PLANT MEMBRANES: STRUCTURE, FUNCTION, BIOGENESIS

Christopher Leaver and Heven Sze, Organizers

February 8 - 13, 1987

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Mitochondria in Higher Plants: Structure and Function

G 001 PLANT MITOCHONDRIA : STRUCTURE AND FUNCTION, Roland Douce and Michel Neuburger,
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One of the major functions of mitochondria from all organisms is to provide ATP as the principal energy source for the cell. This is true also of plant mitochondria and it is therefore no surprise that many basic features of mitochondrial membranes have been conserved between animals and plants despite a billion years of divergent evolution. Thus the morphology of plant mitochondria closely resembles that of their animal counterparts, as do their cyt chain, ATPase complex, energy conservation (H^+ ejection) mechanisms, and membrane phospholipid composition*. However, it is now recognized that there are a number of distinct differences between plant and animal mitochondria. The unique features of plant mitochondria include the following : a) Cyanide-and antimycin A-insensitive respiration ; b) Respiratory-linked oxidation of external NAD(P)H and rotenone-insensitive oxidation of internal NADH ; c) Matrix-located NAD^+-linked malic enzyme ; d) Rapid oxaloacetate transport ; e) Rapid glycine oxidation by leaf mitochondria ; f) The size and complexity of their DNA. In addition, the rate of O_2 consumption on a protein basis is much higher than that in animal mitochondria, while fatty acid oxidation is not detectable in plant mitochondria. At one time these differences were not felt to be real but rather to be artifacts due to difficulties associated with isolating mitochondria from plant tissues. Fortunately this view is no longer widely held. Plant mitochondria isolated from a number of tissues are also capable of actively accumulating NAD^+ from the external medium. The slow rate of NAD^+ accumulation in the matrix space is concentration-dependent, exhibits Michaelis-Menten kinetics and is strongly inhibited by the analogue N-4-azido-2-nitrophenyl-4-aminobutyryl-NAD^+ (NAP_4 - NAD^+). When suspended in a medium that avoided rupture of the outer membrane, intact purified mitochondria progressively lost their NAD^+ content by passive diffusion. This led to a marked decline in the state-3 rate of NAD^+-linked substrates oxidation by isolated mitochondria. The rate of NAD^+ efflux from the matrix space is strongly temperature dependent and is inhibited by the analog inhibitor of NAD^+ transport indicating that a carrier is required for net flux in either direction. It is possible that this carrier has an important regulatory function in vivo by allowing manipulation of matrix NAD^+ concentration and thus regulating the activity of all NAD-linked enzymes. It is obvious that these results emphasize the very flexibility and complexity of plant mitochondria functioning*.

* R. Douce, Mitochondria in higher plants, structure, function biogenesis, 327 pages, Academic Press, Orlando, 1985.

Structure and Function of Energy-Transducing Membranes - I

G 002 THYLAKOID PROTEIN PHOSPHORYLATION, John Bennett, Biology Department, Brookhaven National Laboratory, Upton, NY 11973.
The chloroplast is a major site of protein phosphorylation in plants. Phosphoproteins have been detected in the outer envelope membrane, the soluble phase or stroma, the ribosomes and the photosynthetic membranes or thylakoids. In the case of thylakoids, phosphorylation is catalyzed by one or more redox-controlled protein kinases. Activation of the kinases depends on reduction of a component of the electron transport chain, possibly plastoquinone. Through the use of (1) synthetic oligopeptides resembling the phosphorylation sites of thylakoid proteins and (2) synthetic quinones that are analogs of plastoquinone, we have been studying the number, phosphorylation site and mechanisms of redox control of the kinase(s). Analysis of phosphorylation site specificity in the case of the light-harvesting Chl a/b protein (LHC II) is useful in deciding which of the 10-16 LHC II genes of higher plants are likely to code for phosphorylatable proteins. Evidence is also presented to show that at least one soluble phosphoprotein (12 kDa) binds to thylakoids and is phosphorylated by a redox-controlled kinase prior to release into the stroma. Thus, thylakoid protein phosphorylation may regulate the organizadion and function of both thylakoid proteins and soluble proteins.

G 003 PREDICTION OF BILAYER SPANNING DOMAINS OF HYDROPHOBIC MEMBRANE PROTEINS: APPLICATION TO THE CHLOROPLAST b CYTOCHROMES, W. A. Cramer and W. R. Widger, Dept. of Biological Sciences, Purdue University, West Lafayette, IN 47907.
The chloroplast cytochrome b_6-f complex is a member of the ubiquitous cytochrome b-c_1 family found in mitochondria and photosynthetic bacteria. There is a high degree of primary sequence homology between at least nine members of this family, so that it is of interest to use this case to test predictive methods for the folding of membrane polypeptides. Common criteria for amino acid hydrophobicity are (i) surface accessibility of amino acids in soluble proteins and (ii) free energy of transfer of amino acid side chains from water to a hydrophobic (vapor) phase. At least 7 residues are needed for an average of hydrophobicity at each position in order to avoid excessive noise, while not more than 13 to allow detection of the transition from the hydrophobic membrane-spanning domains to the polar aqueous regions. The unique value of the cytochrome family is that homology of the hydropathy plots can be documented by calculation of a cross-correlation coefficient (CCR) between pairs of functions derived from two relatives and between unrelated proteins. CCR = 0.5-0.9 between cytochrome relatives and is near zero between cyt b_6 and unrelated membrane proteins, showing that the hydropathy function is a highly conserved structural parameter. Five non-polar ~20 residue peptide regions, I-V, were predicted to span the bilayer. The choice of the bilayer-aqueous interface was dictated by the presence of charged amino acids at the termini of the non-polar sequences. The existence of turn regions at the polar boundaries was checked using algorithms that predicted the boundaries of the membrane-spanning helices of the L,M polypeptides of the *Rps. viridis* reaction center. The four conserved histidine residues that serve as heme ligands are distributed as two pairs on spanning peptides II and V, with one His from each pair on either side of the membrane, so that peptides II and V are cross-linked by two hemes oriented perpendicular to the membrane plane. The heme orientation, COOH-terminus on the stromal side, and location of a unique mucidin resistance site (for the yeast cytochrome) on the lumenal side are verified by experiment. The heme orientation is optimal for transmembrane electron transport. The greater heterogeneity in spectral and redox properties of the two hemes of the chromatophore and mitochondrial cyt b compared to those of chloroplasts may result from the one major difference in the sequence, insertion of an extra Thr-184 between the two His in helix V.

For cyt b-559, a heme-crosslinked heterodimer involving two short (MW = 9,162; 4,268) polypeptides, each spanning the bilayer, was inferred from an analysis of the gene sequences, heme ligands, polypeptide stoichiometry and NH_2-terminal sequences, and the presence in each polypeptide of a 25-26 residue hydrophobic domain. (Supported by NSF DMB84-03308.)

G 004 STRUCTURAL ORGANIZATION OF PSI AND PSII COMPLEXES OF CHLOROPLAST MEMBRANES

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The ability to isolate resolved chloroplast membrane electron transfer complexes that retain the native properties of these complexes in situ has greatly aided in studies of structural organization. As an example of this approach are recent studies of the chloroplast PSI complex although a similar approach has been used in the study of the PSII complex.

A native PSI complex has been isolated after treating spinach membranes with low concentrations of Triton X-100, followed by sucrose gradient centrifugation. This complex has a Chl a/b ratio of 5 to 6 and a Chl/P700 ratio of approximately 200. By fractionation of this complex, it has been possible to separate two chlorophyll-containing complexes: 1) a Chl b-containing complex (Chl a/b ratio = 4) having no photochemical activity, 2) a Chl a-containing reaction center core complex that has a Chl/P700 ratio = 100. The function of the Chl a/b complex has been shown to be as an antenna complex since the rate of $P700^{+}$ formation in light-limiting conditions is stimulated by the presence of this complex, presumably due to the ability of the Chl a/b complex to transfer absorbed energy to the PSI reaction center. Fractionation of PSI chlorophyll-protein complexes also demonstrates a separation of PSI polypeptides: the Chl a/b complex contains subunits of 23,22 and 20 kDa while the PSI reaction center core complex has subunits of 62,58,16,14,10 and 8 kDa. Each of these complexes contains approximately half of the total chlorophyll in PSI. It is also possible to demonstrate that all the Chl a in the reaction center core complex is associated with the 62 and 58 kDa subunits by preparation of CPI, a Chl-containing fraction that has a Chl/P700 ratio of approximately 100 and contains only the high molecular weight subunits. Recent results have indicated that in addition to containing P700 and the intermediate electron acceptor, A_0, CPI also contains the $Fe-S_X$ center and phylloquinone, an electron carrier that has been identified as the electron acceptor A_1 in the PSI complex.

The topography of the PSI complex in membranes has been analyzed using these Chl-protein complexes and membrane-impermeant probes. The procedure involved modification of membranes with either 14-C-TNBS or pronase, isolation of the native PSI complex, fractionation into the sub-complexes, and analysis by SDS-PAGE in conjunction with either autoradiography or immunoblotting. This procedure has allowed an organizational model to be developed which indicates the transmembrane organization of several of the PSI subunits. (Supported in part by a grant from the National Science Foundation.)

G 005 **ORGANIZATION OF THE THYLAKOID MEMBRANE.** **Kenneth R. Miller Brown University, Providence, Rhode Island 02912.** The photosynthetic membranes found in both eukaryotes and prokaryotes provide useful experimental systems for the exploration of basic questions of membrane structure and function. For some questions, the most useful systems have been prokaryotic. The semi-crystalline organization of the ***Rhodopseudomonas viridis*** photosynthetic membrane has made it especially useful for studies of membrane protein organization. We have prepared two- and three-dimensional density maps from Fourier techniques of the intact photosynthetic membrane and also of sheet-like crystals prepared from purified ***Rh. viridis*** reaction center. These studies, along with the detailed atomic level map prepared from X-ray diffraction data (1), have provided the most complete structural picture of any photosynthetic membrane to date. We have used the high-resolution STEM (scanning transmission electron microscope) at Brookhaven National Lab to complement these studies with measurements of membrane mass. These measurements, prepared from the analysis of electron scattering from freeze-dried samples, yield a mass per unit area for the membrane of 35.37 d / A^2, or approximately 480,000 d per crystallographic unit cell in the membrane. This value places certain constraints on the arrangement of lipid and light-harvesting protein in the membrane, and these constraints will be explored. Studies on the thylakoid membranes of higher plants are less advanced, due in part to a greater complexity of membrane organization. Work in our laboratory has centered on the localization of specific membrane components, including protein and lipid. Antibodies against the light-harvesting chlorophyll a/b protein complex (LHC-II), the β subunit of the ATP synthetase, and a photosystem II core preparation have been used to localize these components on thin sections and freeze-etch replicas of isolated photosynthetic membranes, and the implications of these localizations will be discussed. Attempts to explore the role of galactolipids in membrane organization have included experiments in which a specific lipase was used to cleave galactosylglycerol from the membrane, leaving free fatty acids behind (2). Polyacrylamide gel electrophoresis of the lipase-treated membrane showed that only minor protein alteration was associated with the treatment. However, major structural alterations occured in the membrane, including the loss of nearly all of the EFs fracture face particles (generally associated with photosystem II) and the apparent dissociation of tetrameric particles on the inner surface of the thylakoid. Besides emphasizing the obvious point that both protein and lipid are important in photosynthetic membrane organization, these studies indicate that the intergration of several experimental approaches may be necessary to solve some of the problems of membrane organization.

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(This work was supported by grant GM28799 from the National Institutes of Health.

Structure and Function of Energy-Transducing Membranes - I

G 006 MOLECULAR GENETIC ANALYSIS OF THE PLASMA-MEMBRANE H^+-ATPase OF NEUROSPORA CRASSA,
Karl M. Hager, Suzanne M. Mandala, James W. Davenport, Lawrence Aaronson, and Carolyn W. Slayman, Departments of Human Genetics and Physiology, Yale School of Mecicine, New Haven, CT 06510.
The plasma membrane of the fungus Neurospora crassa contains an electrogenic H^+-translocating ATPase, which has long been known to be related both structurally and functionally to the [Na^+,K^+]-, [Ca^{2+}]-, and [H^+,K^+]-ATPases of animal cells and the plasma-membrane [H^+]-ATPase of higher plants. It has a single 100kD subunit that reacts with ATP to form a covalent β-aspartyl phosphate intermediate, and it is sensitive to inhibition by vanadate. Using polyclonal antibody against the purified 100kD polypeptide, we have cloned the ATPase gene by immunoscreeing of a λgt11 expression library. Identity of the cloned gene was verified by Northern blotting, hybrid select translation, and the sequencing of several tryptic peptides. The encoded protein contains 920 amino acids (99,886 Da) and is predicted by hydropathy analysis to possess at least eight transmembrane segments. It shows significant amino acid sequence homology with the [Na^+,K^+]- and [Ca^{2+}]-ATPases, particularly in regions involved in ATP binding and hydrolysis.

G 007 BIOSYNTHESIS AND FUNCTION OF PLANT MEMBRANE LIPIDS,
J. Brian Mudd, Steve Thomas, and Kathryn F. Kleppinger Sparace, ARCO Plant Cell Research Institute, 6560 Trinity Court, Dublin, CA 94568.
In higher plants, fatty acid synthesis is localized entirely within the plastids. The initial products of fatty acid synthetase, acyl-acyl carrier proteins (acyl-ACPs) have two principal uses: a) acylation of glycerol-3-phosphate (G3P) in the plastid, or b) export to the cytoplasm by combination of the acyl-ACP thioesterase of the inner membrane of the envelope and the acyl CoA synthetase of the outer membrane of the envelope.

