane and cytoplasmic domains of the vesicular stomatitis virus (VSV) glycoprotein (G). These hybrid proteins were expressed in animal cells and were found to be inserted into microsomal membranes in the expected transmembrane orientation. However, the transport of both proteins to the cell surface required the presence of at least one N-linked oligosaccharide. Deletions in the cytoplasmic domain, which are known to greatly reduce the efficiency of VSV G protein transport to the cell surface, also reduced cell-surface transport of the hybrid proteins. These experiments indicate a role for this cytoplasmic domain in transport that is independent of the nature of the extracytoplasmic domain.

In polarized epithelial cells such as the MDCK line, the VSV G protein and the influenza virus HA protein are known to be transported to the basolateral and apical membranes respectively (Rodriguez-Boulan and Sabatini, 1978, Proc. Natl. Acad. Sci., USA, 75:5071). To examine the location of the signals determining this polarized expression we derived MDCK cell lines expressing G protein or G protein with the cytoplasmic domain of and HA molecule replacing its normal cytoplasmic domain (GHA). We found that G protein was transported to the basolateral membrane while GHA protein was transported to the apical and basolateral domains. These results indicate that cytoplasmic domains may play some direct role in determining polarized transport of plasma membrane proteins.

E 021 ARE THERE INTRACELLULAR SORTING SIGNALS IN THE HUMAN LOW DENSITY LIPOPROTEIN RECEPTOR, by David W. Russell, Michael S. Brown, Joseph L. Goldstein; University of Texas Health Science Center at Dallas

Receptor-mediated endocytosis is a process by which mammalian cells internalize nutrients, viruses, toxins, and hormones. The process begins when specific receptors on the cell surface bind macromolecules and move laterally into clathrin-coated pits. Within minutes the coated pits invaginate into the cell and pinch off to form coated vesicles. After removal of their clathrin coats by an ATP-driven uncoating enzyme, the vesicles fuse with one another to form endosomes whose internal milieu is rapidly acidified by an ATP-driven proton pump. This reduction in pH frequently brings about a dissociation event between the ligand and its specific receptor. Often, but not universally, the ligand is sorted to the lysosome and degraded while the receptor cycles back to the cell surface.

Since its discovery more than ten years ago, the low density lipoprotein (LDL) receptor has served as a paradigm for receptor-mediated endocytosis. Its characterization at the cellular, biochemical, and genetic levels is responsible for much of what we know about this important process. Recently, we have gained new insights into the LDL receptor through the application of the powerful techniques of molecular biology. Biochemical tools developed with the purified LDL receptor protein allowed the isolation of cDNA clones corresponding to the bovine and human receptor mRNAs. DNA sequence analysis of these cDNAs revealed that the receptor protein is composed of at least 5 discrete domains: a ligand binding domain, a domain homologous to the epidermal growth factor (EGF) precursor, a carbohydrate-rich domain, a transmembrane segment, and at the COOH terminus, a cytoplasmic domain of fifty amino acids.

The predicted domain structure of the protein is reflected in the intron-exon organization of the LDL receptor gene. A very strong correlation exists between the position of introns in the gene and subdomains in the ligand binding, EGF-homologous, and carbohydrate-rich regions of the encoded proteins. Furthermore, homologies detected at the protein level between the LDL receptor and five other proteins are mirrored in the gene structure: homologous regions are encoded by discrete exons. Thus, the LDL receptor is a mosaic protein whose gene is built up of shuffled exons.

In an effort to define the roles that predicted protein domains play in receptor-mediated endocytosis, we have begun to analyze at the DNA level naturally occurring mutations in the LDL receptor gene in patients with the prevalent genetic disease, familial hypercholesterolemia. The lecture will review some of these results, and in particular will focus on the role of the cytoplasmic domain and other domains in intracellular transport.

Intracellular Targeting - III: Recycling

E 022 THE LDL PATHWAY: GENES REQUIRED FOR THE SYNTHESIS AND FUNCTION OF A GLYCOPROTEIN RECEPTOR, Monty Krieger, David Kingsley, Karen Kozarsky, Robert Sege, and Lawrence Hobbie, Biology Dept. and Whitaker College, MIT, Cambridge, MA 02139.

Biochemical, immunological, and genetic techniques were used to study the defects in four types of LDL receptor-deficient mutant hamster cells (ldlA, ldlB, ldlC, ldlD). ldlA mutants have defects in the structural gene for the LDL receptor and are analogous to cells from patients with familial hypercholesterolemia. Nucleic acid and immunological probes have identified several different mutant forms of the LDL receptor in ldlA cells. These mutant alleles should be useful for structure-function studies of the LDL receptor.

Normal endocytic recycling of LDL receptors is disrupted in ldlB, ldlC, and ldlD mutants because the receptors are rapidly degraded soon after they reach the cell surface. In each case, receptor instability is associated with defects in the Golgi-associated posttranslational processing of LDL receptors and other glycoconjugates. These defects affect the processing of N-linked, O-linked, and lipid-linked carbohydrate chains. Primary and secondary transfectants of ldlB cells have been isolated after treatment of ldlB cells with human DNA. The transfected cells show wild type levels of LDL receptor activity and normal patterns of glycoprotein processing. These results suggest that all of the abnormal phenotypes of ldlB cells are due to defects in a single gene.

The defect in ldlD cells has been identified as a marked deficiency in the enzyme UDP-glucose 4-epimerase. This enzyme normally catalyzes the reversible isomerization of UDP-glucose to UDP-galactose and of UDP-N-acetylglucosamine to UDP-N-acetylgalactosamine. Human deficiency in this epimerase can lead to a severe form of galactosemia. When epimerase-deficient ldlD cells are grown on glucose as the sole sugar source, they cannot synthesize enough UDP-galactose and UDP-N-acetylgalactosamine to allow synthesis of normal N-linked, O-linked and lipid-linked carbohydrate chains. The abnormal glycosylation patterns and the lack of LDL receptor activity in ldlD cells can be completely reversed by providing the cells with exogenous sources of galactose and N-acetylgalactosamine. These sources can include the pure sugars, glycoproteins containing these sugars or other cells grown in close proximity (transfer via intercellular junctions). The results suggest that O-linked carbohydrate chains play an important role in determining LDL receptor stability.

E 023 MOLECULAR SORTING DURING RECEPTOR-MEDIATED ENDOCYTOSIS, I. Mellman, S. Schmid, S. Green, K.-P. Zimmer, T. Koch, V. Lewis, H. Miettenen, and A. Helenius, Dept. of Cell Biology, Yale University School of Medicine, New Haven, CT 06510.

Endocytosis of receptor-bound ligands is characterized by the internalization of receptor-ligand complexes in coated vesicles and their delivery to endosomes where the acidic pH favors the dissociation of ligand from receptor. Free receptors then recycle back to the plasma membrane for re-use while the dissociated ligands are transported to lysosomes for degradation. Important variations of this scheme are also known, particularly in the case of immunoglobulin receptors. Following delivery to endosomes, macrophage Fc receptors (FcR) are transported along with their ligands to lysosomes while in epithelial cells, FcR can be directed across the cell monolayer. Thus, it is clear that endosomes play a central role in regulating the intracellular traffic of ligands and receptors, and that individual receptors can be transported to and from endosomes in characteristically different fashions. In this presentation, we will review recent work directed towards an understanding of these molecular sorting events at the biochemical level. Using endosomes purified from CHO cells by free flow electrophoresis, we have identified two distinct endosome subpopulations: "early" endosomes which are closely associated with receptor recycling events, and "late" endosomes which collect internalized tracers just prior to their delivery to lysosomes. While both contain electrogenic ATP-driven proton pumps, the pH in early endosomes is slightly less acidic due to the presence of Na+,K+-ATPase activity which establishes an interior-positive membrane potential opposing the activity of the proton pump. In addition to these functional differences, recent experiments also suggest slight biochemical differences between the two endosome subpopulations. Both early and late endosomes are clearly distinct from lysosomes whose membranes are composed of a unique class of heavily sialylated glycoproteins. These lysosomal membrane glycoproteins (lgp's) lack the man-6-P recognition marker and must be targetted polysosomes by a mechanism independent from the man-6-P receptor. lgp's are rapidly transported from Golgi to lysosomes at rates similar to man-6-P-containing lysosomal enzymes and also appear to avoid transport to the cell surface. Finally, we may also discuss the structure and expression of a macrophage-lymphocyte IgG FcR, an Ig-like transmembrane glycoprotein we have recently cloned and sequenced. During endocytosis, the intracellular pathway of the FcR can be modulated: recycling from endosomes when the receptor is bound to monovalent ligand vs. transport to lysosomes following multivalent ligand attachment. Analysis of the primary and oligomeric structure of the FcR should provide insight into how membrane proteins can avoid, or preferentially favor, transport to lysosomes.

E 024 SORTING OF CELL SURFACE PROTEINS AND LIPIDS IN MDCK CELLS, Kai Simons and Gerrit van Meer, European Molecular Biology Laboratory, Postfach 10.2209, D-6900 Heidelberg, Federal Republic of Germany.

Epithelial cells form sealed monolayers, with each cell having its plasma membrane divided into two domains, the apical and the basolateral cell surfaces. With MDCK cells grown on permeable supports, we and others have shown the apical and basolateral virus proteins are sorted from each other intracellularly. Our recent studies suggest that sorting occurs in the trans-most part of the Golgi complex, which we call the trans-Golgi network (1).

Also the lipid composition of the apical and the basolateral surface domains is different. The major difference is in the distribution of sphingoglycolipids, which seem to be concentrated in the apical domain. To study the sorting of lipids in filter-grown MDCK cells we have established an experimental model using C6-NBD-ceramide (2). This lipid added to MDCK cells concentrates into the Golgi complex where it is metabolized to C6-NBD-sphingomyelin and C6-NBD-glucosylceramide. Traffic of the metabolized lipids to the cell surface is inhibited at 20°C. However, if the cell monolayer is warmed to 37°C, the NBD analogues move to the plasma membrane. The appearance of the glucosylceramide is polar, about 4:1 (apical:basolateral), whereas the sphingomyelins are distributed to both cell surface domains in about equimolar ratios.

References:
1. G. Griffiths and K. Simons (1986) The trans Golgi network: Sorting at the exit site of the Golgi complex, Science, in press.
2. N.G. Lipsky and R. Pagano (1983) Sphingolipid metabolism in cultured fibroblasts, Proc. Natl. Acad. Sci.USA 80, 2608-2612.

Processing and Targeting

E 100 CHROMOGRANIN IN LYMPHORETICULAR CELLS, Ruth Hogue Angeletti, Judith Martin, Jiang Qian, Eva Ling and Mary Bilderback, Univ. of Pennsylvania, Philadelphia PA 19104-6079.
Chromogranin is a secretory protein found in neuroendocrine cells and neurons, but not within the vesicles of exocrine cells. This protein has recently been detected in cells of immune tissues. Using monoclonal antibodies and a cDNA probe for chromogranin, we have examined in more detail the association of this protein with granulated cells in lymphoreticular tissues. Both normal cells and continuous cell lines were studied. Chromogranin is not associated with all granulated cells, but is restricted to a small subset of cells. (NS-22697)

E 101 EXPRESSION OF INTRACELLULAR MEMBRANE PROTEINS FROM SYNTHETIC RNA, John Armstrong, Malcolm MacRae* and Alan Colman*, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX and *Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, England.
Some enveloped viruses acquire their envelopes by budding through intracellular membranes of the host cell. We have cloned and sequenced cDNA for glycoproteins from two such viruses, the E1 protein of Coronavirus, which buds at the Golgi complex, and VP10 protein of Rotavirus, which buds at the ER. Synthetic RNA was prepared to both cDNAs and injected into cultured cells and Xenopus oocytes. In cultured cells, the E1 protein was localised in the Golgi complex and the VP10 protein in the ER. In Xenopus oocytes, the E1 protein acquired sialyated O-linked sugars while the VP10 protein acquired Endo-H sensitive N-linked sugars, both consistent with similar localisations. Thus the two proteins can be used as models to study sorting into the ER and Golgi complex. When the RNAs were expressed in meiotic oocytes, the VP10 protein was unchanged, but the E1 protein was processed to a much lesser extent, consistent with a cessation of vesicular transport during cell division.

E 102 ANALYSIS OF A PROTOZOAN SECRETORY PATHWAY, Paul A. Bates, Ilona Bönsch and Dennis M. Dwyer, LPD, NIAID, NIH, Bethesda, MD 20892
Extracellular forms of the parasitic protozoan, Leishmania donovani secreted a soluble acid phosphatase during in vitro culture. Metabolic labelling experiments demonstrated that this glycoprotein was rapidly synthesized, released and constituted the major secretory protein. Both primary glycosylation, presumably in the rough endoplasmic reticulum, and secondary glycosylation, presumably in the Golgi apparatus, occurred during biosynthesis. These resulted in the addition of N-linked oligosaccharide side chains and complex polysaccharides, respectively. Inhibition of either or both processes with tunicamycin and/or monensin altered the electrophoretic mobility of the enzyme but did not prevent its secretion.

Breakage of cells and disruption of organelles by freezing and thawing rendered the intracellular enzyme activity soluble. However, subcellular fractionation revealed latent activity in particulate fractions. Also, indirect fluorescent antibody staining of cells with monoclonal antibodies to the soluble acid phosphatase gave a discrete localized pattern, especially in the region of the flagellar reservoir. These observations suggest that acid phosphatase release occurs via a classical vesicle-mediated secretory mechanism. The extracellular enzyme is presumed to be of survival value to the organism during its intracellular growth and multiplication within the phagolysosomal system of macrophages.

E 103 ANALYSIS OF THE SIGNAL FUNCTION IN DELETION MUTANTS OF NEURAMINIDASE, AN AMINO TERMINALLY ANCHORED GLYCOPROTEIN, Don J. Brown, Brenda G. Hogue, Debi P. Nayak, University of California, Los Angeles, California 90024.

Influenza neuraminidase (NA) is a membrane glycoprotein with a single, amino-terminally located hydrophobic region. The first 40 amino acids of this protein has been previously demonstrated by this laboratory to function both as a transmembrane anchor and as a non-cleaved signal sequence. To determine if these functions were separable, a nested set of amino terminal deletions were created. These were placed into the pGem3 vector 3' to the T7 promoter, transcribed and translated in vitro using wheat germ lysates. Translocation of the proteins was assessed by the inclusion of dog pancreas microsomes and analyzing for NA glycosylation. The retention of anchor function was assessed by the ability to extract the glycosylated protein with ice cold carbonate. We demonstrate that the highly conserved 9 amino acids of the amino terminus are not required for functional SRP binding nor translocation of the molecule.

Sequences of Deletion Mutants

W.T. M N P N Q K I I T I G S I C M V V G I I S L I L Q I G N J I S I W I S H S I Q T
N minus 11 M P A G R N S G S I...
N minus 15 M V V G...
N minus 18 M P A G R D G I I...
N minus 24 M P A G R L Q I...
N minus 27 M P A G R N S G N I I...

E 104 LYSOSOMAL DELIVERY OF THE MAJOR MYELIN GLYCOPROTEIN, Kurt R. Brunden and Joseph F. Poduslo, Depts. of Neurology and Biochemistry, Mayo Clinic, Rochester, MN 55905

Schwann cells (SCs) of rat sciatic nerve respond to injury by altering the levels of biosynthesis of the major myelin glycoprotein, P_0. If SCs are deprived of axonal contact through permanent nerve transection, the level of newly synthesized P_0 is reduced. We demonstrate that much of the down-regulation of the glycoprotein is a result of delivery to lysosomes for degradation shortly after synthesis. If the level of P_0 biosynthesis is evaluated by [^{3}H]mannose incorporation into nerve slices, a marked increase of labeled P_0 is seen in the presence of the lysosomotropic agent, NH_4Cl. Pulse-chase analysis in the absence and presence of NH_4Cl reveals that this increase is due to an inhibition of degradation that occurs 1-2 hours after synthesis. This result is verified when P_0 level is evaluated by [^{3}H]mannose or [^{3}H]amino acid incorporation in the absence and presence of L-methionine methyl ester, a dilator of lysosomes. The oligosaccharide processing inhibitors, deoxymannonojirimycin (dMM) and swainsonine (SW), also block P_0 degradation, presumably through an inhibition of normal glycoprotein processing. The data obtained with dMM and SW indicate that P_0 delivery to the lysosomes occurs after the action of Golgi GlcNAc transferase I and that $Man_{6-9}GlcNAc_2$ processing intermediates are not targeted for degradation. This implies that the mannose-6-phosphate targeting mechanism employed by hydrolases is not involved in P_0 transport. The lack of observable P_0 phosphorylation in the transected nerve either in the absence or presence of NH_4Cl or SW supports this conclusion. The re-routing of the glycoprotein appears to be a mechanism whereby SCs post-translationally regulate the level of P_0 expression in the absence of myelin assembly.

E 105 POST-TRANSLATIONAL TRANSLOCATION OF INFLUENZA VIRUS HEMAGGLUTININ. Chuck C.-K. Chao, Phil Bird, Mary-Jane Gething and Joe Sambrook, Dept of Biochemistry, University of Texas Health Science Center, Dallas, Texas 75235

Influenza virus hemagglutinin (HA) is a trimeric integral membrane protein. We are investigating its post-translational translocation in a cell-free translation system. Wild-type and truncated forms of HA were expressed in a system consisting of rabbit reticulocyte lysate and canine pancreatic microsomal membranes. Four types of HA were used: A+T+, A+T-, A-T+, A-T- (A = membrane spanning anchor region, T = translation termination codon). All four types of HA were efficiently in vitro translated and in each case greater than 95% of the molecules co-translationally translocated into membrane vesicles as reflected by glycosylation and protease-protection assays. However, only the A-T- form was post-translationally translocated into vesicles (approximately 10%); addition of ATP and GTP caused a slight increase of translocation. A similar derivative of HA with a mutated hydrophobic signal sequence was also translated, but not cotranslationally translocated. Hence, at least 4/5 of HA, but not the full-length molecule, can be post-translationally inserted into membrane vesicles.

E 106 THE UPTAKE OF FRUCTOSE BIPHOSPHATE ALDOLASE INTO THE GLYCOSOME OF TRYPANOSOMA BRUCEI.

Christine E. Clayton and Dina Ricereto, The Rockefeller University, 1230 York Avenue, New York, N.Y. 10021-6399.

The glycolytic enzymes of Trypanosomes and related organisms are found in a unique organelle, the glycosome, which may be evolutionarily related to the glyoxisomes and peroxisomes of higher organisms. Fructose biphosphate aldolase is synthesized on free polysomes of trypanosomes and taken up into the glycosomes without detectable covalent modification. The uptake process in vivo is extremely rapid, having a half-time of less than 5 minutes. The protein sequence has no obvious features to differentiate it from cytoplasmic aldolases of other organisms, apart from a preponderance of basic residues (a feature also seen in other glycosomal enzymes). The results of in vitro uptake experiments will be presented.

E 107 OVEREXPRESSION OF ompC CAUSES SELECTIVE DECREASE IN SYNTHESIS OF EXPORTED PROTEINS IN E.coli. Eva Marie Click, Greg McDonald and Carl Schnaitman, University of Virginia, Charlottesville, VA 22908.

OmpC, OmpF, OmpA and LamB are the major outer membrane (OM) proteins of E.coli. The relative amounts of these proteins can be varied genetically or environmentally, yet the total mass of the OM protein/cell remains nearly constant. We have studied the nature of this regulation using cells in which OmpA and LamB are the only major OM proteins being expressed due to deletion of the chromosomal copy of ompC and a mutation eliminating expression of ompF. The cells were transformed with an expression vector containing the ompC gene under control of the trp-lac promoter. In the absence of inducer, the amount of OmpC produced in these cells is less than that produced from a chromosomal ompC gene. When expression of the cloned ompC is induced with IPTG, the OM protein content of the cells is almost entirely OmpC, as shown by SDS-PAGE. Effects on protein synthesis were assessed by pulse-labeling cells with ^{35}S-Met and analyzing whole cell lysates, OM fractions, and immunoprecipitates by autoradiography of SDS-gels. The results showed a large increase in the relative rate of synthesis of OmpC within 10 min of induction. Accumulation of precursors of newly synthesized proteins, indicative of disruption of export, was not detected. Instead, there was a concomitant decrease in synthesis of the other major OM proteins, OmpA and LamB. Synthesis of MalE, a protein which is exported to the periplasm, was not affected. Thus, the cell appears to be able to selectively regulate the synthesis of one class of exported proteins, namely outer membrane proteins. Whether this regulation is at the level of translation or transcription is under investigation.

E 108 SEQUENCES REQUIRED FOR TRANSLATION ARREST MAP DOWNSTREAM OF SIGNAL PEPTIDE WITHIN MATURE BODY OF THE PROTEIN. Nicholas Davis, Marjorie Russel and Peter Model, The Rockefeller University, New York NY 10021.

The gene III protein (pIII), a minor coat protein of bacteriophage f1 (M13 or fd) has an N-terminal, 18-long cleaved signal peptide and is inserted into the E. coli inner membrane (where phage assembly occurs). pIII is normally made in relatively low amounts and attempts at over-expression have failed. Like many other exported or membrane proteins, expression seems to be limited at some post-transcriptional level. Since translation might be coupled to secretion, we made several discrete in-frame deletions of sequences that encode the pIII signal peptide. Instead of relieving a translational block, expression was drastically reduced some 20- to 50-fold in these deletions. Larger deletions, however, that included the signal sequence and extended down into regions encoding the mature portion of pIII, restored expression and resulted in a 20-fold over-expression compared with the original, wild-type starting level. Gene fusion experiments suggest that these effects are on translation. We suggest that the sequences required for coupling translation and secretion (i.e. those which might be recognized by an SRP-like activity) map to sequences in the mature protein, not the signal sequence. The signal sequence may participate at some later stage of the process to release a translational arrest.

E 109 FUNCTION OF THE SACCHAROMYCES CEREVISIAE MNN2 GENE PRODUCT. Cecilia Devlin, Randy Schekman, and Clinton Ballou, University of California, Berkeley, CA. 94720.

The temperature-sensitive lethal sec mutants show that secretion is coupled to cell-surface growth and define the secretory pathway in S. cerevisiae. Addition of N-linked "core" oligosaccharide occurs upon insertion of the polypeptide into the endoplasmic reticulum and addition of the "outer chain" carbohydrate occurs in another compartment, probably the Golgi. The carbohydrate can be a marker for the transit of glycoproteins since it is not required for their secretion or localization in yeast.

All of the mnn mutants have fewer mannose residues in the outer chain but several are not defective in oligosaccharide biosynthesis in vitro. To test the hypothesis that these mutants affect secretion, we screened them for mislocalization of carboxypeptidase Y (CPY) by Western blotting. ProCPY passes through the Golgi on its way to the vacuole; however, when the enzyme is overproduced proCPY is secreted. The mnn mutants do not accumulate or secrete proCPY.

To clone the MNN2 gene by complementation, we screened transformants with fluorescently-labelled antisera and a dye which distinguish wild-type and mnn2 cell-surface carbohydrate. A 3.0 kb subclone of the original 9.0 kb insert directs integration to the MNN2 locus. Strains containing the 9.0 kb insert in YEp24 do not overproduce mannosyl transferase activity. To determine if a null mutant has a growth phenotype we constructed a gene disruption. Genomic Southern blot hybridization experiments will demonstrate whether or not this gene is unique. To monitor overproduction of the MNN2 protein, and for future experiments, we will make antibody to a MNN2-lacZ fusion protein from E. coli.