Acylation of G3P in the plastid gives rise to a product with oleate (18:1) at the sn-1 position of glycerol and palmitate (16:0) at the sn-2 position. This fatty acid distribution is characteristic of glycerolipids synthesized in the plastid.

The fatty acyl moieties exported to the cytoplasm are used in the acylation of G3P in the ER and mitochondria. The end products are characteristic of these organelles: phosphatidylcholine (PC) phosphatidylethanolamine (PE) and phosphatidylinositol (PI). The fatty acid distribution in these phospholipids shows a preference for 18 carbon acids at the sn-2 position. Sixteen carbon acids are found mainly at the sn-1 position.

The diacylglycerol (DG) moiety of the lipids synthesized in the ER can be introduced into the chloroplast glycerolipids. For example a characteristic lipid of the plastid, monogalactosyldiacylglycerol (MGDG) shows fatty acid distribution at the sn-1 and sn-2 position characteristic of both the plastid biosynthetic pathway and the cytoplasmic biosynthetic pathway. Phosphatidylglycerol (PG) of the plastid is unusual in that it is synthesized entirely in the plastid.

Although we understand quite well the lipid biosynthesis in the plastids, mitochondria and ER and we understand interrelationships between these organelles, the syntheses and/or interactions in plasma membrane and tonoplast are much less well understood.

Functions of the lipids in the various membranes is not well documented. A correlation between chilling sensitivity and the fatty acid composition of PG has been pointed out. But the generality and the causality of this relationship has been questioned.

Biogenesis of Membranes

G 008 BIOGENESIS OF THYLAKOID MEMBRANE PROTEINS,
John C. Gray, Paul P.J. Dunn, Christopher J. Eccles, Sean M. Hird, Anna-Stina Höglund, David I. Last, Barbara J. Newman and David Willey, Botany School, University of Cambridge, Cambridge CB2 3EA, U.K.

The thylakoid membrane of higher plant chloroplasts is composed essentially of four supramolecular complexes, photosystems I and II, cytochrome b-f complex and ATP synthase, involved in the light reactions of photosynthesis. Our aim is to understand how the components of these complexes are synthesised and assembled into the membrane complexes to produce a functional photosynthetic membrane. We have located and characterised genes in chloroplast DNA from pea and wheat for seven components of photosystem II, three components of the cytochrome b-f complex and six components of ATP synthase. In several instances, genes for identified components of these complexes are co-transcribed with open reading frames coding for putative integral membrane proteins. The products of these open reading frames are being characterised by immunochemical methods using antibodies raised against the products of gene-fusions expressed in Escherichia coli. Of the identified intrinsic membrane proteins, only cytochrome f is synthesised with a cleavable signal sequence; other integral proteins synthesised on thylakoid-bound ribosomes do not have cleavable signal sequences.

The synthesis of nuclear-encoded components of the thylakoid membrane and its associated electron transfer system is also being studied. cDNA clones for most of the identified nuclear-encoded components have been isolated from libraries in λgt11 and M13 K8.2. Sequence analysis of cDNA clones for plastocyanin and ferredoxin-$NADP^+$ reductase indicates these proteins are synthesised with long N-terminal extensions. The transit peptide for pea plastocyanin differs considerably from that for Silene plastocyanin. Sequence comparison of these transit peptides suggests residues associated with proteolytic processing.

G 009 STRUCTURAL AND FUNCTIONAL PROPERTIES OF MITOCHONDRIAL TARGETING SEQUENCES,
Eduard C. Hurt, Dan Allison, Alison Baker, David Roise and Adolphus P.G.M. van Loon, Biocenter of the University, Klingelbergstrasse 70, CH-4056 Basel, Switzerland.

Most imported mitochondrial proteins are synthesized in the cytoplasm, generally as larger precursors with amino-terminal presequences (1). Employing gene fusion and a physico-chemical analysis of presequence peptides we tested the following questions: (i) Are presequences sufficient to direct attached proteins into mitochondria and their correct intra-mitochondrial compartments? (ii) What are the functional and structural properties of presequence peptides? (iii) Can one build a functionally active artificial presequence based on general structural characteristics of mitochondrial presequences? (iv) How could mitochondrial presequences have evolved? Amino-terminal presequences of imported mitochondrial proteins are sufficient to direct non-mitocondrial "passenger" proteins to mitochondria and into specific intra-mitochondrial compartments. Each mitochondrial "transport" sequence analyzed so far is composed of a "matrix-targeting" domain, and if the translocation of the attached protein across the mitochondrial inner or outer membrane must be prevented, a "stop-transport" domain adjacent to the "matrix-targeting" domain. This suggests that intramitochondrial sorting is determined by the combination of various topogenic signals at the amino-terminus of the imported polypeptide itself. How might presequences function mechanistically during the import process? A peptide representing the presequence of subunit IV of yeast cytochrome c oxidase was chemically synthesized and tested for its interaction with membranes. This peptide, although quite soluble in aqueous solution, spontaneously inserts into lipid membranes. The amphiphilic property of the presequence peptide is consistent with a model where the presequence of subunit IV forms an amphiphilic alpha-helix. Amphiphilic helicity appears to be a fairly general feature of mitochondrial presequences (2). Artificial presequences composed of only three types of amino acid, arginine, leucine and serine (these residues frequently occur in natural mitochondrial presequences and might significantly contribute to the overall amphiphilicity) were sufficient to direct an attached protein into mitochondria, both in vitro and in vivo. What is the evolutionary origin of presequences? Potential mitochondrial targeting sequences also are found in non-mitochondrial proteins, but in an inactive form most likely because they are buried within the folded protein. These cryptic mitochondrial targeting sequences can be activated if placed in front of passenger proteins. Cryptic mitochondrial targeting sequences also occur in the E. coli genome. This suggests that even prokaryotes contain pools of mitochondrial targeting sequences. Such sequences may have been utilized to transport proteins into primeval mitochondria during evolution of eukaryotic cells.
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G 010 TARGETING OF PROTEINS INTO CHLOROPLASTS, Kenneth Keegstra*, Cynthia Bauerle*, Thomas Lubben*, Thomas Moore*, Sjef Smeekens+ and Peter Weisbeek+, University of Wisconsin, Madison, WI 53706* and University of Utrecht, Utrecht, The Netherlands+.

Most chloroplast proteins are encoded in the nucleus and synthesized in the cytoplasm. These proteins contain targeting information that results in their transport into the chloroplast and their segregation to the proper chloroplast compartment. At least part of the targeting information is contained in an amino terminal extension known as a transit peptide, that is removed by proteolysis after the protein acquires its proper location. We have studied the targeting process in vitro using purified intact chloroplasts and radiolabelled precursor proteins. Precursor proteins are prepared by in vitro transcription/translation of cloned precursor genes. Wild type precursor genes have been modified using recombinant DNA techniques allowing the synthesis of altered precursor proteins. Several different chimeric precursor proteins are imported efficiently into chloroplasts demonstrating that a transit peptide is sufficient to direct the import of a protein into chloroplasts. Studies with plastocyanin, an imported protein located in the thylakoid lumen, have lead to the conclusion that targeting to the lumen occurs in two steps. The first is transfer across the envelope membranes and the second is transfer across the thylakoid membrane. The plastocyanin transit peptide has separate domains that direct each step. Current studies are aimed at determining whether this transit peptide can direct other proteins into the lumen.

Dynamic Aspects of Metabolite Transport

G 011 MEMBRANE TRANSPORT SYSTEMS IN CHLOROPLASTS AND MITOCHONDRIA, U. Ingo Flügge, Institut of Plant Biochemistry, University of Göttingen, 3400 Göttingen, F. R. G.

Green plants have two different organelles for the generation of energy, mitochondria and chloroplasts. Both are capable of catalyzing ATP synthesis driven by electron transport. In both organelles the inner membrane is the actual barrier between the organelle and the cytosol and thus the site of specific transport proteins which coordinate the metabolism in both compartments. The inner mitochondrial membrane also contains the enzymes for oxidative phosphorylation whereas in chloroplasts, electron transport is located on a separated membrane system, the thylakoids. The inner envelope of chloroplasts is primarilary involved in metabolite transport as well as in some biosynthetic processes. During photosynthesis, the fixed carbon is exported in form of triose-phosphate from the chloroplasts by the phosphate translocator. In the cytosol, triosephosphate serves as the precursor for sucrose biosynthesis whereby inorganic phosphate is released and shuttled back into the chloroplasts in exchange for more triosephosphate. Inside the chloroplasts, phosphate is used for the formation of ATP catalyzed by the thylakoid ATP synthase. In addition to the triosephosphate/phosphate exchange, triosephosphate can also be exported in exchange with 3-phosphoglyerate. This shuttle provides the cytosol with the products of the light reaction, ATP and reducing equivalents. The cytosol can also be supplied with ATP synthesized during mitochondrial oxidative phosphorylation. This requires the uptake of phosphate as well as of ADP and the export of ATP catalyzed by the mitochondrial phosphate translocator and the ADP/ATP antiporter, respectively. It is still an open question to what extent mitochondria contribute to the cytosolic energy supply during illumination. The oxygenation of the CO_2 acceptor ribulose 1,5 bisphosphate results in the formation of phosphoglycolate. The conversion of phosphoglycolate to 3-phosphoglycerte requires not only several enzyme-catalyzed steps in both the mitochondrial and chloroplast compartments as well as in the peroxisome, but also different passages of intermediates across these organelle membranes, which are expected to be catalyzed by specific translocators. In addition, NH_3 which is released during mitochondrial glycine oxidation has to be refixed by the GOGAT system inside the chloroplasts. This requires the uptake of 2-oxoglutarate into the chloroplasts and the export of glutamate. Both transports are mediated by specific translocators. In addition to these dicarboxylate translocators, chloroplasts contain a specific and highly active oxaloacetate transport mechanism which is also present in mitochondria. Uptake of oxaloacetate coupled to the release of its reduced form, malate, lead to a withdrawl of reducing equivalents from the organelles which can subsequently be used elsewhere e.g. for the reduction of hydroxypyruvate in the peroxisomes. Membranes of chloroplasts and mitochondria contain not only different metabolite translocators but also systems catalyzing protein import since the bulk of the organelle polypeptides is nuclear coded and synthesized on cytoplasmic ribosomes.

G 012 PROTON-COUPLED TRANSPORT SYSTEMS AT THE TONOPLAST, Ronald J. Poole, Centre for Plant Molecular Biology, Biology Department, McGill University, 1205 Doctor Penfield Avenue, Montreal, Quebec, Canada H3A 1B1.

Tonoplast transport plays a role in many physiological functions, including storage of nutrients, osmoregulation, adaptation to salinity, and regulation of cytoplasmic calcium. In many cases, large-scale reversal of transport may be required, as in retrieval of stored nutrients, osmoregulation in stomata, and malate transport in Crassulaceans. In other cases, response to environmental conditions may be called for, as in adaptation to salinity. The mechanism and regulation of tonoplast transport are becoming more accessible, and I will review our present knowledge of the relationship of solute transport to the pH and electrical gradients created by the H^+ pumps, and our fragmentary understanding of transport regulation.

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G 013 MECHANISTIC INFERENCES FROM CHARGE-FLOW MEASUREMENTS ON A PROTON PUMP. Clifford Slayman, Department of Physiology, Yale School of Medicine, New Haven, CT 06510.

A number of conspicuous physiological properties of the plasma membrane proton pump in Neurospora have been described (1-5): a wide span of voltage dependence, extending from membrane potentials near -50 mV to beyond -300 mV; a conductance which, under steady-state conditions, is everywhere positive; kinetic, rather than thermodynamic, dependence on metabolic energy; high sensitivity to intracellular pH, but low sensitivity to extracellular pH; a measured stoichiometry of 1 proton pumped, per ATP molecule split; and a reversal potential which, during chronic energy restriction, shifts from ca. -400 mV to -200 mV.

Except for the last, these macroscopic properties are readily described by simple 2-, 3-, and 4-state reaction-kinetic models incorporating a single voltage-dependent step in which the transported ion must traverse an Eyring-type energy barrier. When constrained to fit the data, these models predict the following kinetic features: a) overall rate-limitation by the step involving energization, i.e., phosphorylation of the protein; b) very rapid charge ejection once ~P and H^+ are bound; c) energy conversion from ~P during the charge ejection; d) preferred steady-state dissociation of the proton binding sites; e) exclusion of ions other than protons from net transport during the proton cycle. Several possible explanations for the metabolism-dependent change in reversal potential are being explored: altered positioning of the membrane energy barrier; modification of parallel (non-pump) membrane processes; and an actual change in stoichiometry of the pump, from 1 H^+/cycle to 2 H^+/cycle.

Examination of partial reaction pathways for the proton pump, by both pulse-relaxation studies and in-vitro chemistry, will be necessary to relate these predicted molecular properties to emerging sequence and structure data.

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Supported by Research Grant GM-15858 from the National Institute of General Medical Sciences.

H^+-ATPASE OF THE VACUOLAR MEMBRANE.