E 110 CHARACTERIZATION OF THE CANINE SIGNAL PEPTIDASE COMPLEX, Emily A. Evans, Gregory S. Shelness, and Günter Blobel, Rockefeller University, New York NY 10021.

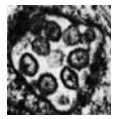
We have previously described the purification of canine microsomal signal peptidase to near homogeneity as a complex of several polypeptides (Evans, E.A., R. Gilmore, and G. Blobel (1986) PNAS 83, 581-585). Two of these polypeptides were shown to contain high mannose oligosaccharide moieties, and were reduced to a single band on SDS-PAGE after treatment with endoglycosidase H. This result, coupled with our new finding that these polypeptides have identical amino acid sequences at their *N*-termini, implies that these bands represent differentially glycosylated forms of the same polypeptide. All the polypeptides which comprise the signal peptidase complex specifically bound to and eluted from a Con A affinity column coincident with signal peptidase activity. This behavior provides convincing supporting evidence for the existence of a signal peptidase complex since only two of these polypeptides were shown to bind Con A. The two forms of the glycoprotein eluted separately during Con A affinity chromatography, suggesting that there is only one form of this polypeptide per complex. The complex cannot be dissociated by high concentrations of salt or non-ionic detergent, but this structural integrity is not maintained by interchain disulfide bonds. The activity of purified signal peptidase was inhibited by *o*-phenanthroline, several sulfhydryl reagents, and high concentrations of chymostatin and elastatinal. Removal of the oligosaccharide moieties did not effect activity. Oligonucleotides specific for the *N*-termini of several polypeptides of the signal peptidase complex were used to screen a MDCK cell cDNA library. The results of sequence analysis of these clones will be presented, as will further biochemical characterization of the signal peptidase complex.

E 111 **THE EFFECTS OF THE NH_2-TERMINAL PROPEPTIDES OF HUMAN APOLIPOPROTEIN A-I AND A-II ON CO-TRANSLATIONAL TRANSLOCATION AND PROTEOLYTIC PROCESSING,** Rodney J. Folz and Jeffrey I. Gordon, Department of Biological Chemistry, Washington University School of Medicine, St. Louis MO. 63110.

The function of NH_2-terminal propeptides are not known. Peters and Reed (*J. Biol. Chem.* 255, 3156-3163 (1979)) have hypothesized that propeptides (e.g. in human serum albumin) may act as intracellular targeting signals for secretory proteins lacking carbohydrate. Of the 8 known human apolipoprotein sequences determined to date, only apolipoprotein (apo) A-I and apo A-II (the principle protein components of human serum high density lipoproteins) are initially synthesized as prepropolypeptides. The hexapeptide prosegment of apo A-I (R-H-F-W-Q-Q) is unusual in the it ends in Gln-Gln. The pentapeptide prosegment of apo A-II (A-L-V-R-R) is more typical of propeptides in that it ends in the dibasic residues Arg-Arg. In an attempt to analyze the affects of these propeptides on early events in their secretion, we isolated full-length cDNAs encoding apo A-I and apo A-II from a human liver λ-gt11 cDNA library. Oligonucleotide-directed mutagenesis was used to construct mutants in which the nucleotides encoding the propeptides were deleted. mRNA was generated from wildtype and mutant cDNAs using an SP6 vector system. Signal peptidase processing was assayed by the co-translational addition of canine pancreatic microsomal membranes, followed by NH_2-terminal sequencing of the primary and processed translation products. Translocation into the microsomal membranes was assayed by the post-translational addition of protease or protease plus detergent. For apo A-II, deletion of the propeptide resulted in a redirection of signal peptidase cleavage, occurring two amino acids into the mature protein sequence, even though the "wildtype" signal peptide was completely intact. This redirected site of proteolytic cleavage is consistent with rules for predicting signal peptide cleavage sites. However, both the wildtype and mutant apo A-II were co-translationally translocated into the lumen of the microsomal vesicles, and interacted with the membranes in an analogous way. Thus, the pentapeptide prosegment of apo A-II may act as an "adapter". This adapter would be necessary when signal peptidase will not cleave the junction formed by a signal peptide and its mature protein. (Supported by NIH grants HL-18577, AM-30292, and MSTP grant GM-07200).

E 112 DOES SEMLIKI FOREST VIRUS RNA REPLICATION OCCUR ON THE LYSOSOMAL MEMBRANE? Susan Froshauer, Klaus-Peter Zimmer and Ari Helenius, Dept. of Cell Biology, Yale University School of Medicine, New Haven CT 06510.

The initial interactions of Semliki Forest virus (SFV), an enveloped, positive-stranded RNA virus with the cell have been well characterized. After internalization by endocytosis, the virus particles are delivered to acidic prelysosomal vacuoles called endosomes. In response to the low pH, viral surface glycoproteins undergo a conformational change and mediate a fusion reaction between the membranes of the virus and the endosome. As a result of the fusion reaction, the nucleocapsid is delivered to the cytoplasm where translation and viral replication begins. There is virtually no information about the early cytoplasmic events that follow penetration (i.e. uncoating of the viral genome, initiation of translation, assembly of the RNA polymerase, relocation of the replicative intermediates to the specialized membrane bounded organelles, called cytopathic vacuoles (CPVIs), where viral RNA is thought to replicate). Our studies are aimed at characterizing the cell biology of these early cytoplasmic events with the major focus on determining the nature and origin of CPVIs (see figure) and the mechanism by which the viral components are targeted to this particular organelle. To address these questions we are using three strategies: 1)immunocytochemical EM analysis of ultra-thin cryosections. 2)cell fractionation by free-flow electrophoresis. 3)bulk delivery of anti-sense oligonucleotides complementary to SFV RNA. We propose that SFV replication occurs on the lysosomal membrane, and the location is determined by interaction of viral components with the endosomal membrane at penetration. According to our model, the SFV genome may become associated with the lysosomal membrane when endosomes fuse with lysosomes. Results from EM studies on infected cells using gold-labeled antibodies specific for the lysosomal membrane protein lgp110 indicate that lgp110 antigen co-localizes with the membrane of CPVIs. This provides initial evidence that CPVIs originate from lysosomes and that the lysosomal membrane may be the site of viral replication.



E 113 IMPORT OF FOREIGN PROTEINS INTO CHLOROPLASTS, Anthony A. Gatenby, Thomas M. Lubben, Paul Ahlquist and Kenneth Keegstra, University of Wisconsin, Madison, WI 53706

Proteins that are imported into chloroplasts possess a transit peptide that enables the nuclear encoded protein to be transported through the organelle membranes by a post-translational mechanism. To examine the types and sizes of proteins that can be imported into chloroplasts we have fused the transit peptide of the soybean ribulose bisphosphate carboxylase small subunit to Brome Mosaic Virus coat protein (20 kDa), cyanobacterial RuBPCase large subunit (52 kDa) and maize beta ATPase subunit (58 kDa). Following in vitro transcription and translation, the radiolabelled precursors were incubated with isolated pea chloroplasts and the import of protease resistant proteins examined. We observed considerable variation in the efficiency of import, ranging from very low levels for the coat protein, to substantial levels for the ATPase subunit, indicating that quite large proteins can be imported efficiently. The addition of extra mature small subunit amino acids at the transit peptide cleavage site did not improve the efficiency of import of coat protein. We obtained similar results in vivo for the coat protein, by fusing its gene to a wheat transit peptide encoded on Brome Mosaic Virus RNA3. The fusion protein could be detected in isolated barley protoplasts infected by viral transcripts, but its import and cleavage in chloroplasts was not observed.

E 114 MEMBRANE GLYCOPROTEIN SORTING: EXPRESSION AND INTRACELLULAR TARGETTING OF CHIMERIC VIRAL GLYCOPROTEINS. H.P. Ghosh, N. Ghosh-Choudhury, M.A. Ali, K.S. Raviprakash and L. Rasile, Department of Biochemistry, McMaster University, Hamilton, Ontario, Canada.

A major focus of this laboratory has been to study the mechanism of sorting and transport of membrane glycoproteins by using enveloped viruses which assemble at different intracellular membrane locations. The glycoproteins G of VSV, gB and gC of HSV-1 and G_1 and G_2 of Bunyamwera virus are targetted to the plasma membrane, nuclear envelope inner membrane and the membrane of the Golgi complex, respectively for virus assembly in those locations. The glycoproteins G of VSV and gB and gC of HSV-1 have been expressed from cloned genes in mammalian cells and found to be localized in the plasma membrane and nuclear membrane, respectively. A number of hybrid glycoproteins containing domains of VSV G and HSV-1 gB and gC have been constructed and expressed in COS cells. The hybrid proteins are glycosylated and immunologically active. Immunofluorescence studies showed that the chimeric protein gB-G is not transported to the cell surface. The hybrid gB-gC, however, is transported to cell surface and secreted into the medium. Expression and localization of Bunyamwera virus glycoproteins G_1 and G_2 using cloned DNA are in progress. (Supported by MRC Canada.)

E 115 PURIFICATION AND PROPERTIES OF GLYOXYSOMAL MATRIX PROTEINS FROM CASTOR BEAN ENDOSPERM, Elma Gonzalez, Suzanne M. Harley and Michael D. Brush, Department of Biology, University of California, Los Angeles, CA 90024.

Glyoxysome function in the endosperm of lipid storing seeds is developmentally regulated. In castor bean (Ricinus communis), glyoxysomes proliferate from non-detectable enzyme levels in the dry seed to maximal enzyme activities at 5 days post-germination. Glyoxysomal-specific enzymes are present in endoplasmic reticulum during the first two days post-germination. This localization is explained by our recent discovery (Plant Physiol 80:950) of the existence of glycoproteins in the glyoxysomal matrix. In this study we have focused on four of the abundant glyoxysomal matrix polypeptides. Four polypeptides, 55 k, 56 k, 62 k and 65 kD in the matrix of isolated glyoxysomes have been purified to homogeneity by reverse phase HPLC and preparative SDS-PAGE. Each polypeptide is unique based on hydropathic properties and comparison of peptide fingerprints (Cleveland et al., J. Biol. Chem. 252:1102). All four of the isolated polypeptides show specific binding to Concanavalin A. Monospecific antisera have been raised in rabbits against each of the glycopolypeptides. Studies are in progress to determine the size of the respective in vitro-sythesized polypeptides. Supported by NSF DMB 85 16995.

E 116 A TOPOGENIC ROLE FOR THE AMINO TERMINUS OF PROALPHAFACTOR, Reza Green[1], Richard A. Kramer[2], and Dennis Shields[3], [1]Mt. Sinai School of Medicine, [2]Roche Institute for Molecular Biology, [3]Albert Einstein College of Medicine.
Expression in yeast of fusion proteins between proalphafactor and somatostain (SRIF), results in the correct processing and secretion of this heterologous 14-amino acid peptide (Green et al., J. Biol. Chem. 261, 7558-7565 (1986)). When the chimeric genes were placed under control of the yeast acid phosphatase (PHO5) promoter, significant amounts of an unglycosylated form of precursor accumulated intracellularly, suggesting a disruption in a RER function. We report here that appearance of this species is due to a minor alteration in the three amino-terminal residues of the chimera, i.e. Met-Arg-Phe in native proalphafactor is changed to Met-Phe-Lys in the hybrids. The unglycosylated precursor represents a population of molecules that are successfully targeted to the endoplasmic reticulum membrane but are incompletely translocated across the lipid bilayer. Our data demonstrate that, even though proalphafactor lacks a cleavable signal peptide, the N-terminus plays an important role in topogenesis. Furthermore these results show that translocation and glycosylation can be uncoupled from protein synthesis in vivo, and therefore can be post-translational events.

E 117 MUTANTS OF HERPES SIMPLEX VIRUS TYPE 1 LACKING THE CYTOPLASMIC DOMAIN OF GLYCOPROTEIN C, Thomas C. Holland, Wayne State University, Detroit, MI 48201.
Herpes Simplex Virus Type 1 (HSV-1) specifies at least six glycoproteins. At least one of these glycoproteins, designated gC, has been shown to be nonessential for virus replication in cell culture. Because it is nonessential, virus mutants expressing mutant gC glycoproteins have been exploited to study a number of the properties of this glycoprotein. The structural domains of gC include an N-terminal signal sequence, a large external domain, a hydrophobic transmembrane domain and a short, highly basic cytoplasmic domain at the C-terminus. In this study, a plasmid was constructed containing a mutant gC gene in which a termination codon was inserted at the end of the transmembrane domain, just upstream of the cytoplasmic domain. The mutant gene was recombined into an HSV-1 mutant with a deletion of the gC gene by cotransfection of plasmid and viral DNA. Recombinant viruses were isolated by plaque hybridization. The recombinant viruses have been characterized to determine the role of the gC cytoplasmic domain in incorporation of gC into virion envelopes, membrane binding of gC, and processing of gC. These experiments have shown that the amount of mutant gC is altered in a number of properties. After overnight labeling, the amount of gC detected in mutant infected cultures was substantially lower than in wild type infected cultures. However, pulse labeling showed that approximately equal amounts were synthesized 9 h after infection, indicating that the mutant gC was less stable than wild type. Also, approximately 20% of the mutant gC was secreted from the infected cells. This may indicate that the charged amino acids in the cytoplasmic domain contribute to the membrane binding of gC, perhaps because it is too energetically unfavorable to transport this domain across the lipid bilayer. Quantitation of gC in mutant virions by virion purification has not been completed, but mutant virions were as susceptible to neutralization by gC specific monoclonal antibodies as wild type, showing that at least some gC was incorporated into virus particles. Additional gC mutants, including some with charged amino acids inserted into the transmembrane domain, are being constructed.

E 118 CHARACTERIZATION OF RIBOSOME-MEMBRANE INTERACTION IN ROUGH ENDOPLASMIC RETICULUM
Michael Hortsch and David I. Meyer, European Molecular Biology Laboratory, Heidelberg, FRG.

Secretory proteins are synthesized on ribosomes bound to the membrane of the endoplasmic reticulum (ER). To identify proteins involved in ribosome binding and protein translocation, beyond the (SRP-DP-mediated) recognition step, controlled proteolysis was used to functionally inactivate rough microsomes that had previously been depleted of DP. Increasing levels of proteases abolished the ability of membranes to be functionally reconstituted with the active cytoplasmic fragment of DP. This functional inactivation did not correlate with a loss of signal peptidase activity, nor with the ability of the DP fragment to reassociate with the membrane. It did correlate, however, with a loss of in vitro ribosome binding to stripped, "rough" microsomes. Ribophorins I and II are two membrane proteins which are restricted to rough microsomes and were postulated to be involved in the binding of ribosomes to the ER membrane. Immunoblots developed with monoclonal antibodies against ribophorins I and II demonstrated that no correlation exists between the protease-induced inability of microsomes to bind ribosomes and the integrity of the ribophorins. Ribophorin I was 85% resistant and ribophorin II 100% resistant to the levels of protease needed to totally eliminate in vitro ribosome binding. Moreover, no direct association was found between ribophorins and ribosomes; upon detergent solubilization at low salt concentrations, ribophorins and other rough ER specific proteins could be sedimented in the presence of absence of ribosomes. These results indicate that ribophorins are predominantly disposed in the ER lumen and are probably part of a rough ER specific supramolecular complex or network structure.

E 119 EXPRESSION OF GLYCOPROTEINS BY EMBRYONAL CARCINOMA CELLS: NOVEL KINETIC COMPARTMENTS DURING GLYCOPROTEIN PROCESSING, Raymond J. Ivatt and John W. Reeder, M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030.

Embryonal carcinoma and early embryonic cells express unusually large and complex carbohydrates. These carbohydrates are copolymers of the sugars galactose and N-acetylglucosamine (polylactosamines). Various structural organizations (from linear to highly branched) and various decorations (fucose, sulfate, and sialic acid) have been described. In embryonal carcinoma cells polylactosamines are prominent members of a large spectrum of glycans assembled on mannose cores derived from a common precursor glycan. Metabolic pulse labeling and structural studies have identified the following: (1) A small proportion of the glycoproteins found extracellularly are actually secreted directly; however, these carry a very different spectrum of glycans from the cell surface glycoproteins. (2) The bulk of the glycoproteins at the cell surface turn over relatively rapidly (half lives 2-4 h) and accumulate extracellularly. (3) There is a large reservoir of unprocessed, high-mannose containing glycoproteins stored in a pre-Golgi compartment that are mobilized to replace the material lost by this cell surface turnover. (4) There is a separate kinetic compartment for branch formation and sulfation and as a consequence, there is a delayed size redistribution following polylactosamine initiation that coincides with a large increase in the formation of highly branched, sulfated glycans.

This work was supported by grants CA-42650 from the National Cancer Institute and 1-972 from March of Dimes Birth Defects Foundation.

E 120 **VITAMIN K-DEPENDENT γ-CARBOXYLATION OF FACTOR IX REQUIRES AN INTACT PROPEPTIDE.**
M.J. Jorgensen, A.B. Cantor, B.C. Furie, C.L. Brown, C.B. Shoemaker, and B. Furie. New England Med Ctr and Tufts Univ, Boston MA and Genetics Inst, Cambridge MA.

The vitamin K-dependent proteins, including Factor IX (FIX), undergo a unique step in post-translational processing during which specific glutamic acid residues are converted to γ-carboxyglutamic acid. The intracellular forms of these proteins contain amino-terminal extensions of 18 - 26 residues, and sequence homology among these propeptides has suggested a role for this region in signaling γ-carboxylation. To test this hypothesis, alterations were made in the propeptide (residues -1 to -18) of FIX and the effects on γ-carboxylation determined. The FIX cDNA coding sequence was modified using oligonucleotide mutagenesis and expressed in Chinese hamster ovary cells. Secreted protein was analyzed by radioimmunoassay. The extent of γ-carboxylation was estimated by determining the ratio of γ-carboxy FIX to total FIX antigen. γ-carboxy FIX antigen was measured using conformation-specific antibodies directed against determinants stabilized by γ-carboxyglutamic acid and metal ions. By this analysis, unmodified recombinant FIX was judged to be 64% carboxylated. Deletion of the 18-residue propeptide completely abolished γ-carboxylation, but not secretion, of FIX. Substitution of Ala for Phe(-16) or Glu for Ala(-10) severely impaired carboxylation, as measured by γ-carboxy FIX to total FIX antigen ratios of 6% and 2%, whereas substitution of Arg for Ile(-7) had no effect. Substitution of Glu for Arg(-4) prevented secretion of any FIX antigen. Thus the amino-terminal portion of the FIX propeptide appears to be a signal for recognition by the carboxylation apparatus and/or targeting of these proteins to appropriate subcellular compartments for γ-carboxylation.

E 121 THE ROLE OF THE 78 kDa GLUCOSE REGULATED PROTEIN (GRP 78) IN THE GLYCOSYLATION AND SECRETION OF COMPLEX PROTEINS. R. J. Kaufman, D. Bole*, and A. J. Dorner, Genetics Institute, Cambridge, MA and *Dept of Pathology, Yale Medical School, New Haven, CT

The role of GRP 78 in the synthesis, glycosylation, and secretion of human factor VIII (fVIII) and human tissue plasminogen activator (tPA) has been studied in Chinese hamster ovary cells. Human fVIII is a 330 kDa protein which is synthesized as a single chain precursor and processed to a two chain form in the Golgi. The protein has an internal domain of 900 amino acids containing 20 N-linked glycosylation sites. Deletion of this domain results in higher levels of fVIII secretion. The degree of GRP 78 association with the wt and deleted forms of fVIII has been studied. Over 50% of the wt molecule is bound to GRP 78 in the endoplasmic reticulum. A majority of this bound protein is never secreted. The deleted form of fVIII exhibits less association with GRP 78 and its association is transient, i.e., it is eventually secreted. Overexpression of GRP 78 results in a decrease in wt fVIII secretion with little effect on the deleted form. These results suggest the number of glycosylation sites and their utilization may affect binding to GRP 78. To test this hypothesis we compared secretion of tPA and of a tPA mutant (3x) that had destroyed the 3 N-linked glycosylation sites by site-directed mutagenesis. Wt tPA is transiently associated with GRP 78 and efficiently secreted. In the presence of tunicamycin, the majority of wt tPA is blocked in secretion and remains complexed to GRP 78. In contrast, 3x is only slightly associated with GRP 78 and is efficiently secreted in the presence and absence of tunicamycin. These results suggest that GRP 78 plays a major role in glycosylation and transport of secreted glycoproteins.

E 122 PDGF RECEPTOR BIOSYNTHESIS IN NORMAL AND SSV-TRANSFORMED CELLS, Mark T..Keating, Thomas O. Daniel and Lewis T. Williams, Howard hughes Medical Institute, University of California, San Francisco, CA 94143.

Oligopeptides predicted by the platelet-derived growth factor (PDGF) receptor cDNA sequence were used to generate polyclonal antisera that specifically immunoprecipitated the receptor. Synthesis of this protein was examined by pulse-chase labeling of cultured fibroblasts with ^{35}S methionine followed by immunoprecipitation. In both Balb/c 3T3 and normal rat kidney (NRK) fibroblasts, the receptor was synthesized as a precursor with apparent molecular weight of 160 kDa and was converted to a mature 180 kDa form within 30-45 minutes. N-linked oligosaccharides were evident on both precursor and mature forms. Terminal processing of these oligosaccharides accounted for much, but not all of the apparent molecular weight shift to the mature form. The half-life of labeled PDGF receptors was 2 hours in the absence, and 45 min in the presence, of PDGF. Fibroblasts transformed by the simian sarcoma virus (SSV) synthesized a comparable 160 kDa precursor. Notably, in SSV-transformed NRK cells, this precursor was converted to a diffuse band of apparent molecular weight 160-180 kDa and no discrete 180 kDa band was detected.

E 123 PRIMARY AND SECONDARY STRUCTURAL CONSTRAINTS ON SIGNAL PEPTIDE HYDROPHOBIC CORE REGIONS. D.A. Kendall, G.A. Laforet and E.T. Kaiser. The Rockefeller University, New York, NY 10021.

Prokaryotic signal peptides all contain a hydrophobic core region which may adopt a secondary structure that is important for signal peptide function. To investigate the role of a hydrophobic α-helix in the E. coli alkaline phosphatase signal peptide, we have constructed a mutant with nine consecutive leucine residues in the hydrophobic core segment. This was accomplished using site-specific mutagenesis to make four amino acid substitutions in the wild type sequence. A multicopy plasmid containing the alkaline phosphatase gene with either the mutant or wild type sequence was used to transform an E. coli host. Transport studies with the mutant strain indicate that mature alkaline phosphatase is correctly targeted to the E. coli periplasm and that processing of the precursor to the mature form of the enzyme is extremely rapid. This indicates that a signal sequence with high potential for hydrophobic α-helix formation is sufficient for alkaline phosphatase transport. However, signal sequences in general remain highly variable and have not converged toward optimization of this structural unit. This may reflect the importance of hydrophobicity rather than conformation or indicate that some sequence specificity is necessary for complementarity with the mature protein. To address this question we have constructed two groups of mutants. One set is designed to assess the relative roles of hydrophobicity and secondary structure in signal peptide function. The other set uses natural core sequences to determine the potential involvement of protein-specific constraints.