G 014 Heven Sze, Stephen K. Randall, Klaus K. Kaestner and Shoupeng Lai, Botany Department, University of Maryland, College Park, MD 20742, U.S.A.

An electrogenic H^+-pumping ATPase acidifies the vacuolar lumen and provides the proton motive force for solute transport. The purified tonoplast ATPase from oat roots is a multimeric enzyme of 400-500 kDa with at least three different subunits of masses 72, 60 and 16 kDa. The structure and function of the various subunits are being investigated using chemical modification by inhibitors and polyclonal antibodies. Nbd-Cl (7-chloro-4-nitrobenzo-2-oxa-1,3-diazole) inhibition of ATPase activity was active-site directed and prevented by MgATP. $[^{14}C]$Nbd-Cl binding to the 72 kDa polypeptide of the purified ATPase was reduced by MgATP or an analog, TNP-ATP. Dithiothreitol reversal of both Nbd-Cl inhibition and $[^{14}C]$Nbd-Cl binding to the 72 kDa subunit suggest the inhibitor modified cysteinyl -SH or tyrosyl -OH groups. Polyclonal antibodies directed against the 72 kDa subunit inhibited ATPase and H^+ pumping activities. The results suggest the 72 kDa contains an ATP- (or nucleotide-) binding site that may constitute the catalytic domain. Inactivation of the ATPase activity by N,N'-dicyclohexylcarbodiimide (DCCD, a site-specific inhibitor) was correlated directly to $[^{14}C]$DCCD binding to the 16 kDa subunit. The 16 kDa could be extracted with chloroform/methanol indicating it is a proteolipid. Using antibodies to the holoenzyme as probes, we found the proteolipid was tightly associated with the membrane while the 72 and 60 kDa subunits could be removed by salt washes. Our current model of the tonoplast ATPase includes integral 16 kDa proteolipids that may form the H^+ channel and catalytic (72 kDa) and putative regulatory (60 kDa) subunits that are peripheral. Together these subunits couple ATP hydrolysis on the cytoplasmic side to H^+ translocation into the vacuole. The complete subunit composition and their stoichiometry, and the molecular structure and function have yet to be determined. (Supported by National Science Foundation PCM-04130.)

Reference: Randall, S.K. & H. Sze (1986) J. Biol. Chem. 261, 1364

Synthesis and Intracellular Transport of Macromolecules

ASPARAGINE-LINKED OLIGOSACCHARIDES OF PLANT GLYCOPROTEINS ARE MODIFIED IN THE GOLGI COMPLEX, BUT DO NOT PLAY A ROLE IN PROTEIN TARGETING,

G 015 Maarten J. Chrispeels, Kenneth D. Johnson, Loic Faye and Arnd Sturm, Department of Biology, University of California, San Diego, La Jolla, CA 92093.

Plant glycoproteins contain both high-mannose and complex oligosaccharide sidechains, linked to specific Asn residues. The synthesis of all such sidechains starts in the rough ER with the en bloc transfer of $Glc_3Man_9(GlcNAc)_2$ groups from dolichylpyrophosphate. Subsequent modifications occur in the ER (removal of Glc) and in the Golgi complex (removal of Man and addition of Xyl, Fuc, GlcNAc and Gal). These modifications, carried out by Golgi-localized enzymes, must occur in a specific sequence. For example, the removal of 4 Man residues is a prerequisite for the addition of a single GlcNAc which in turn is a prerequisite for the subsequent addition of Fuc and Xyl. The protein body proteins phytohemagglutinin and phaseolin acquire terminal GlcNAc residues in the Golgi complex, and these residues are removed again in the protein bodies. Such intricate modifications of oligosaccharide sidechains are reminiscent of the modifications leading to the formation of Man-6-phosphate groups on lysosomal hydrolases. These groups are known to be required for the proper targeting of the lysosomal hydrolases. Much circumstantial evidence leads to the conclusion that the N-linked glycans of plant glycoproteins do not have such an informational role. This evidence can be summarized as follows: although many vacuolar or protein body as well as secreted proteins are glycoproteins, a number of them do not have N-linked glycans. Examples are the vacuolar/protein body proteins legumin, glycinin, pea lectin, and wheat germ agglutinin, as well as the secreted proteins barley aleurone α-amylase (type A or low pI) and PAPI (probable amylase/protease inhibitor). Thus, N-linked oligosaccharides do not seem to be required to direct plant proteins to either vacuoles/protein bodies or the extracellular space. To confirm that this is so, we are introducing a gene for phytohemagglutinin, which has been mutated to remove one or both glycosylation sites, into tobacco and are studying the targeting of the resulting gene products. Secondly, inhibition of glycosylation by tunicamycin does not inhibit the secretion or the transport to vacuoles of proteins which normally have N-linked glycans (e.g. phytohemagglutinin and rice scutellum α-amylase). This work is supported by grants from the National Science Foundation, the United States Department of Agriculture, and the Department of Energy.

G 016 PLANT PROTEIN SECRETION IN YEAST AND E. COLI AND TARGETING OF PLANT PROTEINS INTO CHLOROPLASTS. A. Gatenby[1,3], S. Rothstein[1], C. Lazarus[1], M. Boccara[1], D. C. Baulcombe[1], D. Willey[2], J. Gray[2], T. Lubben[3], P. Ahlquist[3], K. Keegstra[3]. [1]Plant Breeding Institute, U.K.; [2]University of Cambridge, U.K.; [3]University of Wisconsin, U.S.A.
We are interested in the structural features of plant proteins that enable them to be translocated through foreign membranes, either from yeast, bacteria or chloroplasts. Several examples will be considered. The wheat alpha-amylase signal sequence could be recognized in yeast and the enzyme secreted into the medium where it degraded starch(1). An interesting feature of this recognition was that the plant signal sequence was located in an internal position in a phosphoglycerate kinase (PGK)/alpha-amylase fusion protein. Pulse chase experiments indicated that a very slow processing occurred. If, however, the alpha-amylase initiator methionine was fused to that of PGK, such that the signal sequence was at the N-terminus, then processing became far more efficient. We also observed that the alpha-amylase signal sequence is recognized in E. coli (2). Although recognition appears to be less efficient in the bacterium, an active enzyme is secreted into the periplasmic space in a form that indicates specific recognition and clearage of the signal sequence. Cytochrome f is a chloroplast encoded protein that is inserted into the thylakoid membrane. The gene possesses prokarytoic features that allows it to be readily expressed in E. coli. Cytochrome f also appears to contain a signal sequence and preliminary experiments indicated that both a full-length and a cleaved mature protein were present when the gene was expressed in E. coli (3). By fusing various parts of the cytochrome f gene with lacZ, and conducting cell fractionation experiments, it can be demonstrated that the pea signal sequence directs insertion of the cytochrome f fusion protein into the bacterial inner-membrane. This insertion is mediated by the bacterial secA gene product and indicates that the chloroplast signal sequence is being recognized in a very specific manner by the E. coli secretory pathway. A similar pathway may therefore exist within the chloroplast to direct protein to the thylakoid membrane. The ability to target foreign proteins into chloroplasts has also been examined in both an in vitro and in vivo approach. We have constructed gene fusions between the transit peptide of Rubisco small subunit and the genes for the ATPase beta subunit, cyanobacterial Rubisco large subunit, and Brome Mosaic Virus (BMV) coat protein. There is a considerable range in the efficiency of import. Derivatives of BMV have also been constructed to enable studies on the in vivo targeting of proteins into chloroplasts in isolated protoplasts.

(1) Nature 308, 662-665; (2) Gene, "in press"; (3) P.N.A.S. 82, 7955-7959.

G 017 INTRACELLULAR TRANSPORT AND SECRETION OF BARLEY ALEURONE α-AMYLASE, Russell L. Jones, Department of Botany, University of California, Berkely CA 94720.
A family of α-amylase isoenzymes are secrèted from the aleurone layer of barley and we have studied the intracellular route of their transport and secretion. The general pathway of intracellular transport of these enzymes from rough endoplasmic reticulum to Golgi apparatus and to the cell exterior via secretory vesicles is now generally accepted. Because the aleurone cell is a constitutively secreting tissue that does not accumulate secreted proteins and where the pool of Golgi-localized enzymes is particularly small, details concerning the possible modification of these proteins, by glycosylation for example, are lacking.
Evidence from several laboratories suggests that barley α-amylase isoenzmyes are either not glycosylated, or at the most contain only a few sugar residues. Modification of secreted α-amylase is indicated however by the presence of precursor forms of α-amylase having isoelectric points which are different from secreted forms. Both barley aleurone layers and protoplasts contain two enzymatically active α-amylase isoenzymes with low isoelectric points that by pulse-chase labelling and immunochemical evidence are precursors of secreted forms. Furthermore, the sodium ionophore monensin blocks secretion of these isoenzymes and causes the accumulation of precursor forms within the cell. Electron microscopy shows that the Golgi cisterne of monensin-treated aleurone layers and protoplasts become highly distended, while cell fractionation data indicate that the accumulation of α-amylase occurs within the Golgi apparatus. Our attempts to label these precursor isoenzymes with radiolabelled sugar residues have not been successful however. Large pools of sugars within aleurone cells may make radiolabelling of putative glycoproteins difficult. Secreted α-amylase isoenzymes are also resistent to the deglycosylating enzyme endo H. Further progress on this problem will be reported.

G 018 SECRETION BY PLANT CELLS: INTERMEDIATE PLASMA MEMBRANE CONFIGURATIONS VISUALIZED IN ULTRARAPIDLY FROZEN CELLS, L. Andrew Staehelin and Stuart Craig*, MCD Biology, University of Colorado, Boulder, CO 80309-0347; *permanent address: CSIRO, Plant Industry, Canberra, ACT 2601, Australia.

We have employed freeze-fracture electron microscopy in conjunction with propane-jet and high pressure freezing techniques to investigate vesicle-mediated secretion and membrane recycling events in plant tissue culture cells (carrot, sycamore maple) and in intact root tips (pea, corn). Stabilization of the cells by means of ultrarapid freezing has enabled us to visualize new intermediate membrane configurations related to these events. Many of these structures appear to be critically dependent on turgor pressure forces acting on the plasma membrane and thus can only be reliably demonstrated in specimens preserved in their turgid state. Examination of our micrographs has revealed three basic types of plasma membrane configurations related to secretion/membrane recycling events: flattened vesicles with slit-shaped membrane fusion sites, horseshoe-shaped membrane infoldings, and small round membrane infoldings. Individual cells exhibited great variations in the distribution and in the absolute numbers of such membrane profiles as well as in the ratios of the different types of configurations.

Our micrographs suggest that vesicle-mediated secretion in plant cells involves the following steps. Fusion of a secretory vesicle with the plasma membrane leads to the formation of a single, narrow-necked pore that increases in diameter up to about 60 nm. During discharge, the vesicle is flattened, forming a disc-shaped structure perpendicular to the plane of the plasma membrane. As the vesicle is flattened the pore is converted to a slit, the maximum length of which coincides with the diameter of the flattened vesicle. The flattened vesicle then tips over and concomitantly the plasma membrane slit becomes curved into a horseshoe-shaped configuration as it extends along the outer margins of the tipped-over vesicle.

Some coated pits are present interspersed between the above-mentioned structures, but their numbers appear insufficient to account for an exclusively endocytic mechanism of membrane recycling. Instead, our micrographs are more consistent with a mixed mode of recycling of membrane components to the cortical ER and to Golgi cisternae that involves both internalization of membrane by endocytosis and of individual lipid molecules by unknown mechanisms (lipid exchange proteins?). We are currently examining the effects of different perturbants (temperature, low Ca^{2+}, ATP synthesis inhibitors and ionophores) on the different types of membrane profiles. Supported by NIH grant GM 18639.

Membrane Receptors and Transmembrane Signalling

G 019 REGULATION OF CYTOPLASMIC CALCIUM LEVELS AND A BISTABLE CALCIUM DEPENDENT PLASMA MEMBRANE PROTEIN KINASE, D. Blowers, M. Collinge, S. Gilroy, H. Harvey and A.J. Trewavas, Department of Botany, University of Edinburgh, King's Buildings, Mayfield Road, Edinburgh EH9 3JH, Scotland.

We have been examining the salient characteristics of a regulatory system involving calcium ions. A plasma membrane bound calcium/calmodulin dependent protein kinase has been detected in pea shoot. This enzyme seems to consist of two sub-units, 28K and 18K, of which the 18K is the catalytic sub-unit. This catalytic sub-unit autophosphorylates on serine residues. Autophosphorylation above a certain level inhibits the capability for phosphorylating other proteins. This enzyme has the characteristics of a bistable metabolic switch; in one mode it will phosphorylate other proteins after calcium activation, in the other it will only phosphorylate itself. Although the enzyme can be activated by calmodulin our current data suggests that calmodulin binds to the enzyme substrates. Binding of the protein kinase may thus be transient.

Cytoplasmic calcium can be measured by electroporation of Quin 2 into protoplasts. Some of the kinetics of cytoplasmic calcium regulation will be described as will recent attempts to identify calcium channel proteins.