E 124 INSERTION OF M13 PROCOAT INTO THE CYTOPLASMIC MEMBRANE OF E. COLI, Andreas Kuhn, Biozentrum, University of Basel, CH-4056 Basel, Switzerland

The mechanism how M13 procoat inserts into the cytoplasmic membrane of E. coli might differ from that of other membrane or secreted proteins. Although procoat contains a normal, cleavable leader peptide, its assembly into the membrane involves both hydrophobic regions (1) as well as the charged termini of the molecule (2). Mutants that change the characteristic of these regions fail to initiate membrane insertion.
M13 procoat insertion does not depend on the gene products secA and secY, which are essential for most membrane and exported proteins (3, 4). This feature is not simply caused by the length of procoat nor is it a function of its leader peptide. Replacing the procoat leader with the leader of the outer membrane protein A (ompA) still retains secY independence. The procoat leader itself is sufficient to mediate export of ompA, although at a slower rate. The function of the leader is also retained if it is placed into the center of a protein. An amino terminal fusion protein encompassing a "leader peptide" of 18 kd and coat protein is still processed by leader peptidase in vivo.
(1) Kuhn, A., G. Kreil and W. Wickner, EMBO J. (in press) (2) Kuhn, A., W. Wickner and G. Kreil (1986) Nature 322, 335-339 (3) Wolfe, P.B., M. Rice and W. Wickner (1985) J. Biol. Chem. 260, 1836-1841 (4) Michaelis, S. and J. Beckwith (1982) Ann. Rev. Microbiol. 36, 436-465

E 125 POST-TRANSLATIONAL MODIFICATION OF THE INFLUENZA VIRUS HEMAGGLUTININ EXPRESSED IN INSECT CELLS, Kazumichi Kuroda[1], Charlotte Hauser[2], Albrecht Gröner[3], Rudolf Rott[1], Walter Doerfler[2] and Hans-Dieter Klenk[4], Universität Gießen[1], Universität Köln[2], Hoechst AG Frankfurt/M[3], Universität Marburg[4], F.R.G.

The hemagglutinin undergoes a number of post-translational modifications in the course of transport from the rough ER to the plasma membrane. These include removal of the signal sequence, acylation, attachment of carbohydrate, sulphation and proteolytic cleavage of the precursor HA into the fragments HA_1 and HA_2. In order to examine the capacity of insect cells to exert these modifications, the hemagglutinin of FPV (fowl plague virus) was expressed in Spodoptera frugiperda cells, using a baculovirus system. A c-DNA sequence of the hemagglutinin gene has been inserted into the pAc 373 polyhedrin vector. Spodoptera frugiperda cells were co-transfected with this construct and authentic AcNPV (Autographa californica nuclearpolyhedrosis virus) DNA, and then recombinant virus was isolated. In recombinant virus infected cells hemagglutinin was synthesized and transported to the plasma membrane. The attachment of carbohydrate and its processing was shown by endoglycosidase H digestion. Activation of the hemagglutinin by proteolytic cleavage at the sequence Lys-Lys-Arg-Lys-Lys-Arg did also occur. Other modifications of the hemagglutinin in insect cells are under study.

E 126 POST-TRANSLATIONAL FATTY ACYLATION OF SODIUM CHANNELS FROM EEL ELECTRIC ORGAN. S.R. Levinson, W.B. Thornhill, A.W. Pike, and P.V. Fennessey, University of Colorado Medical School, Denver, CO 80262. The sodium channel from the electric organ of Electrophorus electricus consists of a single large (approx. 210k Da) polypeptide with unusually hydrophobic characteristics. For example, SDS binding studies show that the polypeptide can complex with the detergent at a ratio of up to 6g SDS/g protein in a manner that suggests the ordering of extensive micellar phases around hydrophobic channel domains. Electrophoretic mobility studies using SDS-PAGE also demonstrated that this hydrophobicity is post-translationally acquired, since the unprocessed core polypeptide appears to bind near normal amounts of SDS. A cause of the hydrophobic character of the mature molecule might be fatty acylation, a post-translational modification which has been shown to occur for a number of other proteins. We have thus determined the fatty acid composition of the eel channel polypeptide using combined gas chromatography/mass spectroscopy Significant amounts of closely-associated fatty acyl moieties were found by these methods, representing up to 10% by weight of the polypeptide. Most of the fatty acyl material was palmitate, with lesser amounts of stearate and traces of myristate also in evidence. Exhaustive extraction with chloroform/methanol appears to remove about 65% of the fatty acid. The remaining strongly-bound lipid could largely be removed either with hydroxylamine or phospholipase C, suggesting a covalent linkage to the protein core of the channel via a phospholipid head group. Possible roles of such a relatively extensive fatty acyl domain in the function and targeting of eel sodium channels are presently under investigation. Supported by NIH NS-15879 and RR-01152, and the Muscular Dystrophy Assn.

E 127 DYNAMIC NATURE OF THE FATTY ACID MODIFICATION OF $p21^{N\text{-}ras}$, Anthony I. Magee, Lourdes Gutiérrez, Ian McKay*, Alan Hall* and Chris Marshall*. NIMR, Mill Hill, London NW7 1AA, U.K. and *Chester Beatty Institute for Cancer Research, London SW3, U.K.
A cell line (T15) derived from NIH-3T3 cells by transfection and expressing the normal human N-ras proto-oncogene under the control of the steroid-inducible MMTV promoter has been used to study the stability of the fatty acid modification of $p21^{N\text{-}ras}$. Despite the absolute requirement for acylation of p21 for its activity (Willumsen et al. (1984) EMBO J., 3, 2581-2585) it has been observed that microinjected bacterially-expressed non-acylated p21 can transform cells (Feramisco et al. (10984), Cell, 38, 109-117) suggesting the possibility of post-translational acylation of p21. Indeed we find that T15 cells expressing $p21^{N\text{-}ras}$ to ~1% of total membrane protein are able to acylate p21 with palmitate to normal levels after pretreatment with protein synthesis inhibitors. Pulse-chase studies show that while the half life of the $p21^{N\text{-}ras}$ is >24h, the fatty acid moiety is turning over with a half-life of <20 min. These observations suggest a rapid exchange of palmitate on Cys-186 of $p21^{N\text{-}ras}$. Preliminary evidence suggests that the turnover of the fatty acid is dependent on the presence of growth factors. In addition removal of the fatty acid by hydroxylamine treatment results in dissociation of $p21^{N\text{-}ras}$ from membranes. Thus the dynamic fatty acid modification is implicated in the functional cycle of $p21^{N\text{-}ras}$, possibly by allowing transient membrane association-dissociation.

E 128 **REVERSIBLE LIGAND-INDEPENDENT MODULATION OF HEPATIC GALACTOSYL RECEPTOR DISTRIBUTION AND ACTIVITY BY SODIUM AZIDE.** Douglas D. McAbee and Paul H. Weigel, Division of Biochemistry, University of Texas Medical Branch, Galveston, TX 77550
Isolated rat hepatocytes treated with metabolic energy poisons such as NaN_3 in the absence of ligand lose surface galactosyl receptor (GalR) activity. ^{125}I-affinity-purified anti-receptor IgG (IgG^R) and ^{125}I-asialo-orosomucoid (ASOR) were used to quantitate, respectively, GalR protein and activity on cells treated at 37°C with NaN_3. Surface or total (surface and intracellular) binding of these two probes was measured at 4°C, respectively, in intact or digitonin-permeabilized cells. After 30 min, 15 mM NaN_3 maximally depleted surface binding of both ^{125}I-ASOR and ^{125}I-IgG^R by 50-80% with parallel first order kinetics. Virtually all lost surface GalR protein was intracellular, but about 50% of all cellular GalR were inactive. GalR activity loss was dependent on NaN_3 concentration, but only a subset of GalR was inactivated despite ATP depletion to <1-2% of control. After ATP was restored, surface ^{125}I-ASOR binding completely recovered between 1-2 h without protein synthesis, but ^{125}I-IgG^R binding recovered in only 30 min. We conclude that one of two GalR subpopulations, designated the State 2 GalR, constitutively recycles and undergoes an inactivation/reactivation cycle. In the absence of ligand, these GalR are normally internalized, then inactivated. ATP depletion blocks GalR reactivation, prevents GalR appearance at the surface, and redistributes GalR by trapping inactive receptors inside the cell. After ATP recovery, cells externalize inactive GalR and regain their normal surface receptor content before complete restoration of surface GalR activity. (Supported by NIH grant GM 30218)

E 129 YEAST MATING PHEROMONE a-FACTOR AND ITS RELATIONSHIP TO RAS PROTEINS. Susan Michaelis*, Sonia Santa Anna-A*., Scott Powers+, Michael Wigler+, and Ira Herskowitz*. *Univ. of California, San Francisco, CA, and +CSH Laboratory, Cold Spring Harbor, NY.

Yeast mating pheromone a-factor is a secreted oligopeptide, the precise structure of which is unknown. However, DNA and protein sequence analyses indicate that mature a-factor is 11-15 residues in length and is processed from a precursor which lacks a signal sequence and glycosylation sites, suggesting a novel mode of export for this pheromone. In addition, a-factor maturation likely involves an unusual fatty acid modification (see below). Thus a-factor biogenesis appears to differ significantly from that of α-factor.

We have shown that a common step is required for maturation of a-factor and the yeast RAS proteins by isolation of RAM (RAS and a-factor maturation) mutants, which are defective for both RAS and a-factor activity. It is known that RAS proteins are acylated. Interestingly, a-factor shares with all RAS proteins a common C-terminal sequence, Cys-A-A-X_{COOH}, (A is aliphatic and X is any amino acid). The Cys residue is the site at which RAS is modified by palmitate. We have demonstrated that ram mutants are defective in maturation, membrane localization, and acylation of RAS proteins. We propose that the RAM gene product is responsible for acylation. Further, we suggest that a-factor (or its precursor) contains a fatty acid whose addition is mediated by RAM. We speculate for a-factor that this unusual lipid modification may be involved in a novel secretion pathway.

E 130 TOPOGENIC SIGNALS FOR IMPORT OF GLYCOLYTIC ENZYMES IN GLYCOSOMES (MICROBODIES) OF TRYPANOSOMA BRUCEI, Paul Michels, Anne-Marie Lambeir, David Hart and Fred Opperdoes, International Institute of Cellular and Molecular Pathology, 1200 Brussels, Belgium.

In trypanosomes most enzymes of the glycolytic pathway are found in microbody-like organelles, called glycosomes. The enzymes are synthesized on free ribosomes in the cytosol, as polypeptides of mature size, and posttranslationally imported in the organelle (t 1/2 in cytosol = 1-3 min). To determine the topogenic signals responsible for import of the proteins into the glycosome, we have compared the amino-acid sequences of four glycosomal enzymes : aldolase (ALDO), triosephosphate isomerase (TIM), glyceraldehyde-phosphate dehydrogenase (GAPDH) and phosphoglycerate kinase (PGK) with each other, with a cytosolic isoenzyme for PGK and with their counterparts in other organisms, where they are located in the cytosol. A striking feature of all glycosomal enzymes is a high number of positive residues distributed in two or more clusters along the polypeptide chain. Modelling of the three-dimensional structures of TIM, GAPDH and PGK, using the known structural coordinates of homologouos enzymes from other organisms, indicates that all three may have in common two positively charged patches at the outer surface, about 40 Å apart, which themselves include a pair of basic residues separated by a distance of about 7 Å. We propose that this feature plays an essential role in the topogenesis of glycosomal enzymes.

E 131 **FUCOSYLATION OF MAIZE ENDOSPERM SECRETORY PROTEINS.**
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When maize endosperm cells are cultured in liquid medium a portion of the acid hydrolases normally localized within the vacuoles is secreted extracellularly. The zein storage proteins, which are also localized within the vacuoles, are not secreted into the medium. All of the secretory hydrolases bind to immobilized concanavalin A, whereas it is thought that zein is not glycosylated. This dichotomy suggests a possible role for glycosylation in protein targeting. Acid hydrolysis of the secretory enzymes followed by HPLC of the resultant monosaccharides gave three major peaks, with retention times identical to those of authentic N-acetyl-glucosamine, mannose and fucose. Growth of cultures in the presence of ^{3}H-fucose led to labeling of all of the secretory hydrolases. Acid hydrolysis followed by HPLC analysis demonstrated that the radioactivity remained associated only with fucose. Digestion with endo-beta-N-acetyl-glucosaminidase H, or bovine kidney exo-fucosidase failed to release any label from the hydrolases. Digestion with peptide: N-glycosidase F released the radioactivity from the glycoproteins and subsequent digestion of the oligosaccharides with the exo-fucosidase yielded free fucose as the only radioactive product. The characterization of fucosyl transferase activity is in progress.

E 132 PROCESSING AND TARGETING OF MYELOPEROXIDASE IN HL-60 CELLS,
William Nauseef, University of Iowa, Iowa City, IA 52242

Myeloperoxidase (MPO) is a lysosomal enzyme present in azurophilic granules of human neutrophils and is important for optimal oxygen-dependent microbicidal activity. Native MPO is composed of a pair of heavy-light protomers with subunits of 59-kDa and 13.5-kDa. Intracellular processing and transport during biosynthesis were examined in the human promyelocytic cell line HL-60. Endoglycosidase H and F digestion of proMPO (89-kDa) demonstrated the presence of five N-linked high-mannose oligosaccharide side chains and no complex mannose units. Incorporation of the threonine analog ß-hydroxynorvaline produced species ~2.5kDa and ~5-kDa smaller than the fully glycosylated proMPO, suggesting that two of the glycans were in the THR-X-ASN tripeptide sequence. Processing of proMPO occurred within ~5 minutes. Glucosidase inhibitors castanospermine and deoxynojirimycin resulted in synthesis of a 92-kDa glycoprotein rather than the usually identified 89-kDa peptide. Swainsonine, a mannosidase inhibitor, did not alter the size of the earliest synthesized protein, suggesting that proMPO exited the endoplasmic reticulum or cis-Golgi. Intracellular transport and proteolytic maturation of MPO were retarded by weak bases or monensin at concentrations shown to raise intralysosomal pH. However these agents did not qualitatively alter transport nor increase secretion. Thus although MPO biosynthesis resembled that of other lysosomal enzymes, significant differences exist, including only limited oligosaccharide processing and intracellular transport and proteolytic maturation of proMPO that was only retarded by alkalinizing lysosomes without affecting the products or the fraction secreted.

E 133 **MONENSIN DIFFERENTIATES TWO RECEPTOR PATHWAYS IN THE GALACTOSYL RECEPTOR SYSTEM IN ISOLATED RAT HEPATOCYTES.** Janet A. Oka and Paul H. Weigel, Division of Biochemistry, University of Texas Medical Branch, Galveston, TX 77550
Monensin exerts several effects on the Galactosyl receptor (GalR) system in rat hepatocytes either in suspension or in culture overnight. In particular, two effects support our recent proposal that there are two parallel GalR pathways for endocytosis, ligand processing and receptor recycling. (1) Cells exposed to monensin at 37°C in the absence of ligand lose a portion of the surface GalR activity within 20 min. At ≤5 uM, the monensin effect is transient; surface GalR activity decreases and then recovers. Between 5-20 uM monensin, there is a concentration dependent GalR activity decrease. At ≥20 uM, the down modulation plateaus at about a 50% decrease. We have designated these modulatable receptors as State 2 GalR. State 1 GalR, whose activity is not affected by monensin, can still mediate subsequent ligand uptake, diacytosis, and degradation even after the down modulation of State 2 GalR by exposure of cells to 50 uM monensin for 30 min. (2) Monensin (50 uM) blocks the dissociation of internalized surface-bound ligand. Long term kinetics of dissociation, however, show that the inhibition is only transient. Ligand dissociation resumes after about 20 min and reaches a plateau at 4-5 hr of about 60% dissociation. This dissociation is unaffected by a second addition of 50 uM monensin after 20 min. Only ligand bound to State 1 GalR dissociates. Dissociation of State 2 GalR-ligand complexes does not occur. This result suggests that ligand dissociation in the State 1 and State 2 GalR pathways is driven by different mechanisms. The slow dissociation pathway mediated by State 1 GalR may not require low pH. (supported by NIH grant GM 30218)

E 134 MEMBRANE GLYCOPROTEIN SORTING: ROLE OF THE CYTOPLASMIC TAIL AND TRANSMEMBRANE DOMAIN OF HSV GLYCOPROTEIN gB-1 IN INTRACELLULAR TARGETTING. K.S. Raviprakash and H.P. Ghosh, Deptartment of Biochemistry, McMaster Univsity, Hamilton, Ontario, Canada.
The glycoprotein gB of the nuclear envelope budding HSV-1 is required for infection and virus entry and cell fusion. The glycoprotein gB contains a cytoplasmic domain of 109 amino acid and a triple spanning transmembrane domain of 69 amino acid as compared to the 20-30 amino acid long transmembrane and cytoplasmic domains of other viral membrane glycoproteins. In order to investigate the role of these two domains of HSV-gB in intracellular trafficking and its biological activity we have created specific mutations in the gB gene by site-directed mutagenesis. Three mutants which remove different lengths of the cytoplasmic domain and a mutant lacking the entire transmembrane domain have been constructed. As expected the 'anchorless' mutant, secretes the protein into the medium in a transient expression system. Indirect immunofluorescence studies show that most of the cytoplasmic domain of HSV-1 gB can be deleted without significantly affecting its transport to the plasma membrane. However, these mutant proteins appear not to be localized in the nuclear envelope but in the Golgi complex. (Supported by MRC Canada.)

E 135 ANALYSIS OF SECRETORY PROTEIN TRANSLOCATION ACROSS YEAST MICROSOMES, Jonathan A. Rothblatt and David I. Meyer, European Molecular Biology Laboratory, Heidelberg, FRG
In vitro studies of secretory protein translocation across the membrane of the rough endoplasmic reticulum (rER) have principally utilized the canine pancreatic microsome system in conjunction with wheat germ cell-free protein synthesis. This approach has permitted, over the past 10 years, the biochemical and functional characterization of several components required for protein translocation, including Signal Recognition Particle and Docking Protein. Further exploitation of the pancreatic microsome system has been limited by our inability to selectively inactivate and re-activate other membrane-associated translocation components in order to study them functionally in vitro. The potential for utilizing the yeast Saccharomyces cerevisiae to genetically dissect protein translocation led us to develop a cell-free assay of translocation yeast microsomes (Cell 44, 619-628). In a homologous cell-free system, the translation, translocation and processing of prepro-α-factor, pre-invertase, and prepro-carboxypeptidase Y have been reconstituted. A striking feature of the yeast system is the possibility of certain secretory proteins to translocate and undergo modifications post-translationally, i.e., uncoupled from protein synthesis (EMBO J. 5, 1031-1036). This mechanism has allowed us to investigate the energetics of protein translocation as well as the involvement of cytosolic factors that participate in protein translocation. In addition, the structural features of prepro-α-factor that permits its post-translational translocation have been investigated using this system.

E 136 GENETIC IDENTIFICATION OF COMPONENTS REQUIRED FOR SORTING OF PROTEINS TO THE YEAST LYSOSOME-LIKE VACUOLE, Joel H. Rothman and Tom H. Stevens, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.

The yeast vacuole is equivalent to the mammalian lysosome by a number of criteria. As in mammalian cells, yeast vacuolar proteins are transported to the Golgi apparatus together with secretory proteins. Sorting of these proteins occurs in the Golgi, resulting in targeting of vacuolar proteins to the vacuole, and of secretory proteins to the periplasm. To identify the biochemical components responsible for carrying out protein sorting, we have isolated a large number of yeast mutants that are deficient in vacuolar protein sorting. These mutants define more than 16 VPL (vacuolar protein localization) genes. vpl mutants mislocalize large quantities of at least two vacuolar proteins to the cell surface via late stages of the secretory pathway. A vacuolar membrane protein does not appear to be mislocalized in these mutants, suggesting that soluble and membrane vacuolar proteins are sorted via different pathways in yeast. We have cloned several of the VPL genes and are generating antibody to their products to determine the intracellular location and function of these protein sorting components. Genetic, biochemical, and electron microscopic analyses of the vpl mutants should allow us to develop a more complete understanding of eukaryotic protein sorting.

E 137 A GENETIC AND BIOCHEMICAL ANALYSIS OF A HERPES VIRUS INTEGRAL MEMBRANE GLYCOPROTEIN. J. Patrick Ryan, Alan K. Robbins, Mary E. Whealy, and Lynn W. Enquist, E. I. du Pont de Nemours & Company, Central Reserach & Development Department, Wilmington, DE 19898.

The gIII glycoprotein of Pseudorabies virus (PRV) is localized to both the surface of infected tissue culture cells and the viral envelope that is believed to be derived from the inner nuclear membrane of the host. DNA sequence analysis of the gIII gene indicates the existence in the protein of a putative signal peptide, eight potential N-linked glycosylation sites, and a carboxy terminal anchor sequence. We have begun an analysis of gIII glycoprotein export in infected tissue culture cells. The primary translation product in infected cells is glycosylated at all eight available sites, and migrates as a 74 Kd species. This precursor form is posttranslationally modified, resulting in a 92 Kd polypeptide that eventually can be found at the infected cell surface. We are extending our studies to gIII mutants that have been generated by in vitro manipulations. The gIII gene of virus strain PRV2 encodes a glycoprotein that lacks 134 amino acids, and consequently five N-linked glycosylation sites, from the middle one-third of the polypeptide. Still, the mature form is localized to the virus envelope. Virus strain PRV4 produces a truncated form of the gIII glycoprotein, the result of an amber mutation at codon 157 out of 479, that lacks an anchor sequence and six of its N-linked glycosylation sites. Upon infection in tissue culture, the mature form is found almost exclusively in the medium. Therefore, the first one-third of the gIII glycoprotein contains information sufficient for its maturation and release from infected cells, while a membrane or envelope localization requires at least some of the carboxy terminal 186 amino acids.

E 138 THE PUTATIVE SIGNAL SEQUENCE OF CYTOCHROME P-450PBc2 IS NOT EQUIVALENT TO THE SIGNAL SEQUENCE OF A SECRETED PROTEIN. Ella Szczesna-Skorupa, Nancy Browne, and Byron Kemper, University of Illinois, Urbana, IL 61801.

The N-terminal sequence of cytochromes P-450 (P450) resembles a signal peptide but is not cleaved during the insertion of this integral membrane protein into the microsomal membrane. To examine whether this putative signal peptide was functionally equivalent to a signal peptide of a secretory protein, cDNA coding for a fusion protein was produced in which the signal peptide for preproparathyroid hormone was replaced with the putative signal peptide of P450PBc2 in the protein. The four amino acids preceding the cleavage site of preProPTH were retained in the fusion protein. The translational product of RNA synthesized in vitro from the cDNA was not processed nor translocated by chicken oviduct microsomal membranes in reticulocyte cell free system. The translation, however, was arrested by signal recognition particle in the wheat germ system. Unlike most signal peptides, the P450PBc2 sequence does not contain basic amino acids preceding the hydrophobic core. Introduction by oligonucleotide mutagensis of lysine and arginine in the N-terminal region, resulted in a fusion protein that was partially processed with translocation of both processed and unprocessed proteins. The results indicate that charged amino acids preceding the hydrophobic core may be important in determining the membrane conformation of the signal peptide so that it is cleaved in secretory proteins or is not cleaved and retained as an anchor in a membrane protein like P450. Supported by grants AFOSR 84-317 and NIH GM35897.

E 139 PROCESSING OF BACTERIAL TOXINS BY MAMMALIAN CELLS. Catharine B. Saelinger and Randal E. Morris, University of Cincinnati, Cincinnati, Ohio 45267.
Pseudomonas exotoxin A (PE) and diphtheria toxin (DT) both stop protein synthesis in mammalian cells by the ADP ribosylation of cytoplasmic elongation factor 2. Both toxins are internalized by sensitive cells by receptor mediated endocytosis and reach a prelysosomal acidic organelle. Both toxins are produced as proenzymes and must be activated to express ADP ribosylation activity. The site and mechanism of activation in vivo is not known. We have looked at toxin activation in mouse LM fibroblasts, a cell line sensitive to PE and resistant to DT. LM cells were harvested, homogenized and fractionated on a self-generating Percoll gradient. Different fractions were used to activate the toxins. Wheat germ and ^{14}C-NAD were used as source of substrates. Routinely we found that fractions enriched for endosomes (identified with horseradish peroxidase internalized at 15°C; buoyant density approximately 1.04 g/ml) consistently activated PE, while maximum activation of DT was achieved with heavy lysosomes. Activation occurred only at an acidic pH. The data suggest that in a sensitive cell, PE is converted to an enzyme active form in a prelysosomal acidic vesicle.

E 140 SORTING AND EXPRESSION OF MODIFIED MURINE LEUKEMIA VIRUS GLYCOPROTEINS AT RESTRICTED MEMBRANE DOMAINS, Edward B. Stephens and Richard W. Compans, University of Alabama at Birmingham, Birmingham, AL 35294
In polarized epithelial cells, the maturation sites of enveloped RNA virus that form by budding at cell surfaces are restricted to particular membrane domains. Using vaccinia virus expression vectors, the HA glycoprotein of influenza, gp70/p15E of Friend murine leukemia virus (F-MuLV) and G glycoprotein of vesicular stomatitis virus are preferentially sorted and expressed at the same surfaces of polarized cells where the corresponding viruses are assembled. We have now investigated the expression of viral glycoproteins which differ by specific modifications of their membrane anchor sequences. We have compared the expression of a complete gp70/p15E glycoprotein of Friend mink cell focus forming (F-MCF) retrovirus and truncated forms of this glycoprotein lacking either the (1) transmembrane domain, (2) the cytoplasmic domain, or (3) both transmembrane and cytoplasmic domains. Immunofluorescence studies revealed that in Madin Darby canine kidney (MDCK) cells, intact gp70/p15E or gp70/p15E with a deleted cytoplasmic domain was detectable on the basolateral surface from 4-10 hours postinfection. In contrast, the truncated gp70/p15E lacking the transmembrane or both the transmembrane and cytoplasmic domains could be detected intracellularly but not on the surfaces of the MDCK cells. Immunoprecipitation studies using antiserum to gp70/p15E revealed that these truncated F-MCF glycoproteins were readily secreted into the culture medium whereas the intact F-MCF gp70/p15E remained cell associated. The truncated retroviral glycoprotein lacking both the transmembrane and cytoplasmic domains was found to be released from both apical and basolateral surfaces. The results of expression in polarized cells of glycoproteins with other specific modifications will be presented.

E 141 POSTTRANSLATIONAL MODIFICATIONS OF N-LINKED GLYCANS ON PROTEINS WHICH ARE TARGETED TO PLANT VACUOLES, Arnd Sturm and Maarten J. Chrispeels, Department of Biology, University of California, San Diego, La Jolla, CA 92093.
The vacuoles of plant cells constitute an acidic compartment analogous to the lysosomes of animal cells. In addition to the lytic function (hydrolysis of macromolecules), vacuoles also have a storage function, and can store inorganic ions, organic solutes, or proteins. Lysosomal hydrolases are targeted by phosphorylated glycans (mannose-6-phosphate) and corresponding receptors. The phosphorylation of glycans occurs in the Golgi. The phosphate residues are only of transient nature, they are removed by acid phosphatase after the protein reaches the lysosomes.
In seeds, the vacuoles of the storage parenchyma cells store large amounts of protein and are therefore called protein bodies. In the cotyledons of the common bean, the major proteins in the protein bodies are the storage protein phaseolin and the lectin phytohemagglutinin (PHA). Both are glycoproteins with high mannose as well as complex oligosaccharide chains. The modification of these complex chains occurs in the Golgi where a core of $Man_3(GlcNAc)_2$ is formed, Fuc may be attached to the proximal GlcNAc and Xyl to the β-linked Man. The complex chains acquire in addition 2 terminal GlcNAc residues, which are of transient nature. They are removed again by β-N-acetylglucosaminidase after the proteins reach the protein bodies. These transient modifications are similar to the modifications which the glycans of lysosomal hydrolases undergo, and the possibility that the glycans of plant proteins play a role in targeting is presently under investigation.

E 142 A MAJOR GLYCOPROTEIN DEFECT IN THE PRIMARY GRANULES OF A PEROXIDASE-DEFICIENT HUMAN PROMYELOCYTIC LEUKEMIA (HL60) CELL LINE, Arthur K. Sullivan and John Peyman, McGill Cancer Centre, McGill University, and Division of Hematology, Royal Victoria Hospital, Montreal, Quebec, CANADA H3G 1Y6.
A variant (HL60-A7) of the human promyelocytic leukemia cell line HL60 has been derived which bears large, aberrant primary granules, lacks myeloperoxidase but contains a normal quantity of other enzymes, and shows an additional 1q+ chromosomal marker (Sullivan et al, Leukemia Research 10:501, 1986). These granules were compared with those of the parent HL60 cells by density gradient fractionation, and gel electrophoresis after biosynthetic incorporation of 3H-mannose. The granules of the parent HL60 sedimented into two fractions: the heavy, which contained most of the myeloperoxidase and cathepsin G activities, and the light, which contained most of the acid phosphatase and B-glucuronidase activities. The granules from HL60-A7 gave a single broad band of enzyme activities, consistent with their morphological heterogeneity. Analysis of the protein profiles of the purified granule membranes revealed that the major mannose-containing glycoprotein (m.w. 110-170 KDa) of parental HL60 was not visualized in the products of the A7 variant. We conclude that the concurrence of these biochemical and morphological differences suggests that they may be related. If so, they are one of the first structural abnormalities identified in this system of organelles.

E 143 INHIBITOR STUDIES WITH PROCARYOTIC SIGNAL PEPTIDASE I, Joyce Sutcliffe and Glen Andrews, Pfizer Central Research, Groton, CT 06340.
Signal peptidase I (SPase I) is the enzyme in procaryotes responsible for cleaving signal sequences from the N-termini of non-lipoprotein secretory proteins. SPase I is an atypical endoprotease in that the cleavage recognition site does not appear to consist of a linear sequence of amino acids bridging the cleavage site. Rather, recognition of the cleavage site appears to be afforded via the secondary structure of the signal peptide. Conservation among signal sequences of the core hydrophobic region and the spacing between this region and the cleavage site rather than primary sequence homology support this hypothesis. Our cell-free studies with purified SPase I from E. coli and truncated peptides also support the notion that SPase I recognizes a conserved secondary feature of the signal peptide. Peptides containing the same C-terminus (-1 position) and variable lengths of the procoat signal sequence (up to the -16 amino acid position) were synthesized and tested as potential inhibitors of SPase I. Only the 16-mer (-1 to -16) inhibits the enzyme. This peptide includes amino acids of the core region and amino acids up to, but not beyond, the cleavage site. This result demonstrates that amino acids beyond the cleavage site are not necessary for inhibition and that a significant portion of the signal peptide is required, perhaps for the secondary structural recognition requirements. Attempts to classify SPase I using classical protease inhibitors have confirmed that the enzyme is unaffected by most known inhibitors. However, inhibition in the millimolar range was observed with chymostatin, bestatin, and pepstatin.

E 144 THE EXPRESSION OF A PLANT VACUOLAR PROTEIN IN YEAST, Brian W. Tague and Maarten J. Chrispeels, University of California, San Diego, La Jolla, CA 92093.
We are interested in determining the signals that target proteins to subcellular locations and in determining whether these signals are common between yeast and plant cells. To this end, we have isolated a genomic clone of phytohemagglutinin (PHA), the lectin from the common bean, Phaseolus vulgaris. In the bean seed, PHA functions as a storage protein and is localized in vacuole-derived organelles termed protein bodies. Using standard recombinant DNA techniques, the protein coding sequences of PHA were inserted into the yeast expression vector pYe7, which utilizes the yeast acid phosphatase (PHO5) promoter to drive inserted gene transcription. The constructed vector (pYe7:PHA) was transformed into a yeast strain that allows temperature induction of this promoter. At the permissive temperature, PHA is produced at a level of 0.1% of the total yeast protein as estimated by western blotting. About 99% of the yeast PHA is intracellular, consisting of five major bands on SDS-PAGE. Approximately 1% of the PHA is secreted as a single band. The yeast PHA is glycosylated with two high-mannose sidechains as indicated by their sensitivity to endoglycosidase H (endoH) treatment. This is in constrast to bean PHA which has one high-mannose and one complex sidechain. Additionally, endoH treatment resolves the intracellular yeast PHA into two bands on SDS-PAGE, the lower of which comigrates with chemically-deglycosylated bean PHA. As PHA is a vacuolar protein in the bean, we are currently determining the intracellular location of yeast PHA. Supported by a grant from the Department of Energy and a Fellowship (to BWT) from the National Science Foundation.

PROCESSING AND FATTY ACID ACYLATION OF RAS PROTEINS

E 145 Fuyuhiko Tamanoi and Asao Fujiyama, The University of Chicago, Chicago IL 60637.
Products of ras oncogenes are synthesized in cytoplasm and moves to plasma membranes where the proteins reside and function. It is speculated that palmitic acid covalently bound to the protein provides hydrophobic property needed for the anchorage to membranes. However, little is known about the precise structure of the linkage and mechanism of the modification process. To approach these problems, we have been utilizing yeast as a model system.
We have established that yeast RAS proteins are synthesized as precursors in cytoplasm which are then processed to yield species that migrate faster on a SDS gel. The fatty acylation takes place on the processed form and the fatty acylated forms are found exclusively in the plasma membrane. The presence of a step which precedes the fatty acylation is indicated by the identification of a form which is processed but not fatty acylated. This form may represent an intermediate in the fatty acylation reaction. In collaboration with K. Matsumoto (DNAX Research Institute), we have identified *dpr1* mutation which affects the processing of the precursor RAS proteins but not their fatty acylation. Mammalian ras proteins produced in yeast undergo similar processing/ modification events and the initial processing event is also blocked by the *dpr1* mutation. In addition, the mutation appears to affect the processing of a mating pheromone, **a**. Since a sequence CysAAX exists at the C-termini of the two proteins, it has been proposed that proteins having this sequence form a new group of proteins which share a common processing/ modification event.

E 146 **POST-TRANSLATIONAL PROCESSING OF THE VOLTAGE-REGULATED SODIUM CHANNEL FROM EEL ELECTROPLAX.** W.B. Thornhill and S.R. Levinson, Dept. of Physiology, Univ. of Colorado, School of Medicine, Denver, Co 80262. Compositional analysis has shown the eel sodium channel to be a very heavily post-translationally modified protein (M_r 260,000). The channel is 30% carbohydrate by weight and has 8% fatty acyl moieties associated with it even after SDS-gel filtration and extensive dialysis. These non-protein domains are responsible for the unusual behavior the channel exhibits on SDS-PAGE. We have investigated the biosynthesis and post-translational processing of the sodium channel in both the eel electroplax and in the frog oocyte following the injection of electroplax mRNA. Pulse-chase experiments with radiolabelled precusors to the channel followed by immunoprecipitation of the molecule with specific antisera indicate the channel acquires some presumptive acyl domains before it is completely glycosylated. Lectin binding studies and fractionation of subcellular microsomes into rough and smooth components indicate the channel is heavily processed in the smooth membrane fraction. A number of antibotics which specifically block post-translational processing of membrane proteins also have been used to help ascertain which processing events are required for the intracellular transport of the sodium channel to the cell surface.

POLARIZED SECRETION OF AN ECTOPIC PROTEIN IN DROSOPHILA SALIVARY GLANDS IN VIVO

E 147 Shinichiro J. Tojo, Susan Gemeraad*, David King & James W. Fristrom.
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The apical-basolateral polarity of protein secretion in epithelia is presumed to depend on the recognition of specific protein targeting sequences by the secretory machinery. We have examined whether an ectopically synthesized protein is targeted in vivo to the correct cell surface on secretion. Larval cuticle proteins(LCP's) and salivary gland secretion (Sgs) proteins of Drosophila are apically secreted proteins that are produced in the epidermis and salivary glands respectively. The apical surface is the outer surface of epidermal cells and the lumenal surface of the salivary gland cells, whereas the basal surface of these epithelia is bathed by hemolymph. Sgs proteins secreted from the apical surface of the salivary gland cells are stored in the gland lumen during the third instar and are expectorated at the following prepupal stage. We have transformed Drosophila by P-element transformation with a hybrid gene consisting of the sgs-4 promoter sequence and the coding sequence for LCP-f2. Transgenic late third instar larvae produced LCP-f2 in salivary glands. At the prepupal stage, LCP-f2 was found in expectorant but not in hemolymph indicating that the foreign protein is secreted only from the apical surface of the salivary gland cells and thus the polarity of secretion is maintained. These results strongly support the idea that in salivary glands, polarized protein secretion does not require tissue-specific targeting signal.

E 148 THE SORTING DETERMINANT OF THE YEAST VACUOLAR GLYCOPROTEIN CARBOXYPEPTIDASE Y RESPONSIBLE FOR LOCALIZATION, Luis A. Valls, Joel H. Rothman, Craig P. Hunter and Tom H. Stevens, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403. Many lysosomal and vacuolar proteins are synthesized as zymogens having N-terminal propeptide extensions. We have undertaken a mutational analysis of the carboxypeptidase Y (CPY) sorting determinant to test whether, in addition to maintaining enzymatic inactivity during transport, the propeptide also plays a role in vacuolar targeting. We have found that deletions as small as three amino acids (aa 29-31 of the propeptide) or a single amino acid substitution (gln→lys at position 24) result in drastic missorting of the CPY polypeptide. These mutations cause between 50 and 95% of CPY to be secreted as the unprocessed zymogen proCPY, and define a discrete region of the propeptide that is required for proper sorting and vacuolar delivery of CPY.

If vacuolar protein sorting is à receptor-mediated process, the sorting step which is bypassed in these mutants may be the same step which is saturated when CPY is overproduced.[1] We are isolating allele-specific suppressors of these mutations to identify the putative CPY sorting receptor.

[1]Stevens, T.H., Rothman, J.H., Payne, G.S. and Schekman, R. (1986) J. Cell Biol. 102, 1551-1557.

E 149 **RAT HEPATOCYTES HAVE TWO SEPARATE PARALLEL RECEPTOR PATHWAYS FOR GALACTOSYL RECEPTOR RECYCLING AND LIGAND INTERNALIZATION, DISSOCIATION AND DEGRADATION.** Paul H. Weigel, Div. of Biochemistry, Univ. of Texas Medical Branch, Galveston, TX 77550 Isolated rat hepatocytes express two subpopulations of Galactosyl receptor (GalR) activity, that we designate State 1 and State 2 receptors. Each type of receptor functions in a separate pathway. Both pathways are expressed by cells in suspension or in culture overnight. The pathways differ as follows: 1) Surface State 2, but not State 1, GalR activity is modulated in the absence of ligand by colchicine, hyperosmolarity, low temperature, ATP depletion or monensin. 2) The rate of dissociation of internalized State 2 GalR-ligand complexes is 20-fold faster than that of State 1 GalR-ligand complexes. 3) Diactyosis occurs only in the State 1 pathway. 4) Ligand dissociation from State 1 but not State 2 GalR is inhibited at 18°C. 5) At 18°C, State 2 GalR can recycle and accumulate dissociated ligand. Although State 1 GalR-ligand complexes undergo diacytosis to the cell surface, they do not enter the State 2 pathway and dissociate. 6) Monensin completely blocks ligand dissociation from State 2 GalR but only transiently blocks dissociation from State 1 GalR. 7) Degradation of ligand in the State 2 GalR pathway begins after a 20 min lag but begins almost immediately in the State 1 GalR pathway. 8) Ligand degradation in the State 2 pathway is 30 times more sensitive to colchicine than in the State 1 pathway. State 1 and State 2 GalR may or may not be structurally different but they must function in different endocytic pathways. The State 2 GalR pathway corresponds to the extensively studied coated pit pathway. The minor State 1 GalR pathway, previously unrecognized, could be a non-coated pit or a different coated pit pathway. (supported by NIH Grant GM 30218)

E 150 **TARGETING GENES OF THE EUKARYOTIC TRANSLOCATION APPARATUS BY HOMOLOGOUS RECOMBINATION WITH THEIR PROKARYOTIC COUNTERPARTS.** Matthew R. Young and Paul B. Wolfe, University of Maryland at Baltimore, Baltimore, MD 21201.

We are trying to identify genes coding for components of the translocation apparatus of Saccharomyces cerevisiae based on the ability of their bacterial counterparts to integrate into the chromosome by homologous recombination. It is well known that yeast genes carried on integration vectors recombine into the chromosome within regions of homology. This frequency increases when the vectors are linearized. We have exploited this observation by constructing integration vectors which contain bacterial genes coding for components of the bacterial secretory pathway. The lep gene of E. coli, which codes for the leader (signal) peptidase, and the secA gene, whose gene product is essential for the synthesis and translocation of many secreted proteins in E. coli, have each been recombined into plasmids which carry a selectable marker for transformation of yeast (URA3) but lack information for autonomous maintenance in the nucleoplasm. These integration vectors, pU3LEP and pU3SEC, have also been linearized at any of several unique restriction sites within the bacterial sequences, then used to transform diploid ura3- strains to uracil auxotrophy. Recombinant URA3+ strains are obtained with variable frequencies, dependent on the site of linearization within the bacterial genes. These results are consistent with recombination through conserved sequences of DNA. Furthermore, random spore analysis of both the lep-containing and secA-containing integration strains shows a lethal germination phenotype under selective growth conditions, consistent with the disruption of essential genes. We are presently screening a yeast chromosomal DNA library, by complementation of these lethal germination phenotypes, to isolate the analogues of lep and secA.

Transport

E 200 INTRACELLULAR TRANSPORT OF INFLUENZA VIRAL HEMAGGLUTININ IN SACCHAROMYCES CEREVISIAE
M. Abdul Jabbar and Debi P. Nayak, University of California, Los Angeles, C.A. 90024
The hemagglutinin (HA) of influenza virus possesses topogenic domains necessary for the efficient intracellular transport as well as the productive cell surface expression of the protein in higher eukaryotic cells. The mutational analysis of specific domains has elucidated the functional contribution of the domains in the intracellular transport process. In our effort to understand whether these topogenic sequences can be decoded by the heterologous transport machinery of lower eukaryotes we have expressed both wild-type and mutant forms of hemagglutinin in yeast, Saccharomyces cerevisiae. We have shown that the HA signal is efficiently recognized by the yeast translocation machinery and critically required for membrane insertion since signal minus HA fails to be translocated across the rough endoplasmic reticulum (RER). Moreover, the HA signal also processed properly by the yeast signal peptidase, and the conformation of the protein appears to play a role in the movement of protein from one compartment (RER) to another (Golgi). A mutant HA having a deletion at the carboxyl portion (cytoplasmic and anchor domains) of the molecule was found to be blocked in the RER, whereas another mutant, HA_1 (having deleted the HA_2 portion), traversed the entire transport pathway and secreted into the medium. The secreted HA_1 protein is hyperglycosylated. These results demonstrate the specific requirement for the signal sequence and the proper protein topogenesis of viral hemagglutinin in intracellular membrane traffic of Saccharomyces cerevisiae.

E 201 ARTIFICIAL PRESEQUENCES THAT IMPORT A PROTEIN INTO MITOCHONDRIA, Daniel S. Allison, Biocenter, University of Basel, CH-4056 Basel, Switzerland.
Import of proteins into mitochondria usually is mediated by a transient amino-terminal peptide. Although mitochondrial presequences lack significant sequence homology, they generally are rich in hydrophobic, basic and hydroxylated amino acids, and have the potential to form an amphiphilic alpha-helix. In order to test whether these features are sufficient for import function, synthetic oligonucleotides were used to construct three artificial presequences composed of only the initiator methionine and the three amino acids leucine, arginine and serine. The ratio of these three amino acids was adjusted to match that of hydrophobic, basic and hydroxylated residues in natural mitochondrial presequences. In addition, two of these sequences were designed such that they potentially can form an amphiphilic alpha-helix, while the third should not have such a structure. When fused to the N-terminus of yeast cytochrome oxidase subunit IV, all three of these artificial presequences directed subunit IV to its correct intra-mitochondrial location in vivo. They also mediated efficient uptake of subunit IV into isolated mitochondria. In contrast, artificial presequences composed of glutamine, arginine and serine following the initiator methionine were inactive for mitochondrial targeting function. These data suggest that the import function of mitochondrial presequences does not depend on specific amino acid sequences, but instead may depend on the overall balance between hydrophobic, basic and hydroxylated amino acids. This study also shows that having the potential to form an amphiphilic alpha-helical structure is not essential for the targeting activity of mitochondrial presequences.

E 202 LOCALIZATION OF SIGNAL SEQUENCES IN BOVINE OPSIN, Yves Audigier, Martin Friedlander and Gunther Blobel, Rockefeller University, New-York N.Y. 10021.
Opsin, the apoprotein from the visual pigment of rod photoreceptor cells, spans the membrane seven times and therefore represents an interesting model for studying how such a protein assumes this polytopic orientation in the membrane. A cDNA clone of bovine opsin inserted in a vector containing a strong promoter was cut and fragments of interest were religated. The resulting constructs were transcribed and the RNAs translated in a wheat germ cell free system supplemented with canine pancreas microsomal membranes. Resistance to alkali extraction was used as a criterion for integration into the membranes. The removal of all the transmembrane segments (TMs) and the ligation of the N-terminal domain (usually translocated and glycosylated) to the C-terminus (usually untranslocated) yielded a "cytoplasmic" opsin derivative that was neither translocated across nor integrated into microsomal membranes and by this criterion did not contain a signal sequence. When the coding region for either TM 1,2, 4,5, 6, or 7 was inserted back into the junction between the N-terminal and the C-terminal domain of this "cytoplasmic" opsin, we found that TMs 1,2,4,5 and 6, but not 7, contain a functional signal sequence. Insertion of TM 1 yielded a fully integrated protein; addition of TMs 2 or 4 gave rise to fully translocated proteins; insertion of 5 or 6 yielded a mixture of translocated and integrated proteins. These results suggest that multiple signal sequences are involved in the polytopic integration of membrane proteins.

E 203 REDUNDANT MITOCHONDRIAL IMPORT INFORMATION IS PRESENT AT THE NH_2-TERMINUS OF THE F_1-ATPase β SUBUNIT IN YEAST. David M. Bedwell and Scott D. Emr, California Institute of Technology, Pasadena, CA 91125.

Many proteins destined for mitochondria are initially synthesized with an NH_2-terminal leader sequence that directs the import of the protein into mitochondria. In an attempt to define the general characteristics of import signals we have utilized site-directed mutagenesis to introduce a series of mutations into the leader sequence of an imported yeast mitochondrial protein, the β subunit of the F_1-ATPase complex (encoded by the ATP2 gene). Plasmids encoding mutant β proteins were transformed into a Δatp2 strain and β import was examined. We found that constructions containing either of two non-overlapping deletions of 4 and 8 amino acids in the leader sequence which removed separate pairs of basic residues did not abolish β import. In fact, a combination of the two deletions that removed all four basic residues in the β leader sequence (along with 12 of the 19 amino acids in the leader) did not significantly block import into mitochondria. Import was abolished only when these two deletions were combined together with a third deletion that removed two more basic residues within the mature β protein. Examination of various constructions containing different pairwise combinations of the three deletions showed that the combination of any two deletions did not disrupt import. These results indicate that the β protein contains redundant import information and that three separate regions near the NH_2-terminus of the β precursor (including one in the mature protein) are capable of participating in the import of β into mitochondria.