G 020 CALCIUM TRANSIENTS IN PLANT CELLS, Peter K. Hepler and Dale A. Callaham,
Department of Botany, University of Massachusetts, Amherst, MA 01003

Developing plant cells responding to a stimulus may elicit a transient rise in the intracellular free calcium ion concentration ([Ca]) as part of the signal transduction mechanism required to activate essential reactions. If the presumed changes in [Ca] occur, it becomes necessary to detect their timing, magnitude, kinetics and ultimately their location within the target cell. Measurement of intracellular Ca, however, is difficult; firstly, the free [Ca] is very low (0.1μM-1.0μM) while other related ions, for example Mg, are much higher in concentration; secondly, the detection of small changes of Ca-dependent absorbance (arsenazo III), fluorescence (fura-2), or light emission (alquorin) require highly sensitive and specialized instrumentation, and thirdly, the reporting molecules themselves compete for available Ca and thus may modulate the developmental event under investigation.

For studies of Ca transients in single cells we constructed a microspectrophotometer system designed to measure Ca-dependent changes in absorbance of the metallochromic indicator dye arsenazo III (AA-III). Light from a highly regulated tungsten-halogen lamp is passed through a rotating four port filter wheel (1800 rpm) and directed via a light fiber into the condenser of a Zeiss 1M-35 microscope. A cell loaded with dye is sequentially irradiated with light pulses and the transmittance measured by a photomultiplier tube. Using dividing stamen hair cells of _Tradescantia_ we find that the intracellular free [Ca] increases during early anaphase, reaches a peak by late anaphase and declines thereafter. The time at which Ca returns to the basal level correlates closely with the first visible evidence of the arising cell plate during telophase. The timing of the Ca transient suggests that it might participate in regulating anaphase, possibly in stimulating the depolymerization of microtubules which occurs when chromosomes move to the poles. Here and in other systems the elucidation of Ca transients may greatly enhance our understanding of developmental processes.

G 021 COATED VESICLES IN PLANTS: CHARACTERIZATION AND FUNCTION(S), D.G. Robinson,
H. Depta, S. Hillmer, Abteilung Cytologie, Pflanzenphysiologisches Institut, Universitaet Goettingen, D-3400 Goettingen, FRG.

Endocytosis is a well-characterized event in animal systems (1) by which extracellular macromolecules are either taken up nonspecifically (pinocytosis) or selectively (receptor-mediated endocytosis). In the latter case receptor-ligand complexes are internalized at specific sites in the plasma membrane known as coated pits. Coated vesicles, diameter 50-100 nm, form from coated pits by budding. Such vesicles must discard their coat before fusing with an endomembrane compartment (2). Coated vesicles have been isolated from animal tissues on numerous occasions and their structure is well-known (3). Plants also possess coated vesicles (4) and these have now been isolated (5). There remains however, a great problem as to whether they are also involved in endocytosis since none of the substances known to be internalized via coated pits in animal cells are not physiologically relevant in plants. Moreover it has been claimed that the turgescent state of plant cells would prohibit the budding of vesicles at the plasma membrane. Using cell-foreign ligands (cationic ferritin, colloidal gold-lectin conjugates and certain viruses) we have been able to demonstrate a coated vesicle-mediated endocytosis in protoplasts from leaves and suspension-cultured cells. Depending on the type of experiment the internalized ligand can be detected in small vacuoles, multivesicular bodies, the so-called "partially coated reticulum" as well as the peripheral regions of dictyosome cisternae (6). Using lead solutions as a marker we have also been able to follow endocytosis in corn root cap cells.

The coat proteins of plant and animal coated vesicles are not identical and there are differences in terms of their dissociation and reassembly. In addition plant coated vesicle fractions possess a very active glucan synthase II activity indicating an origin at the plasma membrane for at least some of the coated vesicles.

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(3) Harrison, S.C. and T. Kirchhausen (1983) Cell 33, 650-652
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(5) Depta, H. and D.G. Robinson (1986) Protoplasma 130, 162-170
(6) Hillmer, S. et al. (1986) Eur. J. Cell Biol. 41, 142-149

G 022 BLUE LIGHT-ACTIVATED ELECTROGENIC PUMP IN THE GUARD CELL PLASMALEMMA, Eduardo Zeiger, Dept. of Biological Sciences, Stanford University, Stanford, CA 94305.
Stomatal guard cells respond to light with quantitative increases in their solute content ensuing from active ion uptake and endogenous synthesis of organic solutes. This light response is mediated by two photoreceptor systems, one in guard cell chloroplasts and a second photoreceptor specific for blue light (BL). In intact leaves irradiated with fluences of red light (RL) saturating photosynthesis, pulses of BL elicited a transient stomatal response (1). Suspensions of guard cell protoplasts treated with the same protocol extruded protons into their medium in response to BL pulses (2). Proton extrusion was detectable within 25-30 sec from the onset of pulse stimulation and continued for about 10 min; maximal rates, ranging between 2 to 6 pmol H^+ cm^{-2} s^{-1}, were observed within 2 min. The response was fluence-dependent with half-saturation at about 180 μmol m^{-2} of BL. Proton extrusion was insensitive to vanadate but was inhibited by 10 μM CCCP or 50 μM DES. In whole-cell, patch clamping experiments with guard cell protoplasts (3) BL pulses given under high fluences of RL elicited transient, outward electrical currents of up to 0.88 μA cm^{-2}. The close agreement between the lag time of this response and that of proton extrusion by suspensions of guard cell protoplasts, and between the magnitude of the outward current and the proton fluxes indicate that BL activates a proton pump at the guard cell plasmalemma. These findings substantiate a chemiosmotic mechanism in guard cells driving ion uptake and stomatal opening (1-3). Significant rates of proton pumping observed long after pulse application and an unusually high, apparent quantum yield of the response argue against the generation of cellular energy in response to BL and point to a regulatory role of BL in pump activation. Kinetic data indicate that BL photoconverts an inactive component of the sensory transduction chain into an active one with a half time of 9 sec (at a fluence rate of 250 μmol m^{-2} s^{-1}) and that this active component reverts into the inactive one slowly, with a half time of about 9 min (1). These studies show that guard cells are a valuable model system for investigations of the indentity and localization of the BL photoreceptor and the elucidation of sensory transduction of the BL response.

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(3) Assmann, S.M., Simoncini, L., Schroeder, J.I. Nature 318, 285, 1985.

Mutations Modifying Membrane Structure and Function

G 023 THE MOLECULAR BASIS OF CYTOPLASMIC MALE STERILITY, C.J. Leaver, P.G. Isaac, J. Bailey-Serres, I.D. Small, A.D. Liddell and M.J. Hawkesford, Department of Botany, University of Edinburgh, Edinburgh EH9 3JH, Scotland.
The cytoplasmic male sterile phenotype (CMS) is recognised by the failure of the mature plant to produce functional pollen and apparently results from either incompatability between the nuclear genome of one race or species and the mitochondrial genome of another, or a specific mutation in the mitochondrial genome which is not compensated for by specific nuclear genes which restore fertility.

In maize, sorghum and a number of other species, CMS is associated with reorganisation of the mitochondrial genome, as evidenced by restriction endonuclease fragment polymorphism, and synthesis of characteristic variant mitochondrial polypeptides. Evidence from a number of laboratories suggests that the mitochondrial genotypes characteristic of each form of CMS probably arose by aberrant intra-and/or inter-molecular recombination events leading to the generation of chimaeric mtDNA sequences. Such rearrangements can generate new or extended open reading frames, which are expressed as variant polypeptides. The variant polypeptide(s) may contain completely novel amino acid sequences and/or blocks of amino acid sequence homologous to normal polypeptide(s) encoded by the DNA sequences from which they were derived. These domains may endow the variant polypeptide with some of the structural and/or enzymic properties of the progenitor protein(s), which may target it to the inner mitochondrial membrane where it could compete with related native polypeptides and become associated with specific enzyme complexes, e.g. of the electron transport chain. One can speculate that the presence of a variant polypeptide is tolerated during normal vegetative growth and that the native enzyme complexes are capable of meeting the cellular energy demand. However during microsporogenesis, a phase associated with increased rates of mitochondrial biogenesis, either the impaired mitochondrial enzymes are unable to sustain cellular energy demands or the presence of the variant polypeptide interferes with biogenesis of the specific complex. This would be manifested phenotypically as a failure to produce functional pollen.

G 024 ENDOCYTOSIS IN YEAST, Howard Riezman, Yolande Chvatchko, Vjeko Dulic, Maryse Moya and Evert van Tuinen, Swiss Institute for Experimental Cancer Research, CH-1066 Epalinges, Switzerland. Recent results from our laboratory show that yeast a cells take up the pheromone alpha factor by endocytosis and deliver it to the vacuole for degradation. This degradation is defective in the pep4 mutant which lacks active vacuolar proteases. Using this mutant we can show that pheromone degradation is not necessary for pheromone response but this degradation may be important for subsequent recovery from pheromone action. We have isolated several mutants defective in endocytosis and some of these are also somewhat defective in pheromone response. Some of these mutants are defective in maintaining cAMP levels in the cells. One of these is probably adenylate cyclase (CDC35 or CYR1) and another does not complement CDC25 or CYR2. These results suggest that the cAMP dependent protein kinase cascade is important for endocytosis and possibly also for pheromone response. We have also cloned and are sequencing the END1 and END2 genes which correspond to mutations giving an endocytosis-negative phenotype to yeast cells.

G 025 GENETIC MODIFICATION OF MEMBRANE LIPID COMPOSITION IN ARABIDOPSIS, C.R. Somerville, John Browse, Ljerka Kunst, Suzane Hugly, and Anders Lonneborg, MSU-DOE Plant Research Laboratory, Michigan State University, E. Lansing, MI 48824. We have isolated a series of mutants of Arabidopsis with alterations in the acyl composition of membrane lipids. The mutants, which have no obvious phenotype, were isolated by analyzing the fatty acid compositon of leaf samples from thousands of plants in a mutagenized population. They have provided insights into the regulation of membrane lipid composition, and have been used to evaluate the functional significance of trans-C16:1 and the high trienoic acid content of chloroplast membranes. The availability of a mutant which converts Arabidopsis from a "16:3 type" to an "18:3 type" may also be useful in determining why these two groups of plants coexist. Most of the mutants have altered chloroplast ultrastructure, suggesting that lipid composition has a direct effect on the mechanisms which determine the spatial arrangement of chloroplast lamellae.

Membrane Structure and Biogenesis

G 100 CHARACTERIZATION OF THE TRANSPORT PATHWAY OF PLASTOCYANIN IN CHLOROPLASTS IN VITRO,
Cynthia Bauerle(1), Sjef Smeekens(2), Peter Weisbeek(2), and Kenneth Keegstra(1),
1) University of Wisconsin, Madison, 53703, and 2) Dept. of Molecular Cell Biology,
State University of Utrecht, the Netherlands.
Chloroplasts are complex organelles composed of three membrane systems which define three aqueous compartments. Most chloroplast proteins are nuclear-encoded, cytoplasmically synthesized, and post-translationally imported into the organelle. Targeting is involved in the localization of proteins to their proper chloroplast compartment. Plastocyanin (PC) is an imported protein localized in the thylakoid lumen. Thus, PC must traverse threemembranes en route to its proper location. Import of Silene PC into isolated pea chloroplasts has been demonstrated (Smeekens, et el., 1986). A stromally-located processing intermediate is observed in timecourse experiments of PC import. A model of PC import includes two steps: 1) import to the stroma, with partial proteolytic processing, and 2) transportto the thylakoid lumen with final processing to mature PC. One approach to understanding this final step in the pathway is to reconstitue in vitro the transport of PC into isolated thylakoids. Results of an ongoing project to characterize the translocation of PC across thylakoid membranes will be presented.

G 101 COMPOSITION AND FUNCTION OF PHOTOSYSTEM I IN Lemna. Barry D. Bruce and Richard Malkin, Division of Molecular Plant Biology, University of California, Berkeley, CA 94720
A PSI complex has been isolated from Lemna gibba using a sequential solubilization procedure. With this procedure it is possible to initially isolate the cytochrome b-f complex from thylakoid membranes. The cytochrome-depleted membranes are then used to isolate a PSI complex and LHCPII using a second detergent solubilization. The PSI complex has a chlorophyll/P700 ratio = 180 and a chlorophyll a/b ratio of 4.6-5.8. SDS-PAGE indicates that in addition to two high molecular weight subunits, there are several polypeptides with the apparent molecular weights between 22 and 27 kD, one at approximately 20 kD, four between 14 and 17 kD and at least five polypeptides with apparent molecular weights less than 10 kD. These distinct polypeptides are unaffected by the use of several proteolytic inhibitors during the preparation. The use of 32 cm slab gel electrophresis and silver staining has verified these additional PSI polypeptides. In vivo labeling using ^{14}C-glucose has allowed uniform labeling of intact plants. The radiolabeled plants have been used to isolate in vivo labeled PSI, allowing the subnit stoichiometry to be determined. In addition to the wild-type organism, we have investigated PSI in a photosynthetic mutant previously shown to lack the cytochrome b-f complex. The PSI complex from the mutant is completely lacking the 19-20 kD polypeptide as determined by Western blot analysis and silver staining. However, the mutant shows PSI activity and normal amounts of iron-sulfur centers A and B. (Supported in part by grants from NSF and the McKnight Foundation.)