E 204 THE N-TERMINAL SIGNAL SEQUENCE IS NOT NECESSARY FOR TRANSLOCATION OF CARBOXYPEPTIDASE Y IN YEAST, Elizabeth Blachly-Dyson and Tom H. Stevens, University of Oregon, Eugene, OR 97403.

We have constructed a series of deletion mutations in the signal sequence of the yeast vacuolar (lysosomal) protein carbopeptidase Y (CPY). When we transform yeast cells with plasmids encoding these mutant forms of CPY, we see synthesis of unglycosylated proCPY, a portion of which is posttranslationally converted to glycosylated proCPY and mature CPY. These modifications indicate that the protein is entering the secretory pathway and proceeding to the vacuole in a normal manner. Remarkably, a deletion mutation that removes amino acids 2 through 28 of preproCPY, including the entire signal sequence, still allows glycosylation and secretion of more than ten percent of the unglycosylated proCPY initially synthesized in this mutant. The glycosylated proCPY made in this mutant does not reach the vacuole, but is secreted into the culture medium, apparently because the deletion destroys a vacuolar localization tag as well as the signal sequence. The unglycosylated proCPY made in the deletion mutants is not soluble in the cytoplasm, and at least part of it is membrane-associated, suggesting that it may be blocked in translocation rather than in targeting in the ER.

E 205 FACTORS REGULATING INSULIN RECEPTOR RECYCLING IN ENDOTHELIAL CELLS, Donald Bottaro, Harumi Hachiya, George King, Joslin Diabetes Center, Boston, MA 02215.

A novel role for insulin receptors on vascular endothelial cells (EC) is the transport of insulin from luminal to abluminal cell surface. Unlike most cells, EC degrade less than 20% of the insulin they internalize, and transport the balance across the cell to be released undegraded. To study the factors regulating receptor internalization and recycling, EC insulin receptors were labeled by surface iodination, incubated at 37°C, and localized by exposure of the intact cells to trypsin at 4°C. Interestingly, receptor internalization occurs in the absence of ligand, reaches a maximum within 30 min and is subsequently recycled to the cell surface. When 100 nM insulin is added, maximum internalization of receptors occurs within 10 min. Serine phosphorylation of receptors has been implicated in receptor processing, thus we examined the effects of phorbol myristate acetate (PMA) on EC. Cells treated with 160 nM PMA showed an initial rate of insulin-induced receptor internalization 4-fold greater than control and an increased rate of reappearance of internalized receptor at the cell surface. Phosphoaminoacid analysis of the PMA-treated receptor revealed a concommitant stimulation of serine phosphorylation on the β-subunit. We have observed previously that EC uptake of ^{125}I-insulin in the presence of monensin (50 μM) resulted in the accumulation of intact hormone within cells; surface iodination experiments subsequently revealed that monensin treatment decreased the rate of reappearance of internalized receptor at the cell surface. These data suggest that sorting of internalized hormone and receptor occurs through the acidification of endosomes and that serine phosphorylation of the receptor may provide a biochemical signal for these events.

E 206 SECRETION MUTANTS OF TYPE I COLLAGEN, Peter H. Byers, Daniel H. Cohn, Richard J. Wenstrup, Barbra Starman, Marcia C. Willing and Jeffrey F. Bonadio, University of Washington, Seattle, WA 98195.

Type I procollagen contains three chains, two proα1(I) chains and a single but similar proα2(I), which form a trimeric largely triple helical molecule. The normal pathway of secretion includes the RER and Golgi. We have identified mutations in proα1(I) and proα2(I) which abolish or slow secretion of molecules which incorporate the product of the mutant allele; these mutations include deletions from the α1(I) and α2(I) genes, a partial duplication in the α1(I) gene, and single nucleotide substitutions in the α1(I) and α2(I) genes. The deletions [650bp containing exons which encode 81 amino acids in α1(I), and 4.2kb containing exons which encode about 200 amino acids in α2(I)] result in molecules which fail to leave the RER and are slowly degraded as the RER enlarges. Molecules which contain two members of the extended α1(I) chain are retained in the cell but those which contain one are efficiently secreted. Single amino acid substitutions in the α1(I) chain (eg. gly to cys at 988 in the triple helix) and α2(I) chain (eg. gly to arg at 1012 in the triple helix) delay secretion of the molecules which contain the abnormal chain and result in increased post-translational modification. Mutations near the COOH-terminal end of the triple helix have a greater effect on the efficiency of secretion; mutations in α1(I) disrupt secretion more efficiently than those in α2(I). These mutations may interfere with secretion by affecting triple helix formation or by altering recognition sites for transport of procollagen beyond the RER.

E 207 HIGH LEVEL SECRETION OF HUMAN GROWTH HORMONE BY ESCHERICHIA COLI, Chung Nan Chang, Genentech, Inc., South San Francisco, CA 94080.

A gene encoding the mature form of human growth hormone (hGH) was fused to the signal coding sequence of the Escherichia coli heat-stable enterotoxin II (STII). This hybrid gene was expressed in E. coli under the control of the alkaline phosphatase (APase) promoter with two Shine-Dalgarno sequences derived from trp and STII genes. In low phosphate growth media, cells produced 10-20 micrograms of hGH per mililiter at cell density of 1 OD_{550}. This represents 4-8 percent of total cellular protein. The majority of hGH produced (80-90%) was processed correctly and secreted into the periplasmic space located between the inner and outer membranes. Thus, these results demonstrate that E. coli cells may have the capability of producing and secreting high levels of other pharmaceutically important human proteins.

E 208 PROTEIN UNFOLDING IS REQUIRED FOR PROTEIN IMPORT INTO MITOCHONDRIA. Wen-ji Chen and Michael Douglas, Dept. of Biochemistry, UTHSC at Dallas, Dallas, Texas 75235.

Copper metallothionein (CuMT) is a low molecular weight, cystein-rich protein that can bind copper. In yeast, CuMT is encoded by CUP1 gene and has molecular weight of 6573. It has been suggested that metallothionein acquires a protein conformational change after the binding of metal ions to a roughly spherical structure with diameter of 15-20A. This property thus allowed us to study the question that what is the protein conformation, folded or unfolded, required for the post-translationally mitochondrial import.

To approach this, an in-frame ATP2-CUP1 gene fusion was constructed. The product of this gene fusion is a protein (βCuMT) which consists of the first 382 amino acids of F_1-ATPase β subunit precursor and the intact yeast CuMT at its C-terminal. As expected, the in vitro transcribed-translated fusion protein has molecular weight of 53,000. By in vitro import studies the effect of Cu^{++} on the import was examined. These studies show that the import of βCuMT is completely blocked by 50μM Cu^{++}, while this Cu^{++} concentration has no effect on import of $F_1\beta$. In addition, Cu^{++} blocks the translocation of βCuMT which has been previously bound to the mitochondria before exposure to Cu^{++}. This divalent cation has no such an effect on the $F_1\beta$. In a parallel in vivo study it is demonstrated that the βCuMT indeed retains its copper binding activity. How mitochondria are able to import a previously folded $F_1\beta$ subunit precursor and whether this is an ATP-dependent process is currently being studied.

E 209 **MUTATIONAL ANALYSIS OF THE RAT MITOCHONDRIAL MALATE DEHYDROGENASE TRANSIT PEPTIDE**, Tom W. Chu, Paula M. Grant and Arnold W. Strauss, Washington University School of Medicine, St. Louis, MO. 63110.
Most mitochondrial proteins are targeted to mitochondria by N-terminal transit peptides. Mutational analysis can be used to study the features which cause transit peptides to be recognized by the mitchondrial uptake machinery. We have re-engineered the transit peptide coding region of a rat mitochondrial malate dehydrogenase (mMDH) cDNA (Grant et al., **Nuc. Acids Res. 14**, 6053-6066) in order to facilitate creation of site-specific mutants. The new construct allows small restriction fragments to be removed from the transit peptide coding region which can then be replaced with synthetic oligonucleotides. One mutant which has been constructed in this manner was altered at amino acid positions 14 and 15 which were both changed from arginine to alanine residues. This altered cDNA was expressed in vitro and produced a protein which was capable of binding to isolated mitochondria; however, only 11% of the mutant protein was imported into mitochondria and processed to the mature form as compared to 71% of the wild type sequence. An intermediate situation exists in another mutant in which only one arginine (position 14) was changed to alanine. Forty-six percent of this mutant was imported and processed. The results demonstrate that these residues are critical for the import function of the mMDH transit peptide.

E 210 CHLOROPLAST PROTEIN IMPORT: A PRECURSOR PROTEIN CAN INSERT DIRECTLY INTO THYLAKOID MEMBRANES. Kenneth Cline and Donald Fulsom, Fruit Crops Dept., University of Florida, Gainesville, FL 32611
Higher plant chloroplasts contain an internal membrane system, thylakoids, that is separated from the delimiting envelope membranes by an aqueous matrix, the stroma. Many thylakoid membrane proteins are synthesized in the cytosol as higher molecular weight precursors that are post-translationally imported into chloroplasts. Imported precursors must in some manner traverse the stroma, integrate into the thylakoid bilayer, and be proteolytically processed to mature size. We have reconstituted the membrane integration and processing events for the light-harvesting chlorophyll a/b protein (LHCP) independent of transport across the envelope. The LHCP precursor inserts directly into thylakoids, assumes the correct bilayer orientation, and assembles into the photosystem II chlorophyll-protein complex. In addition to thylakoid membranes, integration requires a chloroplast soluble factor as well as some form of energy, e.g. ATP. The soluble factor is heat and protease labile and has a mol wt greater than 5000. Proteolytic processing of preLHCP to LHCP also occurs, but only under conditions that result in membrane integration. The implications of these results for the in vivo events of thylakoid protein biogenesis will be discussed.

E 211 STRUCTURAL IDENTITY BETWEEN THE NH2-TERMINAL DOMAIN OF RAT AND HUMAN ORNITHINE CARBAMYL TRANSFERASE "SORTING" SEQUENCES. C. Côté, G. Dionne and M. Lacroix, Université du Québec and Institut Armand-Frappier, Montreal, (Québec).
Ornithine carbamyl transferase, an enzyme of the urea cycle catalizing the formation of citrulline from L-ornithine and carbamyl phosphate, is localized exclusively to the mitochondrial matrix of hepatocytes and epithelial cells of the intestinal mucosa. The primary structure of the NH2-terminal "sorting" signal sequence of human and rat pOCT is comprised of 32 amino acids including 5 and 8 basic residues respectively and no acidic residues. A comparison of the primary structure of rat and human pOCT reveals that the mature enzyme portion is 93% identical, whereas that of their presequences shows only 69% homology. These results suggest that some components of the segregation system of pOCT are distinct in these two species. We have analyzed the secondary structure of the NH2-terminal domain of the rat and human pOCT "targeting" sequences using a computer program (Protein Information Analyzer System (PRINAS); Mitsui, Tokyo) that included the Robson's method of predicting helices and another that plots helical projections. This analysis revealed the following key features: an hydrophobic patch opposite to an hydrophilic surface characterized by the disposition of basic residues at potentially strategic functional positions. Using synthetic peptides, the functional identity of this domain of rat and human pOCT as well as the role of specific amino acid residues within these two well-defined regions of this amphipatic domain are currently being investigated.

E 212 Murine mitochondrial ferrochelatase is synthesized as a larger cytoplasmic precussor which is processed by isolated mitochondria., Harry A. Dailey and Steven Karr, Department of Microbiology, University of Georgia, Athens, GA 30602.

Ferrochelatase (protoheme ferrolyase, E.C. 4.99.1.1), the terminal enzyme of the heme biosynthetic pathway, is an integral protein of the inner mitochondrial membrane. In vitro translation of isolated mouse hepatocyte mRNA yields a precussor form of ferrochelatase with a molecular weight approximately 3000 larger than the mature enzyme (40,000). This precussor form can be taken up by isolated mouse mitochondria and processed to the mature enzyme. This processing appears to be an energy requiring step since it is inhibited by CCCP.

E 213 CLONING AND CHARACTERIZATION OF THE GENE ENCODING CYTOCHROME c HEME LYASE IN YEAST, Mark E. Dumont, Joachim F. Ernst, and Fred Sherman, University of Rochester Medical Center, Rochester, N.Y. 14642 and Biogen S.A., Geneva, Switzerland.

Extensive mutational analyses have led to the identification of only two loci, CYC2 and CYC3, which, when mutated, cause a specific deficiency of iso-1 and iso-2-cytochrome c brought about by a block in a post-translational step. We have identified one of these loci, CYC3, as the gene encoding cytochrome c heme lyase, the enzyme catalyzing covalent attachment of heme to cytochrome c. Mitochondrial extracts from $cyc3^-$ yeast showed only 10% of the normal heme lyase activity, measured as the ability of mitochondrial extracts of the different strains to attach ^{55}Fe-labeled heme to apocytochrome c. Furthermore, extracts of a strain bearing CYC3 on a multicopy plasmid under control of a constitutive promoter have about eight times the normal activity. The CYC3 gene, which was known to map near the gene PYK1, was cloned from a plasmid containing PYK1 by complementation of $cyc3^-$ strains. The sequence of CYC3 has been determined, allowing identification of a reading frame encoding a 30,000 MW protein. We have demonstrated in vitro uptake of apocytochrome c into yeast mitochondria and are using this system to identify the roles of CYC2 and CYC3 in mitochondrial import.

E 214 BIOGENESIS OF 15 kDa INTEGRAL PEROXISOMAL MEMBRANE PROTEIN, A COMMON ENDOMEMBRANE POLYPEPTIDE, Yukio Fujiki and Toshiro Tsukamoto, Meiji Institute of Health Science, Odawara, 250 Japan.

Peroxisomal matrix, core and membrane proteins are synthesized on free polyribosomes and mostly at their final sizes, without cleavable signal peptides. These newly synthesized polypeptides are imported posttranslationally into preexisting peroxisomes. Thus the peroxisomes grow and ungergo fission to form new peroxisomes(1). To generalize this idea, we have investigated the biogenesis of 15 kDa integral peroxisomal membrane protein(15IMP). This protein was isolated from highly purified peroxisomes, and used to elicit a rabbit antiserum. In immunoblot analysis, the antibody bound not only to peroxisomal 15IMP, but to that of other endomembranes including mitochondria and both smooth and rough microsomes. Affinity-purified antibody from each endomembrane immobilized on nitrocellulose completely crossreacted among these organelle membranes. The in vitro 15IMP translation product comigrated with the mature protein in SDS-PAGE. mRNA coding for 15IMP was found in free polyribosomes. These results are consistent with the biogenesis of 22 kDa integral membrane protein present exclusively in peroxisomal membranes(2), except for the unique nature of 15IMP as a common protein of endomembranes.

1) Lazarow, P.B. & Fujiki, Y. Ann. Rev. Cell Biol. 1, 489-530(1985).
2) Fujiki, Y. et al. Proc. Natl. Acad. Sci. USA 81, 7127-7131(1984).

E 215 MUTATIONAL ANALYSIS OF CARBOHYDRATE SIDE CHAINS ON INFLUENZA HEMAGGLUTININ. Patricia Gallagher, Ian Wilson*, Joe Sambrook, and Mary-Jane Gething, University of Texas Health Science Center, Dallas, TX 75235, and *Research Institute of Scripps Clinic, La Jolla, CA, 92037.

Evidence is accumulating that N-linked glycosylation may be involved in a variety of roles including (i) maintenance of structure and stability of glycoproteins, (ii) direction of proteins to various subcellular compartments and (iii) shielding of potential antigenic and/or protease sensitive sites (1-2). The cloned X31 hemagglutinin (HA) from the A/Aichi/68 strain of influenza is being utilized as a model membrane glycoprotein to study the role of N-linked glycosylation. Site-directed mutagenesis has been used to alter the canonical Asn-X-Ser/Thr sequences at which carbohydrate side chains are normally attached to HA or to introduce these canonical sequences so that oligosaccharide side chains are added at novel sites on the surface of the molecule. These mutants have been expressed in CV-1 cells using SV40 vectors (3) and characterized with respect to their effect on transport kinetics, cellular destination and biological activity. Our results show: (i) no single oligosaccharide group is absolutely required for transport of HA to the cell surface, (ii) that at least some oligosaccharide groups are required for transport of HA, and (iii) that supernumary oligosaccharides can, but do not necessarily alter the localization of HA along the exocytic pathway.

(1) Hughes, R.C. and Butlers, T.D. (1981). Trends in Biochem. Sci. 6:228-230.
(2) Elbein, A.D. (1981). Trends in Biochem. Sci. 6:219-221.
(3) Gething, M.J. and Sambrook, J. (1982). Nature 300:598-603.

E 216 BIOPHYSICAL APPROACHES TO DETERMINING THE ROLE OF THE SIGNAL SEQUENCE IN PROTEIN EXPORT, Lila M. Gierasch, M. Rafalski, C.J. McKnight and D.W. Hoyt, University of Delaware, Newark, DE 19716.

While the signal sequence is clearly essential to protein translocation across eukaryotic or bacterial membranes, the details of its roles in the process are still poorly understood. We have approached this problem by biophysical study of genetically-characterized synthetic signal sequences from exported proteins of E. coli. Circular dichroism data have revealed a correlation between the tendency to adopt an α-helix in hydrophobic environments and the effectiveness of a particular signal sequence as a facilitator of protein translocation in vivo. The affinity of signal sequences for lipid monolayers also correlates with their in vivo function. Through the use of monolayer spectroscopy, we have characterized the conformational interconversions of signal sequences upon interaction with a lipid monolayer first by adsorption and then by insertion. Recent work with doubly fluorescently labeled signal sequences that enable further analysis of the kinetics and nature of the conformational transitions will be presented. All of the data are interpreted in terms of a model for the initial interaction of a signal sequence with a biological membrane that involves a surface associated β structure which inserts into the lipid and then undergoes a spontaneous transition to a transmembrane α-helix, causing the first 10 or so residues of the mature protein to enter the membrane.

E 217 SODIUM CHANNEL TRAFFICKING IN SQUID NEURONS, Wm. F. Gilly and Tom Brismar, Hopkins Marine Station of Stanford University, Pacific Grove, CA 93950.

Voltage-controlled Na channels are an important determinant of neuronal function, but our knowledge of the details of the cellular trafficking of these membrane glycoproteins in neurons is incomplete. Giant axons of squid are formed by the fused axons from hundreds of small neurons in the giant fiber lobe (GFL) of the stellate ganglion. Somata of GFL cells in vivo do not have measurable Na current (I_{Na}) when studied with voltage clamp techniques. If GFL cells are physically separated from the giant axons and maintained in primary culture (15°C), large I_{Na} appears in the soma membrane over 3-4 days and remains for up to one month thereafter. Axon regeneration does not occur under our culture conditions, but cells do grow and become very globular, probably due to synthesis of new membrane. Inclusion of actinomycin D in the culture medium does not affect the initial appearance of large I_{Na} but subsequently causes the amount of I_{Na} to disappear with a time constant of 1 day. Inclusion of demecolcine, a colchicine derivative, totally prevents appearnce of I_{Na}. I_{Na} also fails to appear in GFL somata from stellate ganglia which are cultured 'intact' (i.e., no enzyme treatment and all giant axons ligated) for 4-5 days. Appearance of I_{Na} in GFL cell bodies must depend on synthesis of mRNA and Na channel proteins, intracellular 'axoplasmic' transport and the lack of a definable axon to serve as the sink for Na channels synthesized in culture. Potassium and calcium currents, which are normally present in the GFL cells, do not change in parallel with I_{Na}.

E 218 PROTON IONOPHORES DISRUPT THE ASSEMBLY OF A PEROXISOMAL PROTEIN, Joel M. Goodman and Edward Bellion, University of Texas Health Science Center at Dallas, Dallas Texas 75235

Peroxisomes are induced in methylotrophic yeasts when grown on methanol. A major protein of this organelle is alcohol oxidase, a flavin-containing homo-octamer which forms a crystalline core inside the peroxisome. Monomeric alcohol oxidase is synthesized in the cytosol and octamerizes upon associating with peroxisomes. We now report that the proton ionophores carbonylcyanide m-chlorophenylhydrazone (CCCP) and dinitrophenol disrupt the pathway of assembly of alcohol oxidase. When added to cells containing radiolabelled newly synthesized monomeric protein, octamerization fails to occur and alcohol oxidase is found instead in a membrane-associated complex. This complex is composed of 3 or 4 other newly synthesized components, at least one of which is peroxisomal. The formation of this complex is reversed after removal of drug, and is also transiently observed in the absence of drug. Moreover, if drug is added to cells containing newly assembled octamers, they dissociate into monomers. We postulate that both the complex and labile octamer are intermediates in the normal pathway of assembly. While these data suggest the existence of a protonmotive force across the peroxisomal membrane, other more indirect effects of these drugs, such as depletion of intracellular ATP, cannot be ruled out in their mechanism. (Supported by the NIH)

E 219 PROTEIN SEQUENCES REQUIRED FOR PEROXISOMAL TRANSPORT, Stephen J. Gould, Gilbert Keller and Suresh Subramani, University of California, San Diego, La Jolla, CA 92093. Expression of wild type and mutant luciferase genes has been performed in an African Green Monkey kidney cell line (CV-I). The wild type luciferase gene codes for a protein which is localized to peroxisomes both in firefly lanterns (Photinus pyralis), and in CV-I cells. Unidirectional deletions from both ends of the luciferase gene are being utilized to determine the location of a peroxisomal targetting sequence. Indirect immunofluorescence to detect luciferase is performed on transfected cells to ascertain the subcellular localization of the mutant proteins. Indirect immunofluorescent staining of catalase, a known peroxisomal protein, is used as a control.

E 220 INTRACELLULAR TRANSPORT OF LYSOSOMAL MEMBRANE PROTEINS, Samuel A. Green, and Ira Mellman, Dept. of Cell Biology, Yale School of Medicine, New Haven, CT 06510.

The biosynthesis and intracellular transport of lgp120 and lgp110, two integral membrane glycoproteins of the lysosome, have been studied in the mouse macrophage cell line J774. Newly synthesized lgp120 and lgp110 are transported from the endoplasmic reticulum to the Golgi apparatus with the same rapid kinetics ($t_{1/2}$ = 14 min) as the IgG1/IgG2b Fc receptor, a plasma membrane marker in J774 cells. Delivery of the lysosomal membrane proteins and a lysosomal enzyme to dense lysosomes, and of Fc receptor to the plasma membrane, occur with similar kinetics ($t_{1/2}$ = 45-50 min). The lysosomal membrane proteins are degraded with a half time of 10 hours, also similar to Fc receptor. Similar results have been obtained for the transport of lgp120 in NRK cells. These data demonstrate that lysosomal membrane proteins are stable in the lysosomal environment, and that their transport there is as rapid and efficient as the transport of plasma membrane proteins to the cell surface.

E 221 BIOSYNTHESIS AND TRANSPORT OF MITOCHONDRIAL AND CYTOPLASMIC SUBUNITS OF NADH DEHYDROGENASE, Robin E. Hall and James F. Hare, The Oregon Health Sciences University, Portland OR 97201

The transmembranous mitochondrial enzyme NADH: ubiquinone oxidoreductase (NADH DH) catalyzes the oxidation of NADH to ubiquinone and couples this to the translocation of protons across the inner mitochondrial membrane. Bovine heart as well as immunoprecipitated hepatoma NADH DH has 26-30 polypeptide subunits by one- or two-dimensional gel electrophoresis. Cytosolic and mitochondrial translation inhibitors have shown that 5 of these subunits in cultured hepatoma cells are of mitochondrial origin. Pulse chase studies have revealed that NADH DH assembly is slower than that of cytochrome oxidase, a well characterized mitochondrial enzyme also having products of both mitochondrial and nuclear genomes. We will identify the rate limiting step in NADH DH assembly by measuring the effect of inhibitors of protein transport, and mitochondrial and cytosolic translation on prelabeled polypeptide incorporation into fully assembled immunoprecipitated enzyme. We have also generated subunit specific polyclonal and monoclonal antibodies, which we hope will identify mitochondrial or cytoplasmic pools of unassembled subunits.

E 222 ASSEMBLY OF PROTEOLIPID PROTEIN (PLP) INTO THE OLIGODENDROCYTE PLASMA MEMBRANE, Lynn Hudson, Toby Behar and Monique Dubois-Dalcq, NIH, Bethesda MD 20892.

Oligodendrocytes are multipolar central nervous system cells that synthesize large amounts of glycolipids and myelin-specific proteins which become concentrated in myelin internodes formed by each process. Initial events in oligodendrocyte maturation include acquisition of multipolarity and the myelin specific lipid galactocerebroside (GC) throughout the cell surface, followed 5-7 days later by the expression of myelin specific proteins. PLP, an integral membrane protein with a covalently attached fatty acid, is the major constituent of myelin and probably functions in the compaction of myelin membranes. We analyzed the orientation of this complex myelin component in the oligodendrocyte membrane by immunofluorescently staining cultured oligodendrocytes with anti-peptide antibodies directed against 5 hydrophilic domains of PLP. Most of the hydrophilic portions of the protein, including the amino and carboxy domains, were detected on the extracellular surface of the plasma membrane and were accessible to exogenous protease. The only negatively-charged domain resided on the cytoplasmic face. Of the 4 hydrophobic segments large enough to cross the membrane, only two were membrane spanning domains. Surface labeled PLP was clustered with the myelin lipid GC in a striking patchy pattern on oligodendrocyte processes. Co-localization of PLP with GC or with myelin basic protein was detected only after PLP was transported to the processes from the cell body, where it was first observed in patches, a pattern consistent with synthesis in RER. Another myelin protein synthesized in RER, myelin-associated glycoprotein, moved to the processes a week earlier than PLP. Our results suggest that PLP is transported independently of at least some of the other myelin components to the site of myelin assembly.

E 223 DIFFERENT ROUTES FOR DIPHTHERIA INTOXICATION OF VERO CELLS DETERMINED BY CELL-SUBSTRATE ATTACHMENT, Thomas Hudson and David M. Neville, Jr., Laboratory of Molecular Biology, National Institute of Mental Health, Bethesda, MD 20892.

African Green monkey kidney (Vero) cells in culture normally grow attached to the substratum, and are among the most sensitive cell types to diphtheria intoxication. The kinetics of this DT intoxication system has been studied under the following conditions. Toxin was bound to cell surface receptors at 4°C. Unbound toxin was removed with ice cold washes of medium. The cells were warmed and the progression of intoxication monitored by determining the rate of protein synthesis at various times after warm-up. The kinetic profile of intoxication consists of a [DT] dependent lag followed by a first order decrease in the fraction of control protein synthetic rates. An acidification requirement is shown to be largely fulfilled before any decrease in protein synthesis is observed (i.e., during the lag), and does not result in the immediate translocation to the cytosol. Comparisons of attached Vero cells and those freshly detached by scraping, show little difference in DT sensitivities or kinetic profiles when toxin binding is carried out at 37°C (i.e., efficient intoxication pathways exist in both cell systems). However, binding of DT to detached cells at 4°C followed by removal of unbound toxin prior to warming resulted in virtually no toxicity. This apparent lesion in detached cells was by-passed through binding of DT for 10 min at 37° followed by chilling to 4°C, removal of unbound toxin and rewarming. Under these conditions the acidification step is not limited to an early event in the intoxication process, but rather is a persistent requirement throughout the course of intoxication. DT translocation to the cytosol appears to be a direct consequence of the acidification process in detached Vero cells. The possible cellular basis for the selecting of one highly efficient toxin entry process over another will be examined in the context of cell-substratum adhesion.

E 224 VACUOLAR TARGETING SIGNAL IN CARBOXYPEPTIDASE Y. Lianna M. Johnson and Scott Emr, California Institute of Technology, Pasadena, CA 91125.

We have mapped sequence determinants in the vacuolar glycoprotein carboxypeptidase Y (CPY) that direct the accurate intracellular sorting and modification of this enzyme. Seven hybrid proteins consisting of amino-terminal segments of CPY fused to the secretory enzyme invertase have been studied with respect to their cellular location and the extent of glycosyl modifications they receive. We found that the N-terminal 50 amino acids of CPY is sufficient to direct translocation of the hybrid protein into the endoplasmic reticulum (ER) as well as efficiently target the hybrid protein to the yeast vacuole. Our data indicate that this 50 amino acid segment of CPY contains two distinct functional domains; an N-terminal signal peptide that is cleaved in the ER, followed by a 30 amino acid segment that defines the vacuole sorting signal. Deletion of this putative sorting signal (Δ21-50) from an otherwise wild-type CPY protein leads to secretion of a proenzyme form of CPY into the periplasm and extracellular medium. Furthermore, examination of the extent to which oligosaccharides on CPY and CPY-Inv hybrid proteins are added, indicates that a determinant in CPY mapping distal to the vacuole sorting signal regulates the level to which the protein is glycosylated.

E 225 YEAST VACUOLAR PROTEIN TARGETING, Daniel J. Klionsky and Scott D. Emr, California Institute of Technology, Pasadena, CA 91125.

The vacuole of the yeast Saccharomyces cerevisiae is an organelle that functions in the storage, degradation and recycling of a variety of biosynthetic components. It contains several hydrolases including proteinase A (PrA), carboxypeptidase Y and repressible alkaline phosphatase. These proteins pass through a part of the secretory pathway before they are delivered to the vacuole. We have begun studies utilizing the technique of gene fusion to identify the sorting signal in the enzyme proteinase A that directs accurate delivery of this protein to the yeast vacuole.

Fusion of the gene encoding proteinase A, PEP4, to the SUC2 gene, coding for the normally secreted yeast enzyme invertase (INV), results in the production of a hybrid protein that retains invertase activity. A PEP4-SUC2-encoded hybrid protein containing 138 amino acids from the N-terminus of proteinase A and lacking the invertase signal sequence is efficiently directed to the vacuole. Analysis of additional hybrid proteins has shown that the first 23 amino acids of proteinase A direct secretion of the PrA-INV fusion. Fusions of intermediate size indicate that the vacuolar targeting signal is located within the N-terminal propeptide region of proteinase A. Further analysis of the targeting signal together with similar studies on other vacuolar enzymes should enable us to identify a recognition determinant in these proteins that allows them to be specifically sorted from other secretory proteins prior to their delivery to the vacuole. (Supported by grant #PF-2776 from the American Cancer Society).

E 226 DIFFERENTIAL EFFECTS OF TRANSIENT BILE-DUCT LIGATION ON THE SORTING AND BILIARY SECRETION OF IgA, ASIALOOROSOMUCOID (ASOR), AND TAUROCHOLATE. Thomas M. Kloppel, Timothy C. Hoops and MySan Le. VA Medical Center and University of Colorado School of Medicine, Denver, CO 80220.

The transhepatocellular movement of molecules from blood to bile requires specific targeting events. Delivery of IgA into bile is mediated by secretory component (SC), and involves a vesicular pathway. ASOR gains entry into the hepatocyte via a specific receptor; most is degraded, however, a portion reaches the bile intact. Taurocholate is transported into bile by a pathway that may include vesicular transport. To further characterize these pathways and gain insights into possible sorting mechanisms, we examined the effect of brief periods of bile-duct ligation on biliary transport. Biosynthetically labeled rat monoclonal IgA (3H-MoIgA), 125I-ASOR, or 3H-taurocholate was injected into the portal vein of anesthetized control rats or rats in which the bile duct was ligated for 2 hr and bile flow then was resumed for 20 min. In control animals, biliary transport of 3H-MoIgA was 84.4 +/-10.9 % of the injected dose; in bile-duct ligated animals, biliary transport decreased to 14%. However, secretion of SC into bile continued at normal levels. Biliary secretion of ASOR decreased from 9.1 +/- 0.9% to 5.4 +/- 0.7%. Biliary secretion of taurocholate was identical in control and bile-duct ligated animals (92% efficiency). These data suggest that the transcellular pathways into bile for various substances may be biochemically dissimilar.

E 227 INDUCTION OF NUCLEAR TRANSPORT WITH SYNTHETIC PEPTIDE NUCLEAR TRANSPORT SIGNALS: Robert E. Lanford, Ronald C. Kennedy, Robert G. White and Patrick Kanda, Department of Virology and Immunology, Southwest Foundation for Biomedical Research, San Antonio, Texas.

A system was developed for the analysis of protein transport to the nucleus. A thirteenmer synthetic peptide with seven amino acids homologous to the SV40 T antigen nuclear transport signal was capable of inducing the nuclear transport of a variety of proteins. Synthetic peptides were conjugated to carrier proteins using a heterobifunctional cross-linker. Conjugated protiens were microinjected into the cytoplasm of mammalian cells and were localized by immunofluorescence with specific antibodies to the respective carrier proteins. Nuclear transport was dependent upon the synthetic peptide and was abolished by using a peptide homologous to an SV40 T antigen transport defective mutant. Proteins with molecular weights of up to 465,000 were capable of specific nuclear localization. In some instances, transport was essentially complete within 15 minutes at room temperature. The kinetics of transport appeared to be influenced by the size of the carrier protein and the number of transport signals per molecule of carrier proteins. A number of peptides have been synthesized with modifications of the transport signal. Cellular factors involved in transport recognized the modified signals to varying degrees.

E 228 ISOLATION OF THE YEAST ATP3 GENE ENCODING THE F_1-ATPASE γ SUBUNIT PRECURSOR. Janet E. Lawson and Michael G. Douglas. Dept. Biochemistry, UTHSCD, Dallas, Texas, 75235

In addition to the α and β subunits of the mitochondrial F_1-ATPase of S. cerevisiae, the γ subunit has also been proposed to be important to enzyme function. All three subunits are encoded by nuclear genes, and the products are transported into the mitochondria. Single nuclear genes encoding the α and β subunits have been isolated and sequenced previously by this lab. In this work, a genomic library of yeast DNA cloned into lac Z in the expression vector λgt11 was screened using polyclonal antiserum to the yeast γ subunit. Out of approximately 5 x 10^6 phage screened, nine phage which produced plaques reactive with this antiserum were isolated. One of these phage was found to encode a β-gal - yeast sequence fusion protein which reacts with the antiserum on Western blots. This fusion protein appears to be approximately 5000 Da. larger than β-galactosidase. We therefore estimate the yeast DNA insert to contain at least 150 bp of the coding region of the putative γ gene (ATP3). Analysis of the λgt11 DNA reveals a total yeast insert of 5.8kb. This DNA fragment has been mapped by restriction enzyme digestion, and its orientation with respect to lac Z in λgt11 has been determined. The fragment determined to be adjacent to the lac Z coding region has been subcloned into mp19, and the sequencing of this fragment is in progress. This fragment recognizes a 1.55kb transcript on Northern blots, a size consistent with the size of the gene product (34KDa). Genomic mapping using this fragment to probe Southern blots has been initiated with the intention of isolating the complete gene for sequencing and structure/function studies.

E 229 RADIATION INACTIVATION ANALYSIS OF INFLUENZA AND SENDAI MEDIATED FUSION AND HEMOLYSIS, John Lenard, Suzanne Gibson and Keiko Morita, UMDNJ-Robert Wood Johnson (formerly Rutgers) Medical School, Piscataway, NJ 08854-5634.

The target sizes for inactivation of the fusion and hemolysis activities of influenza and Sendai virus glycoproteins were determined by radiation analysis. Fusion with erythrocyte ghosts was determined after loading virions with octadecylrhodamine B chloride (R18). Fusion with cardiolipin (CL) liposomes was determined by measuring relief of resonance energy transfer between fluorescent donor and acceptor phospholipids present in the liposomes. Influenza had a similar target size for fusion with ghosts and CL liposomes, ca. 75 kDa, corresponding to a single HA monomer. Sendai had a similar target size of ca. 73 kDa for fusion with ghosts, but had a much larger, more complex target size for fusion with CL liposomes: the inactivation curve had a pronounced shoulder, suggesting a functional unit composed of several separate entities. Sendai, therefore, fuses with CL liposomes and ghosts by different mechanisms, while influenza may use the same mechanism for both. Hemolysis target sizes differed from fusion target sizes for both viruses, suggesting that leakage may be actively mediated by an ensemble of viral proteins different from those responsible for fusion.

E 230 IMPORT AND INTRAORGANELLAR TARGETING OF PRECURSOR PROTEINS INTO CHLOROPLASTS, Thomas H. Lubben, Joern Bansberg and Kenneth Keegstra. University of Wisconsin, Madison, WI 53706.

Chloroplasts are structurally complex oganelles with three different membranes and three different aqueous compartments. Most chloroplast proteins are encoded in the nucleus, synthesized in the cytoplasm and imported into chloroplasts post-translationally. Because of the structural complexity of the chloroplast, the import process must also include targeting the protein to the correct organellar location. In order to gain insight into the mechanism of chlorplast protein import, we have created altered precursor proteins and analyzed their behavior in a reconstituted vitro import assay. Several types of altered precursor proteins have been generated. These include 1)deletion mutations, 2)chimeric precursors containing molecules normally not imported into the chloroplast, and 3)precursors containing structural features known to be membrane-interactive. Genes encoding precursor proteins were cloned into plasmids which were then transcribed in vitro to yield mRNAs. These mRNAs were translated in vitro to yield labelled precursor proteins. Using the above in vitro assay, these various precursor proteins have been characterized with respect to efficiency of import and intraorganellar localization.

E 231 THE NUCLEAR LOCALIZATION SIGNAL IN ADENOVIRUS E1A Robert H. Lyons and Martin Rosenberg, Molecular Genetics Department, Smith Kline and French Laboratories, Philadelphia PA

The adenovirus E1a gene product is found in the nucleus of adenovirus-infected cells. When produced in bacteria and microinjected into cultured cells, E1a will also migrate rapidly into the nucleus. This protein translocation is due to the presence at the carboxyl terminus of a pentameric signal sequence, Lys-Arg-Pro-Arg-Pro. Deletions involving these residues abolish the rapid nuclear uptake. The E. coli enzyme galactokinase (GalK), which normally stays in the mammalian cytoplasm when injected there, is redirected into the nucleus if the pentameric signal is present on the molecule. Both E1a and GalK will remain in the nucleus even without a signal sequence if injected directly there, indicating that the signal is not necessary for nuclear retention. The pentamer must therefore influence the nuclear entry rates of these two proteins.

E 232 AN ACIDIC PREVACUOLAR COMPARTMENT OPERATES IN ENDOCYTOSIS IN *Saccharomyces cerevisiae*, Marja Makarow and Leena T. Nevalainen, University of Helsinki, Finland

We have earlier shown that macromolecules like FITC-dextran (MW 70000) and α-amylase (MW 54000) are internalized by endocytosis into *S. cerevisiae* spheroplasts as well as cells with intact cell walls (M. Makarow, 1985, EMBO J. 4, 1861-1866). Now we have identified a novel intermediate compartment, via which FITC-dextran is transported to the vacuole in yeast. The internal pH of this compartment was found to be acidic. The pH could be raised by ammonium chloride (a lysosomotropic agent), by CCCP (a protonophore) and by DCCD and sodium vanadate (ATP'ase inhibitors). We suggest that this intermediate acidic compartment represents the endosomes of yeast. Two independent reversible ways were found to block the internalization of FITC-dextran in the endosomal compartment. This enabled us to study two portions of the endocytic route separately, namely the transport of FITC-dextran from the exterior of the cell to the endosomes, and its transport from the endosomes to the vacuole. The energy-requirements, the kinetics and the temperature- and pH-dependencies of the dissected endocytic pathway were characterized. It appears that the endosomal compartment of *S. cerevisiae* shares many properties with that of the mammalian cell. Thus, yeast should provide a powerful model to study the mechanisms underlying ingoing membrane traffic in the eukaryotic cell.

E 233 SECRETION STUDIES OF CHORIONIC GONADOTROPIN: THE ROLE OF THE CARBOHYDRATE UNITS. Martin Matzuk, Monty Krieger*, Christopher Corless, and Irving Boime, Washington University, St. Louis, MO 63110 and *MIT, Cambridge, MA 02139.

The glycoprotein hormones, chorionic gonadotropin (CG), lutropin, follitropin, and thyrotropin are dimeric hormones that share a common α subunit but differ in their β subunits. The β subunits show extensive homology; however the CGβ subunit has a 29 amino acid carboxy-terminal extension that contains 4 serine O-linked oligosaccharides. We examined the role of N- and O-linked oligosaccharides on the secretion and turnover of hCG subunits using site-directed mutagenesis and a glycosylation deficient mutant cell line. CGα and β genes were inserted into eukaryotic expression vectors and transfected into CHO cells. The cells were incubated with [^{35}S]cysteine and the intra- and extracellular proteins were immunoprecipitated and resolved on SDS gels. CGα contains 2 N-linked glycosylation sites at asparagines 52 and 78. Mutagenesis of position 52 did not alter secretion or degradation. Mutagenesis of asparagine 78 led to an increase in degradation and decreased secretion. Thus, N-linked glycosylation at position 78 is critical for stability and possibly folding of the α subunit. We analyzed the N-linked and O-linked carbohydrate units on CGβ in a mutant CHO cell line, ldlD, defective in synthesis of O-linked oligosaccharides. Transfection of the CGβ gene into these cells revealed no change in the secretion and degradation rates of the hCGβ subunit. Tunicamycin addition delays the $t_{1/2}$ of secretion from 2 to 4 hrs but does not alter the degradation rate. Thus, unlike CGα, CGβ is resistant to proteases in the absence of any sugar. Also, removal of N-linked, but not O-linked, sugar delays CGβ secretion. Mutagenesis and use of ldlD cells has allowed us to analyze specific carbohydrate units on the CG subunits and the data suggest site-specific functions of the oligosaccharides on CG.

E 234 MAD1, A YEAST GENE INVOLVED IN MITOCHONDRIAL ASSEMBLY OR DELIVERY OF F_1-ATPASE PRECURSORS, Mark T. McCammon and Michael G. Douglas, University of Texas Health Science Center, Dallas, Texas, 75235

In order to identify cellular components which are involved in the import of cytoplasmically synthesized precursor proteins into mitochondria, we have isolated mutants of Saccharomyces cerevisiae which are defective in the localization of normal and hybrid molecules within the organelle. The selection was based on the observation that a chimeric protein consisting of the first 382 amino acids of the F_1-ATPase β subunit fused to β-galactosidase rendered yeast mitochondria nonfunctional. At least two of the mutants regained mitochondrial function by either mislocalization or misfolding the hybrid F_1β-βgalactosidase protein. One of the mutants accumulated precursors to F_1ATPase subunits. The mutation, mad1-1, had a temperature sensitive phenotype which allowed for the selection by complementation of the functional MAD1 gene. MAD1 is essential for cell viability and is not allelic with previously identified mas mutants in yeast. Experiments are in progress to assess the function of the MAD1 protein in the assembly or delivery of mitochondrial proteins.

E 235 CELLULAR LOCALIZATION OF A MINOR HEAT SHOCK PROTEIN, Thomas W. McMullin and Richard L. Hallberg, Iowa State University, Ames, IA 50011.

We have purified a 60 kDa protein from Tetrahymena thermophila which is transiently synthesized during the first 2 hours of a 41°C heat shock. Antiserum prepared against this protein (hsp60) has been used to show that hsp60 is always present in cells but that early in heat shock, its cellular concentration increases at least 3-4 fold. Immunocytological analysis of non-heat shocked cells revealed that hsp60 is localized almost exclusively in spherical structures arranged in rows along the longitudinal axis of the organism. This same pattern has been described for the arrangement of mitochondria in T. thermophila (J. Cell Sci. 39, 299-312). Preliminary cell fractionation studies revealed that the vast majority of hsp60 is found in a crude mitochondrial fraction. Further studies on the cellular localization of hsp60 are continuing. We are currently attempting to determine the fate of the hsp60 which accumulates in the cell during heat shock. The hsp60 antiserum reacts strongly with similar sized polypeptides from total cell extracts of yeast and human lung cells.

E 236 MODIFICATION OF CHLOROPLAST SURFACE PROTEINS AND THE EFFECT ON TRANSPORT OF PRECURSOR PROTEINS INTO INTACT CHLOROPLASTS. Steven M. Pomarico and Sue G. Bartlett. Louisiana State University, Baton Rouge, La. 70803.

Proteins on the surface of intact chloroplasts can be modified either chemically or by protease treatment. Transport of the precursor (pS) of the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase has been used as a model system to study the effect of these modifications on the transport process. Intact chloroplasts have been probed using reagents which modify selected amino acid side chains. Chemical modification of sulfhydryl groups by iodoacetamide and of primary amines by sulfosuccinimidyl acetate (or related compounds) results in decreased transport of pS. Proteolytic modification of the surface proteins of intact chloroplasts by thermolysin also results in decreased transport of pS. The inhibition of transport may be due to the modification of a receptor protein. Alternatively, the modified protein may have another role which is essential for protein transport. The polypeptides of the chloroplast envelope are being examined by SDS-PAGE to identify proteins which are modified by these various treatments. We are also studying the effects of these modifications on binding of pS to the chloroplast envelope.

E 237 AMINO TERMINAL SEQUENCE REQUIREMENTS FOR ANCHORING ROTAVIRUS VP7 IN THE ENDOPLASMIC RETICULUM, Marianne S. Poruchynsky and Paul H. Atkinson, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Rotavirus SA11 VP7 is a membrane glycoprotein with two amino terminal hydrophobic domains. In the coding sequences each is preceded by an in-frame ATG. Transiently expressed VP7 product in COS7 cells remains in the endoplasmic reticulum (ER) and exhibits a high-mannose endo-glucosidase H (endo-H) sensitive type of glycosylation. Our previous studies showed that in the absence of the first initiation codon, the second ATG was utilized efficiently for initiation. The resultant altered VP7, lacking the first hydrophobic domain, was translocated into the ER and was not secreted. Deletions were introduced into the coding region of the second of the two hydrophobic domains removing 2, 6 or 7 amino acids from its carboxy terminal end (d.42-61, d.43-61 and d.47-61, respectively). The altered VP7s left the ER, were secreted from cells efficiently and exhibited a complex endo-H resistant form of glycosylation. The role, if any, of the first hydrophobic domains in the secretory behavior of d.42-61, d.43-61, or d.47-61 products were unclear, therefore our current studies compare the products of the same mutants to their counterparts lacking the first ATG (d.42-61/dh1, d.43-61/dh1 or d.47-61/dh1). We found that the sizes and endo-H sensitivity of both the intracellular or the secreted VP7 products were identical, which shows the second hydrophobic domain acts as a signal sequence and as a membrane anchor. It also indicates that the second initiation codon is used in transfected cells, when both are present. The same result was evident in mutant and wild type genes transcribed and translated in vitro. Products translated in vitro, derived from the deletions which removed a portion of the second hydrophobic domain or a region just distal to it, resulted in products apparently initiating at the second ATG, as well as glycosylated and unglycosylated forms.