G 102 CHARACTERIZATION OF PIGMENT-PROTEINS FROM A NOVEL PROCARYOTE CONTAINING CHLOROPHYLL A AND B. George S. Bullerjahn, Hans C.P. Matthijs[1], L.R. Mur[1] and Louis A. Sherman, University of Missouri, Columbia, MO 65211 USA; [1]University of Amsterdam, The Netherlands.
We have analyzed the chlorophyll-protein complexes of a chlorophyll a and b - containing photosynthetic procaryote. This organism lacks bilin pigments, yet is morphologically similar to cyanobacteria (T. Burger-Wiersma, et al. Nature 320:262). We have resolved 5 chlorophyll-protein complexes on non-denaturing gels (termed CPI-CPV), and we have determined the polypeptide composition of each green band. Band CPI likely contains the PSI reaction center, as the protein composition of CPI is that of a purified PSI preparation. Furthermore, CPI quenches long-wavelength UV light. Bands CPII-CPV are UV-fluorescent and therefore likely are the pigment complexes of PSII and the antennae. Immunological studies indicate that the PSII core polypeptides, the homologs of polypeptides 5 and 6 of Chlamydomonas, comprise band CPIV. CPV contains a 34 kDa chlorophyll-protein which crossreacts to antibodies raised against the cyanobacterial 34 kDa PSII chlorophyll-protein (Pakrasi, et al. PNAS 82:6903; Bullerjahn, et al. BBA 810:148). We have not been able to detect strong crossreactivity of any pigment-protein complex to antibody against maize LHC-II. We are currently examining the 77°K fluorescence excitation spectra to determine the complex(es) which bind chlorophyll b. This study will help determine the phylogenetic relationships between this organism and other phototrophs.

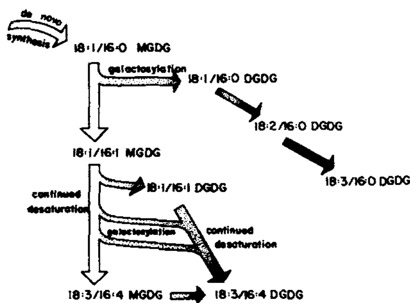
Plant Membranes: Structure, Function, Biogenesis

G 103 PLASMA MEMBRANE AND TONOPLAST VESICLES RESOLVED BY FREE-FLOW ELECTROPHORESIS INTO SUBPOPULATIONS OF DIFFERENT SIDEDNESS. Hervé Canut, Guy Auderset, Andrew Brightman, Claude Penel and D. James Morré, Purdue University, West Lafayette, Indiana 47907 and the University of Geneva, Geneva, Switzerland.

Highly purified fractions of tonoplast and plasma membrane vesicles were obtained from the same homogenates of etiolated hypocotyls of soybean (Glycine max L.) by the technique of preparative free-flow electrophoresis. When different plasma membrane markers for soybean membranes were expressed as total activities, two peaks were seen clearly in the electrophoretic separations. When similar separations were carried out with membranes from soybean cells grown in suspension culture, the vesicles formed from plasma membrane were asymmetric and exhibited a thick outer leaflet and a less thick inner leaflet making possible assignment of absolute orientations to the vesicles obtained. Additionally, the external surface of soybean plasma membranes reacted more strongly with the lectin concanavalin A than did the cytoplasmic surface. Based on these criteria, membranes of fraction "E" (the least electronegative fraction of plasma membrane) were cytoplasmic side out while the cytoplasmic side in vesicles were found in a more electronegative fraction "C." When a similar analysis was done for tonoplast markers, at least two fractions again were observed. By combining sucrose and glycerol gradient centrifugation in conjunction with preparative free-flow electrophoresis, it was possible to obtain a total of four different fractions from the same homogenate. Two of the fractions were isolates of both inside out and right-side out plasma membrane and two of the fractions were, presumably, inside out and right-side out vesicles derived from the tonoplast.

G 104 METABOLIC INTERRELATIONSHIPS BETWEEN GALACTOLIPIDS OF CHLOROPLASTS, Sung Ho Cho and Guy A. Thompson, Jr., University of Texas, Austin, TX 78713.

Although plant galactosyldiacylglycerols are the most abundant polar lipids in our biosphere and the major lipid components of chloroplasts, many questions regarding their biosynthesis and functions remain unanswered. We have explored the metabolism of monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) in the eukaryotic green alga, Dunaliella salina. By pulse-labeling the cells with radioactive fatty acids and analyzing the radioactivity of individual molecular species of the predominantly "prokaryotic" types of MGDG and DGDG, the precursor-product relationships were readily discernible. The figure shows that the first product, 18:1/16:0 MGDG, is further metabolized by two distinct pathways, one leading to DGDGs having both a saturated and an unsaturated fatty acid, and another more heavily utilized pathway leading to highly unsaturated molecular species of MGDG and DGDG. Fatty acids are desaturated while bound to either MGDG or DGDG.



G 105 REGULATION OF CHLOROPLAST-ENCODED CHLOROPHYLL-BINDING PROTEIN TRANSLATION, Robert R. Klein, Hugh S. Mason, Patricia E. Gamble, John E. Mullet, Texas A&M University, College Station, Texas 77843.

Characterization of protein synthesis in etioplasts from 5-day-old dark-grown barley has revealed that the chlorophyll-binding proteins of photosystem I and photosystem II and a 32 KDa membrane polypeptide were not synthesized in dark-grown tissue. (Klein and Mullet, J. Biol. Chem., 1986). Synthesis of the chlorophyll-binding proteins and a 32 KDa membrane protein was significant in plants illuminated for only 15 minutes and was near maximum by 1 hr. Induction of photosystem I chlorophyll-binding proteins was not accompanied by an increase in mRNA for these proteins demonstrating that the synthesis of plastid-encoded chlorophyll-binding proteins is blocked at the translational level. The time course of light-induced chlorophyll-binding protein synthesis agrees closely with the time course of photoreduction of protochlorophyllide to chlorophyll a. Examination of plastid protein synthesis in chlorophyll-deficient barley mutants has shown that the chlorophyll-binding proteins of photosystem I and photosystem II and a 32 KDa membrane polypeptide were not synthesized in either light-grown or dark-grown mutant seedlings. Transcript levels for psbA (encodes for a 32 KDa membrane polypeptide) and psaA-psaB (encodes for photosystem I chlorophyll-binding proteins) in chlorophyll-deficient barley mutants were similar to levels in wild-type plants. We hypothesize, therefore, that the synthesis of plastid-encoded chlorophyll-binding proteins and a 32 KDa membrane polypeptide is regulated by chlorophyll biosynthesis and regulation is at the level of translation.

To determine whether chlorophyll-binding protein synthesis is regulated at the level of translation initiation, elongation or termination, polysomes of plastids from dark-grown and illuminated seedlings were compared and the distribution of psaA-psaB mRNA examined. These results showed psaA-psaB mRNA associated with the polysome fraction of dark-grown plastids indicating that control is at the level of translation elongation and/or termination. Based on these results, a model of light-regulated chlorophyll-binding protein synthesis will be presented.

G 106 A SINGLE GENE MUTATION AFFECTS THE PARTITIONING OF FATTY ACIDS BETWEEN THE TWO PATHWAYS OF LIPID BIOSYNTHESIS, L. Kunst, J. Browse and C.R. Somerville. MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824.

Glycerolipid synthesis in plants is thought to involve two discrete pathways operating simultaneously in two different cell compartments. According to this hypothesis, fatty acids synthesized de novo in the chloroplast may enter the prokaryotic pathway in the chloroplast envelope, or be exported to the endoplasmic reticulum as CoA esters to enter the eukaryotic pathway. In "16:3 plants" such as the small crucifer Arabidopsis thaliana (L.) Heynh. chloroplast membrane lipids are derived in approximately equal amounts from the two biosynthetic routes. We have recently isolated a mutant of Arabidopsis which specifically lacks hexadecatrienoic acid (16:3) due to a mutation at a single nuclear locus. Since this acyl group occurs only on the sn-2 position of MGD in chloroplast membranes, the mutant locus probably encodes an enzyme of the prokaryotic pathway. The lesion does not cause the accumulation of precursors within chloroplasts, but redirects fatty acids towards cytoplasmic sites of lipid synthesis. Therefore the mutant described here is effectively an "18:3 plant", with only the eukaryotic pathway functional in the synthesis of MGD. The plant has no visible alteration in phenotype, but its growth characteristics, chlorophyll concentration and chloroplast ultrastructure are subtly affected by the mutation. Detailed analysis of these differences may provide some insight into how and why "18:3 plants" have evolved, as well as point to the selective disadvantages of "18:3 plants" that allowed for the coexistence of "16:3 plants".

G 107 THE CLONING AND CHARACTERISATION OF A NUCLEAR GENE IN ZEA MAYS INVOLVED IN THYLAKOID MEMBRANE ULTRASTRUCTURE AND PROTEIN ASSEMBLY. Robert Martienssen, Alice Barkan and William C. Taylor, Dept. of Genetics, U.C. Berkeley CA94720.

A number of nuclear photosynthetic lesions have been identified in Zea Mays by virtue of their high chlorophyll fluorescence under u.v. illumination. Many of these are seedling lethal, and have been shown to specifically lack one or other thylakoid membrane protein complex. One such mutant, hcf-Mu106, isolated from a Robertson's mutator background, has been described that is deficient in components of three thylakoid complexes : photosystem I, photosystem II, and the cytochrome f/b6 complex (Barkan et al, EMBO J. 5, 1421 (1986)).

This mutant has a very unusual thylakoid membrane ultrastructure viewed in cross-section, consisting of concentric circular membranes, that may be associated with failure to assemble the three protein complexes. An analysis of the lipid composition of these membranes is presented, as well as further characterisation of thylakoid proteins in the mutant.

Segregation of specific DNA restriction fragments hybridising to the transposable element Mutator is used to identify the nuclear gene responsible for this phenotype. A number of genetic approaches to confirming the identity of cloned DNA fragments are discussed and illustrated.

G 108 CHARACTERIZATION OF PLANT PLASMA MEMBRANE ANTIGENS, David J. Meyer, Claudio L. Afonso, Kristi R. Harkins and David W. Galbraith, University Nebraska-Lincoln, Lincoln NE 68588-0118.

We have developed a monoclonal antibody library directed against crude cellular membrane preparations of heterotrophic Nicotiana tabacum L. cv Xanthi cells grown in suspension culture cells and have used these antibodies to characterize the distribution, localization and expression of antigens recognized by these antibodies. Indirect immunofluorescence analysis of fixed cells and protoplasts indicates that the library includes members directed against antigens located in the plasma membrane, the cell wall or both. Two-dimensional flow cytometric analysis has indicated that these antigens are differentially expressed during plant development. Western blotting has revealed that the different antibodies recognize different epitopes; some of the epitopes are common to many proteins whilst others are restricted to single proteins. One monoclonal antibody has been shown by Western blotting of two-dimensional gels to recognize a cell wall/plasma membrane protein found in a Triton X114-insoluble fraction, having a high pI and a heterogeneous molecular weight on SDS-PAGE (Mr°80-140 kD). This protein is present in heterotrophic suspension cultures but is absent from membranes of mature leaves. We are in the process of purifying these antigens as well as screening a phage expression library in order to analyze the molecular controls that regulate antigen expression in vivo.

G 109 IDENTIFICATION OF PHOSPHORYLATION SITES OF PS II CORE POLYPEPTIDES IN SPINACH, Hanspeter Michel and John Bennett, Biology Department, Brookhaven National Laboratory, Upton, NY 11973.

At least 12 polypeptides of chloroplast thylakoids are phosphorylated. Reversible phosphorylation of the LHC II apoproteins is involved in energy distribution between the two photosystems. Almost nothing is known about the functional aspects of phosphorylation of the other polypeptides. A number of observations have led to the conclusion that several kinases which recognize different substrates, are present in chloroplasts. Identification of phosphorylation sites of different polypeptides would give important information about substrate specificity and about the possible number of kinases. In addition to the LHC II apoproteins four other polypeptides of the photosystem II complex are phosphorylated. After trypsin digestion of photosystem II core complex we isolated the phosphopeptides and compared their amino acid composition with known sequences. In the case of CPa-2 and the herbicide binding protein (D1) the phosphorylated peptide was identified by comparison with the sequence derived from the nucleotide sequence. Another phosphopeptide showed an amino acid composition and sequence which is identical to one found in the 8.3 kD polypeptide. This peptide has some homology to the known sequence of the LHC II phosphopeptide of pea. Less homology to the LHC II peptide is found in the two other phosphopeptides.

G 110 IMPORT OF A CHLOROPLAST INNER MEMBRANE PROTEIN PRODUCED FROM A cDNA CLONE, Thomas S. Moore and Kenneth Keegstra, University of Wisconsin, Madison, WI 53706.

We have begun molecular cloning of nuclear genes encoding chloroplast inner and outer membrane proteins to better understand how intracellular targeting and import is accomplished for these two chloroplast compartments.

Polyclonal antibodies raised against a mixture of pea chloroplast envelope proteins were used to screen a λgt11 cDNA expression library, made with pea poly A^+ mRNA. The cDNA inserts of the 16 positive clones purified ranged from 0.3 to over 3.0 kb. The corresponding gene product of some of the clones was tentatively identified by a novel technique. Each clone, once pure, was used to affinity purify from the polyclonal antibody preparation those antibodies specific to it, thus generating a monospecific antibody preparation. Each monospecific antibody preparation was then used to probe Western blots of chloroplast inner membrane, outer membrane and stromal protein. In most cases, the monospecific antibodies recognized a single protein of either the inner or outer membrane, which was then taken to be the gene product of the clone in question. The cDNA insert of one such clone, tentatively identified as encoding an 86 kDa protein of the chloroplast inner membrane, was subcloned into a plasmid to allow in vitro transcription and subsequent translation. A protein of 96 kDa was produced. Upon incubation with chloroplasts, the protein bound to the chloroplasts and was imported and processed to 86 kDa. Work is in progress to begin sequencing this gene and to further characterize the import and localization of the protein it encodes.