E 238 **SUBCELLULAR LOCALIZATION OF CHOLESTEROL METABOLIZING ENZYMES**
Reinhart, M.P., Billheimer, J.T., Faust, J.R., Gaylor, J.L.
E. I. Du Pont de Nemours and Company, Du Pont Experimental Station, Building 400, Wilmington, Delaware 19898

We have demonstrated that membrane bound enzymes of sterol biosynthesis are located in both smooth and rough endoplasmic reticulum components of rat liver microsomes. The subcellular localization of these enzymes was demonstrated by individual enzyme assay after sucrose density gradient centrifugation. Also, peak fractions of SER and RER were able to catalyze the conversion of radiolabeled mevalonate into cholesterol in the presence of rat liver cytosol. Thus, the final stages of cholesterol biosynthesis occur in the ER. We, and others, have also demonstrated that the enzyme ACAT is restricted to the RER membrane. ACAT activity converts free cholesterol to cholesterol esters thus removing cholesterol from the lipid bilayer. Agents which dissociate ribosomes stimulate ACAT activity. The location of this enzyme suggests that it may be important in maintaining low cholesterol levels in the RER. We propose that free sterols might be inhibitory to ribosomal attachment and that ACAT acts to mitigate the affect of unregulated cholesterol insertion. Steadily increasing sterol content might represent a driving force in maturation of RER into SER.

E 239 NEWLY SYNTHESIZED PROINSULIN AND INSULIN ARE SECRETED ONLY VIA A REGULATED PATHWAY DESPITE RELEASE IN PREFERENCE TO STORED INSULIN, Christopher J. Rhodes and Philippe A. Halban, Joslin Diabetes Center, Boston MA 02215.
It has been proposed that secretory cells are equipped with both regulated and constitutive release pathways. We wished to know whether this applies to a highly differentiated cell, such as the pancreatic B-cell, by studying release of newly synthesized prohormone/hormone versus that of stored processed hormone. Rat pancreatic islets were pulse-labeled ([^{3}H]leucine, 5 min, 16.7mM glucose, 37°C) and then chased at either basal (2.8mM) or stimulatory (16.7mM) glucose at 37°C for a further 175 min. At set times aliquots of incubation medium and islets were removed to analyze the fate of total immunoreactive insulin (IRI) and newly synthesized [^{3}H]proinsulin (PI*) and [^{3}H]insulin (I*; by quantitative HPLC analysis). Release of IRI was stimulated approx. 8-fold by 16.7mM glucose throughout the chase. By 180 min. 15% of IRI islet content was released at 16.7mM glucose and 2% at 2.8mM glucose. After a 30 min. lag period release of PI* and I* was detected; as from 60 min such release was stimulated up to 11-fold by 16.7mM glucose. At 180 min 60% of initial islet PI* had been released at 16.7mM glucose and 6% at 2.8mM glucose. The specific radioactivities of released PI* and I* indicated preferential release of newly synthesized products. This was equally true at both low and high glucose. Further analysis revealed; 1) release of both PI* and I* was sensitive to glucose stimulation; 2) 90% of the newly synthesized products were released as insulin, 10% as proinsulin; 3) only 0.5% of PI* was released in a glucose independent fashion (between 30-60 min). These observations lead us to conclude that 99.5% of release hormone (whether new or old) released form pancreatic B-cells is directed through a regulated (glucose-sensitive) pathway.

E 240 SECRETION OF APOLIPOPROTEINS FROM CULTURED INTESTINAL EPITHELIAL CELLS. Michael J. Rindler, Herbert J. Kayden, and Maret G. Traber, Departments of Cell Biology and Medicine, New York University Medical Center, New York NY 10016.
The ability of the intestinal epithelial cell line Caco2 to synthesize lipoprotein particles and to export them from the cell in a polarized fashion has been investigated. Cells radiolabeled with ^{35}S-methionine secreted specifically immunoprecipitable products corresponding to apolipoproteins B100 and B48 (500 and 250 Kd, respectively), as well as apo A1 (28 Kd) and apo E (36 Kd). When cultured in Millicell chambers, Caco2 formed an intact monolayer with transepithelial electrical resistance of 250-350 ohm·cm^2 and consisted of tall columnar cells in the EM. Radiolabeled apolipoproteins, which were major secretory products, were released in a polarized fashion, predominantly into the basolateral chamber. When fed ^{14}C-oleic acid the cells incorporated it into triglyceride. More than 80% of the triglyceride subsequently released into the medium was also found on the basal side of the monolayers, consistent with the notion that it was in a lipoprotein particle. The secreted apolipoproteins were found in the lipoprotein fractions (density 1.006-1.21 g/ml) when analyzed by ultracentrifugation. These results indicate that intestinal cells in vitro can secrete lipoprotein particles and provide evidence that polarized epithelial cells can target secretory products to the basolateral surface. They further suggest that the sorting machinery for apical and basolateral proteins recognizes signals residing in the intraluminal space of the intracellular organelles involved in protein transport. Supported by grants from the NIH and the Irma T. Hirschl Charitable Trust.

E 241 MUTATIONS AT THE N-TERMINUS OF INFLUENZA VIRUS HEMAGGLUTININ CAUSE MALFOLDING AND PREVENT TRANSPORT OF THE MOLECULE FROM THE ENDOPLASMIC RETICULUM OF MAMMALIAN CELLS. Chuck C.-K. Chao, Mary-Jane Gething, and Joe Sambrook, Dept of Biochemistry University of Texas Health Science Center, Dallas, Texas 75235
We are investigating the role of protein folding and oligomerization in the transport of a membrane-bound protein (influenza virus hemagglutinin) along the exocytotic pathway. In previous work (Gething et al. 1986 Cell 46, 939) We have shown that a subset of alterations to the carboxyterminal sequences of the molecule (the cytoplasmic tail) inhibit folding and trimerization of HA in the endoplasmic reticulum and prevent its transport to the cell surface. The current studies establish that the integrity of the N-terminal sequences of the mature HA of the Japan strain is essential for assembly and transport of the trimer. Mutants lacking just one or two amino acids immediately carboxyterminal to the signal cleavage site are translocated and, core-glycosylated but not fold correctly or assemble into native trimers (as assayed by protease-sensitivity and chemical cross-linking). The mutant molecules do not become terminally glycosylated and are confined to the endoplasmic reticulum. Hypotheses will be presented to explain why sequences at opposite ends of the HA molecule are essential for assembly of native structures and why correct folding should be necessary for transport along the exocytotic pathway of mammalian cells.

E 242 MDCK monolayers devoid of the apical surface: a system amenable for reconstitution of intracellular protein transport. Yula Sambuy and Enrique Rodriquez-Boulan. Department of Cell Biology, Cornell University Medical School, 1300 York Ave, New York, N.Y. 10021. MDCK cells constitute the paradigm of a polarized epithelial cell line currently used in trying to elucidate the mechanisms responsible for the biogenesis of epithelial cell polarity. Previous studies have demonstrated the existence of intracellular sorting mechanisms at the level of the Golgi apparatus that account for the vectorial delivery of apical and basolateral plasma membrane proteins to their respective surface domain. As a preliminary step to reconstitute transport between Golgi apparatus and the cell surface we have developed a procedure that removes the apical surface of confluent MDCK monolayers. Briefly, MDCK monolayers grown on nitrocellulose filters are covered with 3 alternate coats of cationic colloidal silica and a polyanion and the apical surface is removed with a polylysine coated coverslip. S.E.M examination of the material on the coverslip reveals a honeycomb pattern given by the smooth intracellular aspect of the apical surface and the cell borders; the material on the filters is composed of the bottom 2/3 of the cells including nuclei, most of the basolateral membrane and part of the E.R. and the Golgi apparatus. We are currently using this system to attempt to reconstitute the transport of VSV G protein between the Golgi apparatus and the basolateral membrane. Supported by NIH and NSF grants and an Established Investigator Award from the American Heart Association to ERB.

E 243 ACIDIFICATION OF THE CYTOSOL INHIBITS ENDOCYTOSIS FROM COATED PITS. EVIDENCE FOR AN ALTERNATIVE PATHWAY OF ENDOCYTOSIS. Kirsten Sandvig[1], Sjur Olsnes[1], Ole W. Petersen[2] and Bo van Deurs[2]. 1. Institute for Cancer Research, The Norwegian Radium Hospital, Montebello, Oslo 3, Norway. 2. The Panum Institute, University of Copenhagen, DK-2200 Copenhagen, Denmark.

Acidification of the cytosol strongly reduced endocytic uptake of transferrin and EGF, while it had little or no effect on the uptake of ricin and the fluid phase marker, lucifer yellow. Furthermore, the number of transferrin receptors at the cell surface was increased, and there was less aggregation of the binding sites in coated pits. The number of coated pits at the cell surface was increased indicating that the pinching off of coated vesicles was inhibited. Ricin endocytosed by cells with acidified cytosol exhibited normal toxic effect on the cells. The data demonstrate that at internal pH < 6.5 endocytosis by the coated pit/coated vesicle pathway is strongly inhibited, and that an alternative endocytic pathway exists.

E 244 CLONING OF CYC2, A GENE INVOLVED IN CYTOCHROME C MATURATION, Julia Schlichter Fred Sherman, University of Rochester Medical Center, Rochester N.Y. 14642. CYC1 and CYC7 are the structural genes in yeast for apo-iso-1 and apo-iso-2-cytochrome c CYC3 encodes cytochrome c heme lyase. The only other gene that has been discovered which, when mutated, causes a specific deficiency of iso-1 and iso-2-cytochrome c is CYC2. The presence of apo-cytochrome c in cyc2 mutants indicates that the CYC2 gene product is required for a post-translational step in the production of the iso-cytochromes c. The preponderance of chain terminating mutations indicates that cyc2 may encode a structural protein. We hypothesize that CYC2 encodes a recptor on the outer mitochondrial membrane which is responsible for transport of apo-cytochrome c into the mitochondria. The following experiments are being carried out to determine if our hypothesis is correct. The CYC2 gene was cloned by transforming a yeast DNA bank into a cyc2-deficient strain and selecting for growth on lactate media. Genetic mapping confirmed the identity of the clone. The clone is currently being sequenced. Future studies will involve production of the CYC2 protein from an expression vector, antibody production and subcellular localization.

E 245 SYNTHESIS AND SECRETION OF NORMAL AND MUTANT HUMAN ALPHA-1-ANTITRYPSIN IN TRANSGENIC MICE, R.N. Sifers, J. A. Carlson, J. L. DeMayo, S.M. Clift, B. Rogers, M. Finegold, F. J. DeMayo, D. Bullock and S. L. C. Woo, Howard Hughes Medical Institute, Depts. of Cell Biology and Institute of Molecular Genetics, Gastroenterology and Pathology, Baylor College of Medicine, Houston, Texas, 77030.

Alpha-1-antitrypsin (AAT) is a plasma protease inhibitor of hepatic origin. The substitution of lysine for glutamate at position 342 in the mutant PiZ AAT protein results in its accumulation within the rough endoplasmic reticulum (RER) of hepatocytes and its decreased secretion into the circulation. Individuals homozygous for the mutant PiZ allele are at risk for development of emphysema and liver cirrhosis.

The normal PiM and mutant PiZ human AAT genes were cloned and microinjected into mouse embryos to generate transgenic mice. The synthesis of human AAT mRNA was detected in livers of transgenic mice by an S1 nuclease protection analysis. Immunoblotting demonstrated the presence of either normal or mutant human AAT proteins in their plasma. Immunohistochemical analysis of liver sections from the PiZ mice showed the characteristic accumulation of mutant human AAT protein within the RER of hepatocytes. Only a very diffuse staining was observed in liver sections from mice expressing the normal human PiM AAT mRNA at a level similar to that in humans. However, mice expressing elevated levels of the PiM mRNA exhibit accumulation of the normal human AAT protein in the RER of hepatocytes. Based on these data, we hypothesize that the intracellular transport of AAT from the RER to the Golgi may involve a saturable mechanism that has different affinities for various ligands.

E 246 ADENINE NUCLEOTIDE TRANSLOCATOR OF S. CEREVISIAE : MITOCHONDRIAL IMPORT SIGNALS, Cynthia S. Smagula and Michael Douglas, University of Texas Health Science Center at Dallas, Dallas TX 75235. The yeast adenine nucleotide translocator is a 309 amino acid protein predicted to fold into at least four transmembrane regions in the mitochondrial inner membrane. The first 115 amino acids correspond to a hydrophilic N-terminal region as well as a hydrophobic region predicted to be a transmembrane domain. A series of pT7-2 based vectors has been constructed that place various lengths of the 5' coding region of the S. cerevisiae pet 9 gene encoding the translocator upstream of the gene encoding mouse cytosolic dihydrofolate reductase (DHFR). In vitro transcription-translation of the constructs results in a series of protein products consisting of approximately 36, 51, 56, 71, 86, and 115 amino acids of the amino terminal region of the translocator in frame with DHFR. In vitro mitochondrial import and submitochondrial fractionation studies will address the question of whether separable intramitochondrial sorting domains exist within the amino terminal region of the translocator.

E 247 STUDIES OF INTRACELLULAR PROTEIN TRANSPORT IN TOBACCO PLANTS, Antje von Schaewen, and Lothar Willmitzer, Max-Planck-Institut fuer Zuechtungsforschung, Department Jeff Schell, Cologne, Federal Republic of Germany.
Using *in vitro* DNA techniques, chimeric genes were constructed in order to target the resulting protein products to certain subcellular compartments in higher plants. To this end three different amino-terminal portions of a vacuolar protein of potato tubers (Proteinase Inhibitor II) were fused to the bacterial Neomycinphosphotransferase reporter gene under the control of the strong constitutive 35S promoter of Cauliflower Mosaic Virus. From transgenic tobacco plant tissue, fusion proteins with different electrophoretic mobility were obtained. *In vitro* uptake studies using the rabbit reticulocyte translation system in the presence of canine pancreatic vesicles revealed that protein processing occurs also *in vitro*. Experiments are currently performed to determine the localization of the different fusion proteins.

E 248 EXPORT OF CYTOCHROME C_3 AND HYDROGENASE FROM *DESULFOVIBRIO VULGARIS* (HILDENBOROUGH)
G. Voordouw, Division of Biochemistry, Department of Chemistry, The University of Calgary, Calgary (Alta), Canada, T2N 1N4.

Hydrogenase (two subunits, 46 kDa, 13.5 kDa, three iron-sulfur clusters, $H_2 \rightleftharpoons 2H^+ + 2e$) and cytochrome c_3 (12 kDa, four hemes) are both thought to reside in the periplasm of *D. vulgaris*. I have cloned and sequenced the genes encoding these redox-proteins: cytochrome c_3 has an *E. coli*-type signal sequence of 21 amino acid residues[1]. However, the 46 kDa subunit of hydrogenase lacks a signal sequence, indicating a cytoplasmic location [2]. Work by Prickril *et al* [3] suggests that both polypeptides are exported: the 13.5 kDa subunit has a 34 amino acid signal sequence and an internal sequence required for export is proposed for the 46 kDa subunit. The merits of this proposal will be discussed in the light of of recent data on the expression of *D. vulgaris* hydrogenase polypeptides in *E. coli*.

1) Voordouw, G. and Brenner, S. (1986) Eur. J. Biochem. 159, 347-351.
2) Voordouw, G. and Brenner, S. (1985) Eur. J. Biochem. 148, 515-520.
3) Prickril, B.C. *et al* (1986) J. Bacteriol. 167, 722-725.

E 249 EFFICIENT POSTTRANSLATIONAL TRANSLOCATION OF PREPRO-α-FACTOR INTO YEAST MICROSOMES REQUIRES A SOLUBLE FACTOR. M. Gerard Waters, William J. Chirico and Gunter Blobel, Laboratory of Cell Biology, The Rockefeller University, New York, NY 10021.

It has previously been shown that in vitro translocation of prepro-α-factor across the Saccharomyces cerevisiae microsomal membrane can occur posttranslationally in an ATP dependent fashion. To identify protein components of the translocation apparatus we employed a heterologous translocation system. We found that posttranslational translocation of prepro-α-factor into yeast microsomes is efficient when the protein is synthesized in a yeast translation system but inefficient when made in a wheat germ translation system. The efficiency of translocation of wheat germ synthesized prepro-α-factor can be significantly increased by the presence of a yeast cytoplasmic fraction during posttranslational incubation with yeast microsomes. These data indicate that yeast possess a soluble activity that is required for efficient posttranslational translocation of prepro-α-factor into yeast microsomes. The activity is not bound to yeast microsomes in a salt-extractable manner and is not associated with ribosomes, but is found in a post-ribosomal supernatant fraction. Stimulation of translocation is not due to a non-specific effect on ATP levels. The activity is destroyed by N-ethylmaleimide, protease or heat treatments and is therefore, at least in part, proteinaceous. RNase treatment does not affect the activity. The activity has a sedimentation coefficient of about 9.6 S, but it is possible this is the result of peak overlap from two or more essential factors having S values above and below 9.6 S. We are currently purifying the factor(s) in order to further characterize its function in the yeast secretory process.

E 250 MULTIPLE NUCLEAR ENVELOPE LAYERS IN YEAST THAT OVERPRODUCE HMG-CoA REDUCTASE, Robin Wright and Jasper Rine, University of California, Berkeley, CA 94720.
The rate limiting step of sterol biosynthesis is catalyzed by HMG-CoA reductase. In mammalian cells, localization of reductase in the ER membrane is required for sterol-mediated control of the enzyme half-life. We have begun studies to examine whether or not a similar interplay between localization and regulation of reductase operates in the yeast, Saccharomyces cerevisiae. This organism carries two genes that encode reductase isozymes with essentially identical catalytic domains but different membrane domains. Differential subcellular localization of reductase isozymes may be important for reductase regulation in yeast. Mammalian cells that overproduce reductase 100-fold display a characteristic proliferation of the ER that has been described as "crystalloid". We have examined a yeast strain producing 10-fold the wild-type level of one of the reductase isozymes (encoded by HMG1) and observe analogous, but less extensive, ultrastructural abnormalities. The nuclear membrane in these cells consists of stacks of paired bilayers, forming a lamellated structure reminiscent of the layers of an onion around the nucleus. In many instances, intact portions of these membrane stacks ("karmellae") have apparently detached from the nucleus and are found within the cytoplasm, often near the plasma membrane. Immunolocalization experiments using affinity purified anti-reductase antibodies demonstrate the presence of reductase in karmellae, both at peri-nuclear and detached locations. Currently, we are involved in experiments to biochemically characterize karmellae and to determine if its formation is a result of overproduction of the reductase protein or of increased reductase activity.

Additional Poster Abstracts E300 - E318

E 300 A MICROTUBULE-BINDING PROTEIN ASSOCIATED WITH MEMBRANES OF THE GOLGI APPARATUS, Victoria J. Allan and Thomas E. Kreis, European Molecular Biology Laboratory, Meyerhofstrasse 1, 6900 Heidelberg, F.R.G.
The microtubule network appears to be involved in maintaining the correct positioning of the Golgi apparatus within the cell. When the cytoplasmic microtubules are depolymerised (during mitosis, or by drug treatment for example) the Golgi apparatus becomes scattered throughout the cell. We have shown that after removal of nocodazole, the Golgi apparatus begins to recluster within 10 minutes, and is fully reclustered within 60 minutes. We have identified a protein which may be involved in linking the Golgi apparatus to the microtubule network. This protein has a molecular weight of 110,000, and is immunologically related to MAP-2. Like MAP-2, the 110k protein interacts with taxol-polymerised microtubules *in vitro* . In contrast to MAP-2, however, the 110k protein is located in the Golgi apparatus in tissue culture cells, as shown by immunofluorescence. The 110k protein is a peripheral membrane protein which is washed off the membrane by high pH treatment, and its sensitivity to proteases in the absence of detergent suggests that it is located on the cytoplasmic face of Golgi vesicles. We are currently investigating the role of the 110k protein *in vivo*.

E 301 NUCLEAR PORES IN YEAST, Jerry Lynn Allen and Michael G. Douglas, Biochemistry, UTHSCD, Dallas TX 75235. Nuclear pores form an intracellular gap junction regulating in an undefined manner the continuity of discrete nuclear and cytoplasmic compartments. To understand the assembly and function of this organelle, we have initiated a molecular genetic analysis of pores in Saccharomyces cerevisiae. Yeast nuclei are prepared from spheroplasts in the absense of detergents using 18% Ficoll 400 to stablize nuclear integrity. Extraction of the isolated nuclei in sequential steps with DNaseI/RNase A, 2% Triton X-100, and 1 M NaCl yields a peripheral karyoskeleton consisting of a fibrous lamina connecting pore complexes. Intact nuclear pores can be separated from this "lamina-pore complex" subfraction using the combination of ionic detergent and reducing agent. Depending on the method, the pore (enriched in higher molecular weight proteins) is isolated with varying amounts of associated proteins. The yeast pore is approximately 80 nm in size, exhibits an octagonal symmetry about a central annulus and is often seen containing a central "plug". The "core pore" appears to be a skeletal structure which displays fibrous attachments resembling a specialized domain of the lamina. These data show that the structure of the yeast nuclear pore and its organization within the "lamina" is the same as that documented in the higher eukaryotes.

E 302 EVOLUTION OF MITOCHONDRIAL TARGETTING SEQUENCES. A. Baker and G.Schatz, Biozentrum Universitat Basel, Switz. What was the evolutionary origin of mitochondrial presequences? Mitochondria are thought to be the descendents of formerly free-living prokaryotes which entered into symbiosis with other cell types, but they are now dependent on nuclear genes for over 90% of their proteins. Many coding functions have therefore been delegated to the nucleus, and mechanisms evolved to return the gene products to mitochondria, or to permit novel proteins to enter mitochondria. These mechanisms included the accquisition of amino terminal presequences. Mitochondrial presequences do not have a high degree of primary sequence homology, but characteristically contain a high proportion of basic and hydroxylated amino acids, are poor in negatively charged amino acids and have an overall hydrophobicity near to neutral.These residues are often arranged such that the sequences could potentially form amphipathic alpha helices. DNA sequences encoding short peptides with these characteristics would be predicted to occur within the genomes of all organisms, purely on a random basis. Pools of such "cryptic" mitochondrial targetting sequences would have existed within the genomes of primitive eukaryotes. Such sequences could have become activated by DNA rearrangements which placed them upstream of reading frames for existing polypeptides.In order to test this hypothesis the genome of E.coli, and a eukaryotic cytosolic protein were analysed for sequences which can function to import proteins into yeast mitochondria. A number of such sequences have been found.The existence of these kinds of cryptic mitochondrial targetting sequences even within the genomes of organisms which lack these organelles, may have been an important prerequisite for the development of protein traffic within primitive eukaryotic cells.

E 303 SPECIFICITY OF CO-TRANSLATIONAL AMINO-TERMINAL PROCESSING OF PROTEINS IN YEAST.[1]
L.C. Blair, R.A. Bradshaw*, K. Bryan, R.C. Elliott, P. Ghosh-Dastidar, R.L. Kendall, R.K. Koduri, S. Huang, J.-H. Lee, P.-S. Liu, K. Saito, and J.L. Weickmann, INGENE, Inc., Santa Monica CA 90404 and *Dept. of Biol. Chem.-College of Medicine, UCI, Irvine CA 92717.

Protein translation in eucaryotes initiates at methionine (Met) codons; however, most mature proteins lack N-terminal Met residues. In some cases the fate of these residues is obscured by the removal of leader (or extension) peptides. Most proteins that lack leader sequences, particularly cytoplasmic proteins also start from an initiator Met, which is subsequently removed. The specificity of the processing enzyme, methionine aminopeptidase, has been described based on compilations of known sequences and naturally occurring mutants. In general, the N-terminal Met is removed when the penultimate amino acid is a small aliphatic residue. In some cases, the initiator Met is either retained or may be susceptible to additional modification. Further modification of the Met or the exposed penultimate residue is catalyzed by a ribosomal N_{α}-acetyl transferase. This enzyme, which uses the CoA derivative as the acetyl donor, also favors but is not restricted to small aliphatic residues, as judged by similar lists of known sequences. A synthetic gene for the plant protein thaumatin, expressed in yeast, was used to systematically analyze the effect of the penultimate residue (to the initiator Met) on the specificity of these two enzymes. Sequence analysis of the twenty possible proteins revealed four classes of N-terminal amino acid structures: (1) unmodified; (2) acetylated penultimate residues; (3) modified and (4) unmodified, uncleaved N-terminal Mets.

[1] This work was supported by Beatrice Companies, Inc. Chicago IL

E 304 TWO NOVEL BASIC PAIR SPECIFIC ENDOPROTEASES PROCESSING SOMATOSTATIN-28 AND PRO-OCYTOCIN-NEUROPHYSIN PEPTIDES : PURIFICATION AND FUNCTIONAL PROPERTIES.
P. Cohen, P. Gluschankof, Ch. Clamagirand, S. Gomez, M. Camier, Ch. Créminon, A. Morel, H. Boussetta and P. Nicolas, Univ. P. et M. Curie, 75006 PARIS, France.

The processing of basic doublets in prohormones was studied using a strategy based upon the use of synthetic substrates either reproducing or mimicking the precursor sequence around the cleavage loci. In the case of somatostatin-28(S-28) a precursor for both the NH_2- and COOH-terminal fragments (S-28(1-12) and S-14 respectively) an undecapeptide was used together with the octaeicosapeptide prohormone. A S-28 convertase was detected in lysates of secretory granules isolated from either hypothalamus or rat brain cortex. This system, weakly associated to the granules membrane, appears to combine an endoprotease cleaving before the doublet, the Glu ArgLys bond, and an aminopeptidase B removing the extra Arg and Lys residues. For proocytocin-neurophysin (pro OT/Np) a convertase was found associated with granules isolated from the neurohypophysis and the bovine corpus luteum. This endoprotease cleaves the substrate, a peptide 18 or 20 aminoacid long reproducing the entire NH_2-terminal sequence of the prohormone, after the doublet at the $Gly^{10}Lys^{11}Arg^{12}Ala^{13}$ bond. It appears to be co-located with a carboxypeptidase B which excises sequentially the Lys Arg moietie. Both endoproteases appear to possess the properties of thiol enzymes, divalent cations sensitive, which do not cleave lone Lys or Arg residues and are unsensitive to serine-proteases inhibitors. Their rather restricted selectively toward the substrate was shown by use of peptides selectively modified at either residues of the cleavage sequence. These observations raise questions about the possible involvment of multiple processing systems.

E 305 BASEMENT MEMBRANE SYNTHESIS IN DROSOPHILA EMBRYOS, Liselotte I. Fessler, Andrew G. Campbell, Bruce Blumberg, Albert MacKrell and John H. Fessler, Molecular Biology Institute, UCLA, Los Angeles, CA 90024.

Procollagen IV, laminin, entactin and proteoglycans are components of vertebrate basement membranes. Closely similar Drosophila glycoproteins have been isolated and characterized. The cDNAs coding for a Drosophila procollagen IV chain have been sequenced and a high degree of conservation of the junctional domain has been observed between vertebrates and Drosophila. Drosophila and vertebrate laminins have the same cross shape, but show only weak immunological cross reactivity. Antibodies to these Drosophila proteins localize to Drosophila basement membranes of the internal organs and nervous system. Immunofluorescence staining of whole mounts and sections and in situ hybridization with cDNA probes was observed at the beginning of organogenesis at about 8 hrs of development. The nervous system showed a segmental topology for the basement membrane envelope of the ventral nerve cord. Due to the bilateral derivation of the nerve cord, its envelope is the equivalent of two, fused, side-by-side cylinders. At segmental intervals the separating, fused walls have disappeared, leaving a single enclosure. But at each segment boundary the juxtapositioned walls form a conical tube or stalk with a ventro-dorsal axis. These stalks penetrate the whole nerve cord and form prominently staining segmental markers. From the two median cells in the dorsal opening of each stalk, two processes extend to the longitudinal muscles where they attach specifically at the segment borders. Segmental abnormalities of the central nervous system in ftz mutants are accompanied by corresponding changes in the segmental pattern of the basement membranes of the CNS.

E 306 FUNCTIONAL ANALYSIS OF MITOCHONDRIAL PROTEIN IMPORT IN YEAST, Scott M. Glaser and Michael G. Cumsky, Dept. of Mol. Biol. and Biochem., Univ. of California, Irvine.
Recent results, from several laboratories, have now clearly established the critical role of the leader peptide in the selective targeting of proteins translated in the cytosol, to, and into, the mitochondrion. However, it is still not clear whether this sequence is also responsible for correct intra-mitochondrial localization (sorting), and whether the product(s) of additional genes are obligatorily required for import. In order to address both questions, we have been using an experimental system which employs a new vector, which we call an LPS (for leader peptide substitution) vector, and a functional assay. The LPS vector uses a modified yeast *COX5a* gene (the structural gene for subunit Va of cytochrome c oxidase, and an essential component of the enzyme), in which the sequences encoding the wild-type Va leader peptide can be precisely deleted and replaced with a given test sequence. Following transformation of the recombinant plasmid into a yeast host carrying a null mutation of the endogenous *COX5a* gene, we can assay the ability of that sequence to direct subunit Va to the inner mitochondrial membrane, (i.e., to target, and to sort), by asking for complementation of the mutant. Thus far, we have tested the ability of several different sequences to target subunit Va in this system. Of special note is the finding that a leader peptide consisting of the NH_2-terminal 17 amino acids from the yeast *COX4* gene effectively targets subunit Va, and yields a fully functional holoenzyme. This result suggests that a portion of the leader peptide derived from a peripheral membrane protein can efficiently localize an integral membrane protein to its proper location within the inner membrane. We are now in the process of testing several other sequences for their ability to function in this system. Included are mutant leader peptides, which fail to target. We hope to use these mutants to select chromosomal suppressors which restore the ability of the transformants to respire, and hence, the ability of the leader to target and sort. These studies are in progress.

E 307 GENE EXPRESSION DURING SEED GERMINATION AND POST-GERMINATIVE GROWTH OF BRASSICA NAPUS, J.J. Harada, C.S. Baden, L. Comai, and R.A. Dietrich, Department of Botany, University of California, Davis, CA 95616.
We are studying the regulation of gene expression during seed germination and seedling growth in Brassica napus to gain insight into plant developmental control mechanisms. To obtain an overview of gene expression during these developmental stages, we identified cloned mRNA sequences which accumulate primarily during post-germinative growth. Blot hybridization studies with RNA isolated from different seedling parts have shown that these genes can be grouped into those which are abundantly expressed in: 1) cotyledons, 2) seedling axes, 3) both seedling parts. In situ hybridization studies with seedling tissues confirmed these results and demonstrated their cellular sites of expression. The genes prevalently expressed in cotyledons show similar temporal expression patterns. Our recent studies focusing on genes encoding the glyoxylate cycle enzymes malate synthase and isocitrate lyase have shown that they are members of the cotyledon-abundant gene class. Each enzyme is encoded by a gene family. Our work shows the temporal and spatial regulation of germination-induced gene sets. Current studies are directed towards characterizing the cis-acting DNA sequences and trans-acting factors regulating these genes.
(Supported by NSF Grant No. DCB-85 18182).

E 308 RECONSTITUTION OF AN ENDOCYTIC FUSION EVENT IN A CELL-FREE SYSTEM: SOME CHARACTERISTICS OF FUSION IN VITRO, Stella Hurtley, Gareth Griffiths and Graham Warren[1], European Molecular Biology Laboratory, Heidelberg, West Germany. [1]Department of Biochemistry, Dundee, Scotland, UK.
In order to study the mechanisms involved in receptor-mediated endocytosis we designed an assay to reconstitute stages of this process in a cell-free system[1]. Fowl Plague Virus, which has neuraminidase activity, is internalised into endocytic vesicles in one set of cells, and sialic acid labelled SFV is internalised into a second set of cells. Fusion of the separate vesicle populations can be monitored by the release of sialic acid in an energy-dependent cell-free fusion reaction.
Some characteristics of the endocytic fusion event, as revealed by the cell-free system, will be presented. Efficient fusion is independent of the low pH found inside energised endocytic vesicles, is partially inhibited by the addition of μM free Ca^{2+} and is dependent upon the presence of soluble cytosolic components, which can be inactivated by heat treatment. In addition, immunocytochemistry of thin frozen sections of cells prior to homogenisation, and of the homogenates used in the assay show that the conditions used in the preparation of components for the assay maintain a high degree of membrane-bound organelle integrity.
[1]Davey, J., S.M. Hurtley, G. Warren, 1985. Reconstitution of an endocytic fusion event in a cell-free system. Cell 43 643-652

E 309 PROTEIN TARGETING TO THE YEAST MITOCHONDRIA: ANALYSIS OF MAS2, AN ESSENTIAL COMPONENT OF THE IMPORT PATHWAY, Robert Jensen, Kitaru Suda*, Gottfried Schatz*, and Michael Yaffe, Department of Biology, University of California, San Diego, La Jolla 92093 and *Biocenter, Basel, Switzerland.

Most mitochondrial proteins are encoded on nuclear genes, synthesized in the cytoplasm, and post-translationally imported into the mitochondria. To determine how these proteins are targeted specifically to the mitochondria, we have isolated mutants in the yeast Saccharomyces cerevisiae that are blocked in import. One of these mutants, mas2, is defective for the import of a number of mitochondrial proteins and appears to act at an early step in the import pathway.

To determine the function of the MAS2 gene product, we have isolated the MAS2 gene and determined its sequence, raised antisera to a B-galactosidase-MAS2 fusion protein, and used this antisera to localize MAS2 in the yeast cell. In preliminary studies we have found the 44.7 kDa MAS2 protein in the cytoplasm. Ongoing investigations are aimed at determining the role of MAS2 in the import process.

(1) Does MAS2 bind to mitochondrial precusor proteins or to the outer mitochondrial membrane?
(2) Do antibodies to MAS2 inhibit mitochondrial import in vitro?
(3) Is cytosol isolated from mas2 mutants defective in promoting import in vitro?

E 310 Premature Addition of Heme Inhibits Translocation of In Vitro Synthesized Cytochrome c Peroxidase into Yeast Mitochondria

Jim Kaput, Sandra Kirchner, Denise Ekberg, and Teresa Prussak-Wieckowska

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Results from experiments designed to examine targeting and sorting sequences of the intermembrane space protein, cytochrome c peroxidase (CCP), unequivocally demonstrated that in vitro-synthesized wild type-and three signal sequence-mutants of preCCP were not translocated into isolated mitochondria. However, CCP proteins lacking amino acid residues +125 to +189 were imported into mitochondria as judged by proteolytic processing of the precursor, protease protection, mitochondrial association, and submitochondrial fractionation experiments. Since this region of the mature CCP contains amino acids involved in binding the heme prosthetic group, the results suggest that artifactual and premature addition of heme prevents translocation of wild type preCCP into the intermembrane space and prevents import of signal sequence mutants of preCCP into the matrix. The inhibition of CCP translocation across the outer and inner mitochondrial membranes may result from the inability of the protein to unfold after the precursor has bound to the mitochondria. Experiments examining the nature of the membrane components that interact with translocating proteins will also be discussed.

E 311 POST-TRANSLATIONAL MODIFICATIONS AND cDNA CLONING OF D- -HYDROXYBUTYRATE DEHYDROGENASE (BDH). Norbert Latruffe, Arlette Kante and Anne Bailly, Laboratoire de Biochimie (UA CNRS 531), Université de Franche Comté, 25030 Besançon France. Mitochondrial BDH, encoded in the nucleus and located in the inner membrane, exhibits a lecithin requirement for activity. The biogenesis of the rat liver enzyme has been undertaken by using "in vitro" translation of mRNA from polysomes and cDNA cloning techniques.

The "in vitro" translation, followed by an immunoprecipitation with an antibody directed against the mature BDH (31.5 Kd), shows that the enzyme is synthetized on free polysomes as a larger precursor of 34.5 Kd size. Freshly isolated mitochondria in an energetized state are able to convert preBDH into its mature size. A cytosolic factor is needed for import and processing of preBDH by mitochondria since the filtration of the labelled lysate through sephadex G25 abolishes the processing.

The molecular cloning of cDNA encoding for BDH has been initiated in order to deduce the aminoacid sequence of the protein, especially the presequence, and also to prepare a cDNA probe for the gene study (structure and expression). The selected methodology was the immunoscreening (using antiBDH) of a cDNA library from rat liver total mRNA inserted in the lac z gene through the Eco RI site of the phage gtII, an expression vector. The results are the followings:

I - E.Coli strain YI089 lysogenized with the recombinant showed no -galactosidase activity. 2 - The recombinant DNA contains an insert of 350 bp, which does not actually correspond to the full length cDNA. 3 - The size of the hybrid protein (-gal-BDH) is estimated by western blotting.

E 312 Secretion of H-2/Q10^b recombinant gene products by transformant L cells, James McCluskey, Richard Lopez, [S]Anna L. Ramsay, Lisa F. Boyd and David H. Margulies, Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20892 and [S]Department of Immunology and Medical Microbiology, University of Florida College of Medicine, Gainesville, FL 32607

Class I major histocompatibility antigens are integral membrane glycoproteins which comprise a 45Kd heavy chain non-covalently assembled to a 12Kd secreted light chain, β_2 microglobulin. Analysis of crystalline structure as well as the binding interactions of these molecules with T cell receptors depends upon the ability to generate large amounts of highly purified protein. For this purpose we have constructed recombinant class I genes in which 5' sequences of H-2D^d, L^d and K^b are linked to the 3'-half of a Qa subregion gene, Q10^b. The wild type Q10^b gene product is secreted because of a deletion within its transmembrane exon. Following DNA-mediated gene transfer into mouse L cells endogenous radiolabelling and immunoprecipitation from H-2D^d/Q10^b, L^d/Q10^b and K^b/Q10^b transformant cell supernatants was carried out. This confirmed the presence of predicted α1/α2 polymorphic H-2 determinants specific for each of the hybrid proteins. All hybrid molecules were secreted in associated with β2-microblobulin and the H-2D^d/Q10^b and H-2K^b/Q10^b proteins have been purified in milligram amounts from culture supernatants by affinity chromatography. Conditions have been established under which purified H-2D^d/Q10^b protein activates an H-2D^d-specific T cell hybridoma indicating preservation of structural determinants necessary for T cell recognition of the soluble molecules.

E 313 A CANDIDATE GLYCOPHOSPHOLIPID PRECURSOR OF TRYPANOSOMA BRUCEI VARIANT SURFACE GLYCOPROTEINS. A.K. Menon, M.A.J. Ferguson*, M. Duszenko**, S. Mayor and G.A.M. Cross, The Rockefeller University, New York, N.Y. 10021, * Dept. of Biochemistry, Oxford University, Oxford, U.K., **Physiologisch chemisches Institut der Universitat, Tubingen, FRG.

Trypanosoma brucei variant surface glycoproteins (VSGs) are synthesized with a hydrophobic carboxy terminal peptide that is cleaved and replaced by a glycophospholipid membrane anchor within 1 min. of completion of VSG synthesis. The rapidity of this C-terminal post translational modification suggests the existence of a pre-fabricated glycolipid that is transferred en bloc to the VSG polypeptide (Ferguson et. al. JBC 261 356 (1986), Bangs et. al. PNAS 82, 3207 (1985)). We report the isolation from [^{3}H]-myristic acid labelled trypanosomes of radiochemically pure polar material that has the expected characteristics of a VSG-linked glycolipid precursor. The glycolipid can be labelled with [^{3}H]myristic but not [^{3}H]palmitic acid and mild alkali releases 100% of the fatty acid label. Treatment with S. aureus phosphatidylinositol-specific phospholipase C or the endogenous trypanosome glycan-phosphatidylinositol-specific phospholipase C releases 100% of the label as dimyristoyl glycerol; concomitantly released non-lipophilic material inhibits antibody binding to the inter-VSG C-terminal immunologically cross-reacting determinant. Nitrous acid deamination generates dimyristoyl phosphatidylinositol and periodate oxidation releases phosphatidic acid. Studies on the detailed composition, structure and bonding of the glycolipid are in progress.

E 314 DISTINCT STEPS OF MITOCHONDRIAL PROTEIN IMPORT, Nikolaus Pfanner & Walter Neupert, Inst.f.Physiologische Chemie, D-8000 München 2, FRG. We have trapped distinct translocational intermediates during transport of the precursor to the ADP/ATP carrier from the cytosol into the mitochondrial inner membrane (1). The precursor interacts with a protease sensitive component on the mitochondrial surface (2). Then it is directed to protease protected and saturable sites in the outer membrane, where it can be extracted at alkaline pH. This translocation intermediate seems to be associated with the outer membrane by a so far unknown proteinaceous component of the import apparatus. Then the precursor enters the inner membrane. This step requires the membrane potential (3,4) and occurs at contact sites between outer and inner membranes. The completion of translocation and the assembly to the functional dimer can take place in the absence of $\Delta\psi$. A cytosolic activity which is present in reticulocyte lysate seems to be required for the specific binding of precursor to mitochondria. The import of ADP/ATP carrier and of F_1-ATPase subunit ß (5) into mitochondria depends on the presence of both $\Delta\psi$ and nucleoside triphosphates. (1) Pfanner, N. & Neupert, W., in preparation. (2) Zwizinski, C., Schleyer, M. & Neupert, W. (1984) J. Biol. Chem. 259, 7850-7856. (3) Schleyer, M., Schmidt, B. & Neupert, W. (1982) Eur. J. Biochem. 125, 109-116. (4) Pfanner, N. & Neupert, W. (1985) EMBO J. 4, 2819-2825. (5) Pfanner, N. & Neupert, W. (1986) FEBS Lett., in press.

E 315 AN INTERNALIZED AMINO-TERMINAL SIGNAL SEQUENCE RETAINS FULL ACTIVITY IN VIVO, Peter J.M.Rottier, Robert Z.Florkiewicz and John K.Rose, Yale University, New Haven CT 06510.

Internalization of the signal sequence of the vesicular stomatitis virus (VSV) glycoprotein (G) was accomplished by extending the N-terminal coding sequence with sequences derived from pBR322. Such constructs were then expressed in eukaryotic cells. It was found that amino-terminal extensions consisting of 20, 61, or 102 amino acids totally unrelated to any signal peptide affected neither the function nor cleavage of the signal sequence. Subsequent transport of G protein was also not affected. Although the internalized signals functioned with wild-type efficiency in vivo, in vitro membrane insertion, claevage and glycosylation in a wheat germ systen supplemented with dog pancreas microsomes could only be achieved when the amino-terminal presequences were short.

E 316 PROTEOLYSIS IN THE MEMBRANES AND PERIPLASM OF ESCHERICHIA COLI, Kathryn L. Strauch and Jon Beckwith, Harvard Medical School, Boston, MA 02115.

Mutants deficient in proteolysis in the periplasm and membranes of E. coli were isolated with a general technique based on the properties of fusions of alkaline phosphatase (AP) to a membrane protein. Alkaline phosphatase is normally a soluble periplasmic enzyme, but if the phoA gene is fused to the tsr gene (encoding an integral membrane protein) a hybrid protein is produced which has alkaline phosphatase enzymatic activity and is tightly bound to the membrane via an amino-terminal moiety derived from the Tsr membrane protein. The AP moiety of the Tsr-AP hybrid faces the periplasmic side of the inner membrane. Some of these membrane proteins are proteolytically unstable. Degradation of the hybrid protein leads to the accumulation of a smaller species containing the AP moiety. This breakdown product is soluble and located in the periplasm. In a strain with a leaky outer membrane (due to absence of murein lipoprotein) this soluble breakdown product can diffuse away from bacterial colonies leading to a colored halo in the presence of a histochemical stain for AP. Mutants deficient in proteolytic breakdown of the Tsr-AP hybrid were obtained be screening for absence of the AP halo around colonies. One of these mutants is completely defective in breakdown of the Tsr-AP hybrid and of two maltose binding protein-AP hybrids.

E 317 YEAST ACID PHOSPHATASE CAN ENTER IN THE SECRETORY PATHWAY WITHOUT ITS N-TERMINAL SIGNAL SEQUENCE. Rosine Haguenauer-Tsapis and Sandra Silve, Institut Jacques Monod, Université Paris VII - 75005 Paris - FRANCE.

The repressible *S. cerevisiae* acid phosphatase (APase) coded by the PH05 gene is a cell wall glycoprotein that follows the yeast secretory pathway. We have constructed by *in vitro* mutagenesis a deletion (SP) including the entire signal sequence and 4 amino-acids of the mature sequence of APase. mRNA obtained by *in vitro* transcription of PH05 or PH05/ SP genes were translated *in vitro* in the presence of dog pancreatic microsomes. PH05 gene product was efficiently translocated and core-glycosylated, whereas PH05/ SP was not translocated nor glycosylated. The signal peptide is therefore an absolute requirement for *in vitro* translocation in this heterologous system. An APase deficient yeast strain has been transformed with a high copy number plasmid carrying PH05/ SP gene. PH05/ SP gene product accumulated predominantly as an inactive, unglycosylated form located inside the cell. But 10-20 % of this gene product underwent core-glycosylation and outer chain glycosylation before reaching cell wall. Pulse chase experiments have shown that a large part of the unglycosylated precursor underwent proteolytic degradation, while 10-20 % of this precursor was core-glycosylated at least with a 10 to 20 fold decreased rate, as compared to wild type PH05 gene product. It appears therefore that *in vivo* for APase, the signal sequence is important for efficient and rapid translocation, but is not absolutely necessary for entry of the protein in the yeast secretory pathway.

E 318 BIOSYNTHESIS, GLYCOSYLATION, MOVEMENT THROUGH THE GOLGI SYSTEM AND TRANSPORT TO LYSOSOMES BY AN N-LINKED CARBOHYDRATE INDEPENDENT MECHANISM OF THREE LYSOSOMAL INTEGRAL MEMBRANE PROTEINS (LIMPS), Ignacio V. Sandoval, Javier G. Barriocanal, Juan S. Bonifacino and Lydia Yuan, National Institutes of Health, Bethesda, MD 20892.

The biosynthesis, glycosylation, movement through the Golgi system, transport to lysosomes and turnover of three lysosomal integral membrane proteins have been studied in NRK (normal rat kidney) cells using specific anti-LIMP monoclonal antibodies. Immunoelectron microscopy studies revealed the presence of LIMPs in secondary lysosomes, Golgi cisternae, coated and uncoated vesicles located in the trans-Golgi area. Pulse-chase experiments recorded LIMP precursors of 27 kDa (LIMP I), 72 kDa (LIMP II) and 86 kDa (LIMP III) and mature LIMPs of 35-50 kDa (LIMP I), 74 kDa (LIMP II) and 90-100 kDa (LIMP III). Time course studies on the acquisition of Endo H resistance by LIMPs indicated that all three LIMPs moved from the site of their synthesis in the endoplasmic reticulum to the medial-Golgi within 30 to 60 min after their synthesis. All three LIMPs were fully glycosylated before leaving the Golgi system, process during which LIMP I was retained in the trans-side of the organelle. LIMP I reached the lysosomes with a half-time of 2 hours and LIMPs II and III with half times of 1 hour after their synthesis, by a mechanism that was independent of N-linked carbohydrates. LIMPs free of N-linked carbohydrates displayed much shorter half-lives than fully glycosylated LIMPs, suggesting an important role of the sugars in protecting LIMPs against proteolytic degradation. Double immunofluorescence microscopy experiments showed that LIMP I, LIMP II and LIMP III are localized in the same lysosomes.