G 111 DEVELOPMENT OF THE PLASTID ENVELOPE MEMBRANE: ITS BIOCHEMICAL AND PROTEIN TRANSPORT CAPACITY, Jürgen Soll, Botanisches Institut der Universität München, F.R.G.

The envelope membrane is a feature common to all members of the plastid family. It is conservatively retained as a two membrane barrier, consisting of inner and outer envelope, at all levels of plastid development, e.g. proplastids, etioplasts, chromoplasts and chloroplasts. Studies on chloroplasts envelopes have revealed its essential function in the biochemistry of the organell. No data are available so far on the role of the plastid envelope in chloroplast development. We have therefore developed a method to purify etioplasts from dark grown pea plants and to isolate envelope membranes of these plastids.

The results demonstrate that the envelope membranes from etioplasts contain already the enzymes responsible for galactolipid and prenylquinone biosynthesis as well as a proteinkinase and a galactolipid-galactolipid-galactosyltransferase, thus indicating that we have both the inner and outer envelope membrane present. The analysis of protein, lipid and pigment pattern shows significant differences to envelopes from mature chloroplasts. We have studied the capacity of the etioplast, and thus the envelope membrane, to transport precursor proteins in vitro. The etioplast are able to recognize, transport, process and assemble proteins at this developmental level. This process was analyzed in detail, and data will be presented.

G112 DCCD BINDING TO THE CYTOCHROME b_6f COMPLEX, Sallie G. Sprague and Diana S. Beattie, West Virginia University, Morgantown, WV 26506

DCCD, the potent inhibitor of proton-translocating ATPases and the respiratory chain cytochrome bc_1 complex, has been reported to inhibit proton and electron flow in chloroplasts (Sane, et al., 1979, FEBS Lett. 108, 136-140). Under appropriate conditions it is possible to inhibit proton movement without inhibiting electron flow in cytochrome bc_1 complexes from various organisms, and in chloroplasts (unpublished). DCCD binds covalently to glutamic or aspartic acid residues that are presumed to be involved in proton translocation. Comparison of published sequences of cytochrome b showed two highly conserved acidic residues in otherwise hydrophobic regions of the polypeptides (Widger, et al., 1984, Proc. Nat. Acad. Sci., USA, 81, 674-678). Positions 160/162 and 229 have been implicated as possible DCCD binding sites (yeast numbering). Position 160/162 in the cytochrome b sequence is in the carboxyl 20% of the chloroplast cytochrome b_6, while position 229 is only 10 residues from the amino terminus of the 17kD subunit IV of the chloroplast cytochrome b_6f complex. Our preliminary data indicate that $[^{14}C]$-DCCD binds to the 17kD subunit IV in a crude preparation of cytochrome b_6f, thus implicating the asparate at position 229 in mitochondrial cytochrome b. We are currently optimizing conditions to increase binding of DCCD.

G113 GLYCOPROTEIN PROCESSING ENZYMES IN PLANTS. T. Szumilo, G.P. Kaushal and A.D. Elbein, University of Texas Health Science Center, San Antonio, Texas 78284.

In plants, some of the oligosaccharide structures of the N-linked glycoproteins have been elucidated, and some of the biosynthetic reactions have been shown. However, relatively little is known about the processing of glycoproteins in these tissues. We have been studying these reactions in mung bean seedlings and in suspension cultured soybean and sycamore cells. We have purified the processing glucosidase I about 200-fold from mung beans and shown that this enzyme removes only the terminal α1,2-linked glucose from $Glc_3Man_9GlcNAc$. This enzyme is very sensitive to inhibition by castanospermine and deoxynojirimycin and less sensitive to 6-epicastanospermine and 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine. During purification of glucosidase I on DE-52, glucosidase II was separated. This enzyme was purified several hundred fold and shown to remove the two α1,3-glucoses from Glc_2Man_9-GlcNAc. Mung bean extracts also contain mannosidase I, first reported by Forsee. We purified this enzyme 115-fold from microsomes and found it to be a specific α1,2-mannosidase that converts $Man_9GlcNAc$ to $Man_5GlcNAc$. In contrast to animal Mannosidase I, the plant enzyme is not inhibited by deoxymannojirimycin. A GlcNAc transferase I, partially purified from mung beans then adds a GlcNAc to the α1,3-mannose and then mannosidase II removes the α1,3- and α1,6-mannoses to give a $GlcNAc$-$Man_3GlcNAc$. Mannosidase II has also been partially purified and its properties studies. This enzyme is very sensitive to inhibition by swainsonine but not deoxymannojirimycin. A second GlcNAc transferase (II) then adds a GlcNAc, also in α1,2-linkage, to the α1,6-mannose to give a $GlcNAc_2$-$Man_3GlcNAc$ (Supported by NIH AM-21800 and the Robert A. Welch Foundation).

G114 THE TOPOGRAPHY OF THE REACTION CENTER POLYPEPTIDES OF PS II Achim Trebst, Dept. of Biology, Univ. Bochum, D-4630 Bochum 1, FRG

Among others I have proposed a description of the binding niche for the primary acceptors of PS II, Q_A and Q_B, based on the homology of amino acid sequences of the plant polypeptides to the bacterial reaction center polypeptides and on the amino acid changes in herbicide tolerant plants. We can report another fifth such amino acid change in a new mutation. The model predicts that the D-1 and D-2 polypeptides of PS II are carrying also the reaction center of PS II. This has just been directly supported by the isolation of a reaction center complex by Satoh. I wish to report on the results with site directed antisera against the D-2 polypeptide, compared with antisera against the D-1 and the 47 kDa polypeptide, in trypsination experiments with photosystem II. The results are relevant for the architecture of photosystem II and the functional role of the individual polypeptides in PS II reactions.

G 115 SITE-SPECIFIC MUTATIONS IN THE D2 POLYPEPTIDE OF PHOTOSYSTEM II IN THE CYANOBACTERIUM *SYNECHOCYSTIS* 6803, John G. K. Williams and Dexter A. Chisholm, E. I. Du Pont de Nemours & Co., E402/2229, Wilmington, DE 19898.

We are exploring structural and functional features of the Photosystem II reaction center complex by constructing site-specific mutations in the D2 polypeptide component. The D2 polypeptide, when paired with the D1 polypeptide (the 32-kDa herbicide-binding protein), is believed to bind members of the photosynthetic electron transport chain, including the reaction center chlorophyll, the quinone Q_A, and the non-heme ferrous iron. This idea is based on the three-dimensional structure of the bacterial photosynthetic reaction center, which has some functional characteristics and amino acid sequences in common with Photosystem II (H. Michel et. al.(1986) EMBO J. 5, 1149-1158). To explore the validity of this idea, and to learn about the roles of specific amino acids in the Photosystem II protein complex, we have developed a genetic transformation system in the cyanobacterium *Synechocystis* 6803 which makes possible the construction of site-directed mutations in Photosystem II genes. The D2 polypeptide is encoded by the psbD gene. *Synechocystis* 6803 has two copies of psbD, called psbDI and psbDII. Both copies have been deleted from the cyanobacterial chromosome by gene replacement techniques. The psbD double-deletion strain has no Photosystem II function and it requires glucose for growth. Photosynthesis is restored by transformation with the wild-type psbDI gene. To make mutant Photosystem II centers, the double-deletion mutant is transformed with psbDI genes containing site-specific mutations. Several mutations are being constructed and characterized: a) a mutation that introduces an acidic amino acid residue into the putative binding pocket for Q_A, b) a mutation that "straightens" a kink in an important trans-membrane alpha helix by replacing a proline residue with alanine, and c) mutations that replace a histidine residue at the carboxy terminus of the D2 protein, to look for involvement of the D2 polypeptide in water oxidation.

Transport Systems

G 200 ON THE EXISTENCE OF A PROTON PUMPING ATPase IN TONOPLAST
B. ARRIO, A. DUPAIX, M. HILL and P. VOLFIN

In order to account for the accumulation of metabolites in plant vacuoles, the existence of a proton-pumping ATPase has been widely suggested in the litterature. The demonstration of such a tonoplast-bound ATPase was merely based on the characterization of a nitrate-sensitive microsomal fraction. In some examples, this ATPase activity has been evidenced on vacuole preparations obtained under conditions which were criticized by Boller. The application of the reverse phase high-performance liquid chromatography method (RP-HPLC) to the simultaneous separation of adenine nucleotides, in the presence of tonoplast vesicles isolated from Catharanthus roseus, showed results not necessarily correlated with the ATPase hypothesis. but with an adenylatekinase like activity. Immunological data are also in agreement with this observation. Moreover, in light of the H^+ -quenching of quinacrine fluorescence observed during ATP hydrolysis by vacuoles or tonoplast vesicles, the existence of a proton-pumping ATPase may be questioned.

Poster session : 10

G 201 IMPORT OF IN VITRO SYNTHESIZED ACETOLACTATE SYNTHASE INTO ISOLATED PEA CHLOROPLASTS. Newell F. Bascomb, Steven Gutteridge, Julie K. Smith and Kenneth J. Leto. Central Research and Development Department, Experimental Station, E.I. du Pont de Nemours & Co., Wilmington, DE 19898

Acetolactate synthase (ALS), the target site of the sulfonylurea herbicides, is the first enzyme common to the synthesis of the branched chain amino acids. Several lines of evidence indicate that plant ALS is encoded by the nucleus, synthesized on cytoplasmic ribosomes and is ultimately localized in the chloroplast stroma. Analysis of available ALS gene sequences (Barbara Mazur, personal communication) suggests that plant ALS possesses an N-terminal extension atypical of chloroplast transit sequences examined thus far. To study the uptake of ALS by intact chloroplasts, the full length Arabidopsis ALS gene was cloned behind the SP6 polymerase promotor in an RNA expression vector. Translation of the resulting RNA in rabbit reticulocyte lysates results in the production of a major 76kDa (on gels) ALS precursor protein which is post-translationally imported by isolated pea chloroplasts and processed to a mature 70kDa polypeptide found in the chloroplast stroma. ALS is the largest post-translationally transported chloroplast protein examined to date and despite its low abundance exhibits rates of in vitro import which are at least as high as those reported for Rubisco small subunit. Studies on the rate and efficiency of import and steps involved in the import process will be presented.

G 202 TABTOXININE-ß-LACTAM TRANSPORT INTO CULTURED CORN CELLS: UPTAKE VIA AN AMINO ACID-PROTON SYMPORT. DANIEL R. BUSH, R.D. DURBIN, & PAT J. LANGSTON-UNKEFER. LOS ALAMOS NATIONAL LABORATORY AND UNIVERSITY OF WISCONSIN, MADISON

Tabtoxinine-ß-lactam (TßL) is a toxin produced by several chlorosis-inducing pathovars of *Pseudomonas syringae*. TßL is an irreversible inhibitor of glutamine synthetase (GS), and the inactivation of this enzyme has been correlated with the development of chlorosis in infected tissue. We have examined the time- and concentration-dependent *in vivo* inactivation of GS by TßL in cultured corn cells and, we have quantified TßL transport using ^{14}C-labeled toxin. The kinetics of *in vivo* GS inactivation by TßL (50-1000 μM) was non-first order with progressively slower rates of inactivation. Although these results reflect the complexity of two separate processes, e.g. toxin uptake and subsequent enzyme inhibiton, the kinetics of *in vitro* inactivation of purified GS were similar to the *in vivo* results, suggesting enzyme inactivation becomes the rate limiting step in the plant.

Since TßL uptake is a required step for toxin action, we examined TßL transport by following *in vivo* inactivation of GS and with ^{14}C-labeled toxin. *In vivo* inactivation of GS by TßL was inhibited by methionine, alanine, and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), a protonophore. The pH optimum of the treatment solution for TßL activity was 5.0. These results suggest TßL, a novel amino acid, may be transported via an amino acid-proton symport system. This hypothesis was supported with quantitative results using radiolabeled toxin. ^{14}C-TßL uptake exhibited saturable kinetics and, in less than 1 mM solutions, a Lineweaver-Burk transformation of the data yielded an app. K_m of 550 μM. CCCP and N-ethylmaleimide inhibited TßL uptake by 90%. One mM methionine, alanine, and tyrosine also inhibited toxin (300 μM) uptake by 90% and the pH optimum for uptake was 5.0. These results are consistent with the notion TßL is transported into the plant cell via an amino acid transport system. We have initiated experiments using plasma membrane vesicles to examine TßL transport in more detail.

G 203 IAA ACCUMULATION IN MICROSOMAL VESICLES: INFLUENCE OF SURFACE AND MEMBRANE POTENTIALS. KA Clark & MHM Goldsmith. Biology Dept, Yale University, New Haven CT

We are using membrane vesicles as a model system for studying the mechanisms involved in the transport and accumulation of auxin. In particular, we are interested in determining the involvement of a transmembrane pH gradient ($pH_i > pH_o$) and membrane potential (K^+ diffusion potential, $[K^+]_i > [K^+]_o$) in IAA uptake. In response to a pH gradient, ^3H-IAA accumulation into zucchini hypocotyl vesicles is 2-3x that of the ΔpH probe, ^{14}C-butyric acid (BA). NPA further enhances IAA uptake, but only when $[IAA]_o$ is low. Several auxins at 1 μM decrease the accumulation of IAA (10 nM) to the same level as BA without affecting ΔpH. When the intravesicular volume is reduced osmotically, BA uptake decreases accordingly, but saturable IAA uptake is only slightly affected. These results indicate that a saturable, intravesicular binding site contributes to the enhanced, ΔpH-dependent accumulation of IAA. A membrane potential, negative inside (as determined with the fluorescent cation, $diSC_2(5)$), is generated upon addition of valinomycin to vesicles when $[K^+]_i > [K^+]_o$. If a pH gradient is also present, a transient stimulation of IAA accumulation occurs. When the pH gradient has been dissipated by nigericin, however, the membrane potential alone does not cause IAA accumulation. We conclude that the ΔpH-dependent accumulation of IAA is not sensitive to the transmembrane potential. Since increasing the external ionic strength with 100 mM monovalent (K^+, Na^+, Cs^+ or choline) or 10 mM divalent (Ca^{2+} or Mg^{2+}) cations decreases saturable IAA accumulation, without affecting either the membrane potential or the pH gradient, the interaction of the amphipathic IAA molecules with membranes may be influenced by the electrostatic forces at the membrane surface.

G 204 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

In higher plant chloroplasts, the internal thylakoid system is separated from the delimiting envelope by an aqueous stroma. Since many thylakoid membrane proteins are synthesized as higher molecular weight precursors on free polysomes in the cytosol, these proteins must be post-translationally imported across the chloroplast envelope, then traverse the stroma, integrate into the thylakoid bilayer, and be proteolytically processed to mature size. We have reconstituted thylakoid integration and proteolytic processing for the light-harvesting chlorophyll a/b protein (LHCP) independent of transport across the envelope. LHCP precursor (preLHCP) inserts directly into the thylakoids, orients correctly in the bilayer, and assembles into the photosystem II chlorophyll-protein complex. Proteolytic processing of preLHCP to mature LHCP occurs only under conditions that result in membrane integration. Integration of preLHCP into thylakoids also requires an energy source, e.g. ATP, and a soluble stromal factor that is heat and protease labile and has a molecular weight greater than 5000. The implications of these results in lieu of in vivo events of thylakoid protein biogenesis will be discussed.

G 205 OPTIMIZING THE ASSAY CONDITIONS TO DIFFERENTIATE BETWEEN THE TONOPLAST AND PLASMA MEMBRANE H^+-ATPASES OF BARLEY ROOTS, Frances M. DuPont, United States Department of Agriculture, Agricultural Research Service, Western Regional Research Center, Albany, CA 94710. Active transport by the plasma membrane and tonoplast H^+-ATPases of barley roots was measured in-vitro. Transport was measured as quench of acridine orange fluorescence. The optimal conditions for assay of the two enzymes were very different. Transport by the plasma membrane H^+-ATPase had a temperature optimum between 30 and 40°C. The initial rate of proton transport was increased 2 to 3 fold by assaying at 30°C instead of 20°C and the rate was increased further by addition of 1 µM valinomycin. Rates were similar in the presence of 50 mM KCl or KNO_3. Transport by the tonoplast H^+-ATPase had a temperature optimum between 10 and 20°C. The initial rate of proton transport was decreased 2 to 3 fold by assaying at 30° instead of 20°C. Rates were decreased by addition of 0.33 µM valinomycin and the pH gradient was dissipated completely by 1 µM valinomycin. Initial rates of 200 to 400% quench/mg.protein.min were obtained for the plasma membrane enriched fraction at 32°C with 50 mM KCl; and rates of 500 to 900% quench/mg.protein.min were obtained for the tonoplast enriched fraction at 20°C with 50 mM KCl. Addition of 0.2 mM Ca^{2+} to either fraction caused the pH gradient to dissipate rapidly. PPi dependent-proton transport was associated with the tonoplast membranes but not with the plasma membrane fraction, and transport by the PPiase showed a similar sensitivity to NO_3^-, valinomycin and Ca^{2+} as that of the tonoplast H^+-ATPase.

G 206 INTERACTION OF THE SPINACH CHLOROPLAST ENVELOPE PHOSPHATE TRANSLOCATOR WITH CALMODULIN, H. Michael Harrington and Robert H. Suehisa, University of Hawaii, Honolulu, HI 96822.
Previous research by others (Roberts et al., J. Cell Biol. 97:1644, 1983; Roberts et al., In: Molecular and Cellular Aspects of Calcium in Plant Development, p 11, 1986) suggests that the phosphate translocator is the major calmodulin-binding protein (CaM-BP) in the chloroplast and in the envelope. We have labeled the phosphate translocator at the active site with [^{3}H]-$NaBH_4$ and shown that the apparent molecular weight of translocator and the ^{125}I-CaM-BP are identical (29,000D). In addition, the labeled translocator and the ^{125}I-CaM-BP co-purify in a procedure which is reported to yield the translocator in up to 70% purity. Analysis of whole envelope and translocator enriched fractions by ^{125}I-CaM overlay analysis on 2-D (O'Farrel) gels indicates that the CaM-BP has an apparent molecular weight of 34,000D in SDS-PAGE after isoelectric focusing. The 34,000D protein is the major protein by both silver and Coomassie staining in the 30,000D range in both of the fractions. The results add support to the idea that the phosphate translocator protein is the major CaM-BP in spinach chloroplast envelope. We are currently comparing the location of the [^{3}H]-labeled translocator with that of the ^{125}I-CaM-BP and determining the effects of calcium on phosphate uptake in intact chloroplasts.

G 207 PHOTOAFFINITY LABELING OF SUCROSE BINDING, MEMBRANE PROTEINS, W.D. Hitz, K.G. Ripp, and P.V. Viitanen. Central Research and Development Dept., E.I. du Pont Co., Wilmington, DE 19899
Active sucrose accumulation occurs in selected plant cells and cellular compartments. Such accumulation may be accomplished by sugar carriers in the membranes of those compartments which co-transport protons and sucrose and thus use metabolic energy to create solute concentration imbalances. While such carriers have been postulated, none have been identified or isolated from plants. The sucrose photoprobe, 6'-deoxy-6'-(2-hydroxy-4-azido)benzamidosucrose (6'-HABS) was prepared from sucrose via 6'-deoxy-6'-aminosucrose and 4-amino-salicylic acid to aid in the identification of sucrose carrier proteins. The parent compound was radiolabled by treatment with lactoperoxidase and Na^{125}I to give ^{125}Iodo-6'-HABS at about 600 Ci/mmole. 6'-HABS is a competitive inhibitor of sucrose influx into protoplasts from developing soybean cotyledons with a K_i of 75mM, and photolyzes very rapidly at wavelengths between 290 and 350 nm. Photolysis of ^{125}Iodo-6'-HABS with microsomal preparations made from developing soybean cotyedons produces labeling primarily in a moderately abundant membrane protein of about 62 kD. The 62 kD protein is only partially protected from labeling by inclusion of carrier substrates into the photolysis medium. Both the 62 kD protein and the photolabeling of the 62 kD band appear in the same developmental time course as does the ability of the cotyledons to actively accumulate sucrose.

G 208 HEAT SHOCK TRIGGERS TRANSMEMBRANE SIGNAL OF Ca^{2+} AND THERMOPROTECTION, CHU-YUNG LIN, Botany Department, National Taiwan University, Taipei, Taiwan, ROC.
We showed previously that when soybean seedlings were preexposed to 40°C for induction and accumulation of heat shock (HS) proteins the seedlings acquired thermotolerance to withstand the 45°C lethal HS treatment. HS proteins were also shown localized within the organellar fractions during HS in temperature dependent manner. This suggests the acquisition of thermotolerance is possible via the association of HS proteins with the organelles. In order to test this possibility we used EGTA at the concentration of 15 mM between the HS treatments of 40°C and 45°C. We found the seedlings lost the viability and died by the EGTA treatment otherwise the seedlings were thermoprotected. When 15mM $CaCl_2$ was added to the incubation medium at the same time or after the EGTA treatment the seedlings restored the growth. At 28°C although EGTA inhibited amino acid incorporation in soybean seedlings by 40% the seedling growth was not inhibited. The inhibitory effects of EGTA on seedling growth and amino acid incorporation at 40°C was much greater. These results indicate there is an increase of EGTA-sensitive Ca^{2+} level during HS which is important for cell metabolic processes. The removal of this Ca^{2+} during HS is detrimental to the seedlings.

G 209 ENDOMEMBRANE H^+-ATPASES FROM PLANTS AND ANIMALS SHOW IMMUNOLOGICAL CROSS-REACTIVITY, Morris F. Manolson[1], Judith M. Percy[2], David K. Apps[2], Xiao-Song Xie[3], Dennis K. Stone[3], and Ronald J. Poole[1], [1]Center for Plant Molecular Biology, Biology Department, McGill University, 1205 Dr. Penfield Ave., Montreal, Quebec, Canada, H3A 1B1, [2]Department of Biochemistry, University of Edinburgh Medical School, George Square, Edinburgh, EH8 9XD, U.K., [3]Department of Internal Medicine, University of Texas Health Science Center, Southwestern Medical School, Dallas, Texas, 75235, U.S.A.
Comparison of structural studies on endomembrane H^+-ATPases from various systems suggest that certain polypeptides are common to all H^+ pumps of this type. Here we show that antibodies specific to two subunits of a plant tonoplast H^+-ATPase cross-react with the corresponding subunits of H^+-ATPases from chromaffin granules and clathrin-coated vesicles.

G 210 PROTEIN SYNTHESIS INHIBITORS STIMULATE K^+ RELEASE FROM ROSE CELLS, Terence M. Murphy, Dept. of Botany, University of California, Davis CA 95616
The application of cycloheximide (CHX, 10 ug/ml), emetine (30 ug/ml), or cordycepin (CPN, 1 ug/ml) stimulates a rapid efflux of K^+ from washed cultured rose cells (<u>Rosa damascena</u>). The efflux follows a lag of 30 minutes (CHX) to 60 minutes (CPN) and is inhibited reversibly by chilling (0°C) and irreversibly by heating (40°C). The efflux is inhibited by carbonylcyanide m-chlorophenylhydrazone and, in the case of CPN but not CHX, by a mixture of KCN and salicylhydroxamic acid. The effect of CHX is unusual in that efflux stops after two hours and the cells become insensitive to further applications of CHX. The results are discussed in terms of one or more proteins that regulate K^+ flow and that turn over rapidly in untreated cells.

DUNALIELLA - A MODEL SYSTEM FOR CELLULAR ION REGULATION IN PLANTS AND ALGAE

G 211 Uri Pick, Adriana Katz and Mordhay Avron, The Weizmann Institute of Science, Rehovot 76100, Israel

Dunalialla salina is a unicellular green halotolerant alga. The absence of a rigid cell wall and of large internal vacuoles make this organism an excellent choice both for transport studies in vivo and for biochemical studies. We have developed sensitive methods for studying ion transport in intact cells and we have purified plasma membrane vesicles which have an extremely low permeability to protons from Dunaliella cells. We have identified and characterized a vanadate sensitive H^+-ATPase, a Na^+-H^+ antiporter and two specific and distinct K^+ and Ca^{2+} channels in the plasma membrane of the cells. An integrated mechanism for Na^+, K^+ and H^+ transport and regulation in Dunaliella will be presented.

DCCD BINDING TO 16 KDA SUBUNIT INHIBITS TONOPLAST H^+-ATPASE ACTIVITY.

G 212 Stephen K. Randall, Klaus Kaestner, Shou-peng Lai, & Heven Sze. Botany Dept, University of Maryland, College Park, Maryland 20742

The tonoplast ATPase from higher plants pumps protons into the vacuole generating a proton motive force that can be used by various secondary active transport systems. One of our objectives is to understand the structure/function relationship of the subunits of the ATPase. The role of the 16 kDa component of the ATPase was probed by studying the inhibition of membrane-bound ATPase by N,N'-dicyclohexylcarbodiimide (DCCD). Kinetic analysis showed a first-order rate of inhibition (k_{inact} = 0.11 min^{-1} at 50 nmole DCCD applied/mg membrane protein) suggesting a single type of DCCD reactive site on the ATPase. A close correlation (0.996) between [^{14}C]DCCD binding to the 16 kDa polypeptide and the inhibition of ATPase activity suggests that DCCD binding is responsible for the inhibition of the ATPase. When ATPase activity is completely inhibited, 4 to 8 moles DCCD were bound to the 16 kDa polypeptide per mole ATPase. Assuming one DCCD reactive site per 16 kDa polypeptide, each ATPase holoenzyme would consist of at least four 16 kDa subunits. The 16 kDa (DCCD-binding) polypeptide is a proteolipid and chloroform/methanol extraction results in at least 16-fold purification. The purification of the proteolipid is the first step towards understanding the proposed role of the 16 kDa subunit in proton transport (Randall and Sze, J. Biol. Chem. 261:1364 (1986) and its molecular structure. (Supported by National Science Foundation PCM-04130)

REGULATION OF Ca^{2+} TRANSPORT ACROSS THE TONOPLAST OF OAT ROOT CELLS.

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In eukaryotic organisms, cytoplasmic Ca^{2+} concentrations are modulated by coordinating passive fluxes and active transport across the plasma membrane and several organellar membranes. Ca^{2+} is accumulated in the vacuole via a H^+/Ca^{2+} exchange system using either the proton motive force from the tonoplast H^+-ATPase (Schumaker & Sze (1985) Plant Physiol. 79, 1111) or an imposed pH gradient (Schumaker & Sze (1986) J. Biol. Chem. 261, 12172). Ca^{2+} uptake is specifically inhibited by ruthenium red and N,N'-dicyclohexylcarbodiimide. H^+/Ca^{2+} exchange generates a membrane potential, interior positive (suggesting one Ca^{2+} taken up for one H^+ lost), as shown by [^{14}C]SCN accumulation. The exchange transport is reversible, as a Ca^{2+} gradient ($Ca^{2+}_{in} > Ca^{2+}_{out}$) was effective in forming a pH gradient (acid inside). We are attempting to isolate and purify the exchange system to determine its molecular properties. Ca^{2+} accumulated in tonoplast vesicles could be released transiently by inositol 1,4,5-trisphosphate ($InsP_3$) (Km = 0.6 µM), but not by myo-inositol 1-phosphate or fructose-2,6-bisphosphate. A Ca^{2+} antagonist, 8-(N,N-diethylamino)-octyl 3,4,5-trimethoxy benzoate-HCl (TMB-8), blocked $InsP_3$-induced Ca^{2+} release. These results suggest that $InsP_3$ may function as a second messenger in plant cells to mobilize Ca^{2+} from intracellular stores, and that the vacuole may be one major source of $InsP_3$-released Ca^{2+}. (Supported by DOE Grant FG05-86ER13461 to H.S.)

Receptors and Signalling

G 300 TRANSMEMBRANE SIGNALING IN PLANT CELLS INDUCED BY ELICITORS FROM PHYTOPATHOGENIC FUNGI, Izydor Apostol, Philip S. Low and Peter Heinstein, Purdue University, West Lafayette, IN 47907.

Elicitor induced transmembrane signaling in plant cells can be monitored using fluorecent molecular probes. Pyranine, a membrane associated, pH sensitive dye showed a fluorescence change five minutes after the addition of an elicitor preparation from Verticillium dahliae or -glucan from Phytophthora megasperma f.sp. glycinea. The fluorescence change was correlated with phytoalexin formation in cotton and soybean cells. Citrate was found to be a specific inhibitor of both the elicitor-induced fluorescence transition as well as phytoalexin formation. Since the citrate concentrations required to obtain 50% inhibition of the fluorescence transition actually stimulated plant cell mass accumulation by 15%, the effect of citrate cannot be a toxic effect. Using the intracellular pH-sensitive probes, diacetoxyphthalonitrile and carboxyfluorescein, we are able to monitor the subcellular locations of the proton fluxes using fluorescence microscopy. By temporarily correlating the fluorescence intensity changes seen in sequential fluorescence micrographs with the kinetic phases of the fluorescence transitions measured in the fluorimeter, the chronological order of proton fluxes in the major cellular compartments has been assigned.

G 301 BACTERIAL PHYTOTOXIN, SYRINGOMYCIN , STIMULATES A PROTEIN KINASE MEDIATED PHOSPHORYLATION OF RED BEET PLASMA MEMBRANE POLYPEPTIDES. Ashok P. Bidwai and Jon Y. Takemoto. Dept. of Biology, Utah State University, Logan, Utah,84322-0300.
Pseudomonas syringae pv. syringae,is a bacterial phytopathogen, which produces a peptide toxin, syringomycin. The toxin stimulated the phosphorylation of several plasma membrane proteins of red beet storage tissue. Among these was a Mr 100K polypeptide which corresponds in size to the catalytic subunit of the plasma membrane ATPase. The phosphorylations were insensitive to hydroxylamine indicating that the phosphorylated intermediates were not acyl phosphates but rather ester phosphates. Phosphorylation of the 100Kda polypeptide and of most of the other polypeptides was eliminated by treatment of the membranes with 0.1% Na deoxycholate,which also eliminated the ability of the toxin to stimulate the ATPase activity. Phosphorylations of several polypeptides was affected by EGTA. Addition of excess Ca^{2+} restored the phosphorylation of most of the polypeptides. We propose that syringomycin acts by stimulating an endogenous membrane - associated protein kinase which results in the phosphorylation of several plasma membrane polypeptides.One of the phosphorylated polypeptides has a Mr of 100K, which corresponds in size to the plasma membrane ATPase.

G 302 PURIFICATION OF AN AUXIN-STIMULATED NADH OXIDASE FROM PLASMA MEMBRANE OF SOYBEAN. Andrew Brightman, Rita Barr, F. L. Crane and D. James Morré, Purdue University, West Lafayette, Indiana 47907
An auxin-stimulated NADH oxidase(NADH-ascorbate free radical oxidoreductase) activity has been described (1). The enzyme is stimulated by 1-10 µM. auxin and may use monodehydroascorbate as an electron acceptor. The enzyme was isolated from plasma membranes of 4 day old etiolated soybean hypocotyls (Glycine max L.). Plasma membrane was purified by aqueous two-phase partitioning using a 16 g system of 6.4% polymers (polyethyleneglycol/dextran). Solubilization was by incubation with the zwitterionic detergent CHAPS and the solubilized enzyme retained approximately 50% of its original NADH oxidase activity. The enzyme was purified by ion exchange chromatography (DEAE-cellulose), followed by gel filtration. Highly active fractions were analyzed by polyacrylamide electrophoresis under both denaturing and non-denaturing conditions. Identification of the catalytic polypeptide was by direct localization of the NADH oxidase activity in a native gel. Final purification was by affinity chromatography using a 5'AMP-sepharose column. Experiments are underway to test if the enzyme reconstituted in phospholipid vesicles is active in proton transport and to probe the relationship between the activity and a putative auxin receptor.

1. Morré, D. J., P. Navas, C. Penel and F. J. Castillo (1986) Protoplasma 133:195-197.

G 303 MODULATION OF PHOTOSYNTHESIS IN THE GUARD CELL CHLOROPLAST BY BLUE AND GREEN LIGHT.
Bruce T. Mawson and Eduardo Zeiger. Stanford University, Stanford, CA 94305.
Slow, chlorophyll a fluorescence transients are diagnostic for photophosphorylation (early quenching, OPS) and processes associated with carbon fixation (M phase) in chloroplasts. Application of this technique to guard cell chloroplasts (GCCh), using actinic blue light (BL), has shown transients with poor quenching and devoid of the M phase. We now report that use of actinic green light (GL) changes the transients from GCCh, rendering them qualitatively identical to those from mesophyll chloroplasts (MCh). In contrast, transients from MCh were indistinguishable under actinic BL, or GL. Microfluorospectrophotometry was used with single mesophyll cells or a guard cell pair from Vicia faba or Commelina communis, kept in 10 mM KCl and 20 mM HEPES-NaOH buffer, pH 7.1. Under BL, transients from GCCh from both species showed limited quenching and no M phase, as reported previously. Under GL, the same guard cell pair showed an enhanced quenching; subsequent GL irradiations increased both rate and magnitude of the quenching, and also induced typical M oscillations. Mesophyll-like transients were also induced by BL upon addition of 1 mM phosphate to the medium. The sequential transformation of typical BL-induced transients by GL and the phosphate effect indicate that the differential response to light quality is not the result of an unusual pigment composition in GCCh. We postulate that the GL-induced transients from GCCh reflect typical photophosphorylation and photosynthetic carbon fixation capacity and that the rudimentary BL-induced transients result from changes in the GCCh environment ensuing from the specific BL response of guard cells. The GCCh appears as a valuable model system to study intracellular photosynthetic regulation.

G 304 INVOLVEMENT OF LIPID PEROXIDATION IN ELICITOR INDUCED HYPERSENSITIVE RESPONSE,
Kim Rogers and Anne J. Anderson, Utah State University, Logan UT 84322-4500.
Metabolic changes termed hypersensitivity are induced in incompatible cultivars of Phaseolus vulgaris (bean) upon challenge by the fungal pathogen Colletotrichum lindemuthianum. Symptoms resembling hypersensitivity such as the production of phytoalexins can be triggered by treatment of plant cells with certain fungal components termed elicitors. Treatment of Dark Red Kidney bean suspension-cells with mycelial wall components (10 μg carbohydrate/ml) from the incompatible α race of C. lindemuthianum resulted in increased levels of the phytoalexin phaseollin. Products of lipid peroxidation including malonaldehyde and fluorescent "lipofucin like" compounds were also observed. Levels of phaseollin and fluorescent "lipofucin like" compounds above the controls were first apparent 9 hr after treatment, however, significant malonaldehyde increases were only observed after 24 hr. Elicitor-induced increases in fluorescent products of lipid peroxidation, malonaldehyde, and phaseollin were inhibited by the hydroxyl radical scavenger, 25 mM mannitol. These data are consistent with free radical-initiated lipid peroxidation involvement in the symptoms characteristic of hypersensitivity.

G 305 PHOSPHORYLATION OF THE OAT PLASMA MEMBRANE PROTON PUMP BY A CALCIUM-STIMULATED PROTEIN KINASE,
G. Eric Schaller and Michael R. Sussman, Univ. of Wisconsin, Madison WI 53706
We are testing whether the plasma membrane H^+-ATPase is regulated by a phosphorylation/dephosphorylation process. When plasma membranes isolated from oat root cells are incubated with [γ-^{32}P]-ATP, protein and lipid components are phosphorylated by endogenous kinase activity. By glycerol density gradient centrifugation and high pressure liquid chromatography, we have demonstrated that the ATPase is one of the major phosphorylated proteins. The ATPase is phosphorylated at both serine and threonine residues, but not at tyrosine residues. Phosphorylation of the ATPase is stimulated three-fold by calcium, with maximal stimulation occuring at less than 7 uM free calcium. Other membrane proteins also show calcium-stimulated phosphorylation. In contrast, phosphorylation of membrane lipids is unaffected by calcium, suggesting the presence of separate lipid and protein kinases in oat plasma membranes. The ATPase exhibits a unique pH profile for phosphorylation; the amount of incorporated phosphate drops sharply between pH 6.2 and 7.8, a region where the phosphorylation of other membrane proteins decreases only a small amount if at all.

G 306 ELECTRICAL RESPONSES OF THE PLASMA MEMBRANE TO AUXIN AND FUSICOCCIN.

A Senn & MHM Goldsmith. Biology Dept, Yale University, New Haven CT 06511

Selected coleoptiles from 5 day old Avena sativa L. seedlings were incubated in a basal salt medium (1 mM MES, 0.1 mM KCl, 0.1 mM $CaCl_2$, adjusted to pH 6 with BTP) for 1-5 h. Rapid responses to the growth regulators IAA and FC were studied as the external ionic composition of cations (K^+, Ca^{2+}, H^+) or anions (Cl^-) was varied. Membrane potential, proton secretion and growth were monitored in parallel. Electrical properties of the plasmalemma were differentiated from those including the tonoplast by selecting impalements with low resistance and highly negative membrane potentials. Under low salt conditions, the kinetics of IAA- or FC-induced hyperpolarization are closely related to the changes in proton secretion and growth rates, supporting the idea that the cells hyperpolarize as the rate of the electrogenic proton transport increases. As $[KCl]_o$ is raised, the cells depolarize and progressively lose their ability to hyperpolarize in IAA or FC; however, IAA-induced proton secretion and growth remain unaffected. Similarly, as $[H^+]_o$ is raised by varying the pH from 8 to 4, control cells depolarize and become less responsive to IAA. The loss of the electrical response to IAA at high $[KCl]_o$ or low pH appears to be due to a release of calcium from cell walls (as monitored with $^{45}Ca^{2+}$), and the consequent increase in membrane conductance. This can be prevented by raising the concentration of Ca^{2+} to 1 mM. In conclusion, the electrical response to IAA or FC depends on both the electrogenic proton pump and the conductance of the diffusive pathways.

G 307 IDENTIFICATION OF A LECTIN-LIKE PROTEIN IN THE ENDOPLASMIC RETICULUM OF DEVELOPING BEAN COTYLEDONS, Alessandro Vitale, Monica Zoppè, Roberto Bollini, Istituto Biosintesi Vegetali, CNR, via Bassini 15, 20133 Milano, Italy.

We are interested in the synthesis and transport of plant glycoproteins. Developing Phaseolus vulgaris cotyledons synthesize large amounts of phytohemagglutinin (PHA). PHA is synthesized by ER-bound polysomes, cotranslationally glycosylated and transported to the protein bodies via the Golgi complex. Developing bean cotyledons contain also mRNA for a lectin-like protein (LLP) with 45% amino acid sequence homology with respect to PHA. Up to now LLP has been identified only by hybrid-selected in vitro translation using an LLP cDNA clone, while nothing is known about the synthesis and subcellular localization of this protein in vivo. We report that using the Xenopus oocyte expression system we have identified the newly synthesized form of LLP. This form of LLP is present in rather high amounts in the ER of developing cotyledons. LLP is heavily glycosylated and this results in a higher mol. wt. with respect to PHA, while at the amino acid level LLP is slightly smaller than PHA. Experiments are in progress to identify the final intracellular localization of LLP in bean cotyledonary cells.