PLANT CELL BIOLOGY: MECHANISMS, MOLECULAR MACHINERY, SIGNALS AND PATHWAYS

Organizers: Natasha V. Raikhel and Christopher J. Lamb

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Plant Cell Biology: Mechanisms, Molecular Machinery, Signals and Pathways

Keynote Address

A3-001 PHYTOCHROME PHOTOTRANSDUCTION PATHWAYS: BIOCHEMICAL AND GENETIC DISSECTIONS, Chris Bowler, Hiroshi Yamagata, Gunther Neuhaus*, and Nam-Hai Chua, Laboratory of Plant Molecular Biology, The Rockefeller University, 1230 York Avenue, New York, New York 10021-6399; *Institute for Pflanzenwissenschaften, ETH-Zurich, Universitatstrasse 2, CH-8092 Zurich, Switzerland.

Three signal transduction pathways, dependent upon cGMP and/or calcium, are utilized by phytochrome to control the expression of genes required for chloroplast development and anthocyanin biosynthesis in plant cells. For example, *chs* is controlled by a cGMP-dependent pathway, *cab* is controlled by a calcium-dependent pathway, and *fnr* is regulated by a pathway that requires both cGMP and calcium. Using a soybean photomixotrophic cell culture and microinjection into the cells of a phytochrome-deficient tomato mutant, we have studied the regulatory mechanisms acting within and between these three signalling pathways. We provide evidence that changes in cGMP levels mediate the observed induction and desensitization of *chs* gene expression in response to light and demonstrate that high cGMP concentrations cause negative regulation of both the calcium- and the calcium/cGMP-dependent pathways. Conversely, high activity of the calcium-dependent pathway can negatively regulate the cGMP-dependent pathway. We have termed these opposing regulatory mechanisms "reciprocal control". In all cases, the molecules that are involved appear to be downstream components of the signal transduction pathways, rather than calcium and cGMP themselves. Furthermore, we have found that the calcium/cGMP-dependent pathway has a lower requirement for cGMP than does the cGMP-dependent pathway. The role of these phenomena in the regulation of plant photoresponses will be discussed.

Cell Surface/Extracellular Matrix

A3-002 A TALE OF TWO ENZYMES IN CELL-CELL RECOGNITION: S-RIBONUCLEASE AND RECEPTOR KINASE, Teh-hui Kao, Hyun-Sook Lee, Shihshieh Huang, and Balasulojini Karunanandaa, Department of Biochemistry and Molecular Biology, Penn State University, University Park, PA 16802.

We are studying cell-cell recognition between pollen and the sporophytic tissue of pistil during gametophytic self-incompatibility (GSI) interactions, and between developing pollen and the tapetal cells of anther during pollen development. GSI displayed by solanaceous species is controlled by the highly polymorphic *S* locus. Pollen bearing an *S* allele identical to one of the two *S* alleles carried by the pistil suffers growth arrest in the style, and only pollen bearing an *S* allele different from those carried by the pistil can effect fertilization. The two key questions we are addressing are: 1. What is the molecular basis for self/nonself recognition between pollen and pistil? 2. What is the biochemical mechanism for inhibition of self-pollen growth? Using Petunia inflata we have shown that 1. S-RNases, pistil proteins that cosegregate with *S* alleles, are necessary and sufficient for the pistil to recognize and reject self-pollen (1); 2. the ribonuclease activity of S-RNases is essential for rejection of self-pollen, indicating that the biochemical mechanism of GSI involves their cytotoxic activity (2); 3. the carbohydrate moiety of S-RNases is not required for SI interactions, indicating that their *S* allele determinant lies in the amino acid sequence. We have constructed chimeric S-RNase genes and are in the process of examining the *S* allele specificity which each displays in order to identify the *S* allele determinant. We are also using several strategies to identify the pollen *S* gene in order to understand the molecular basis of self/nonself discrimination between pollen and pistil. Pollen development requires intimate interactions between developing pollen and tapetal cells. We have identified a pollen-specific receptor kinase of P. inflata, PRK1, whose cytoplasmic domain autophosphorylates on serine and tyrosine (3). To investigate whether PRK1 plays a role in pollen development, we have generated transgenic P. inflata plants that express an antisense cDNA encoding the extracellular domain of PRK1 in developing pollen. We have found that the transgene does not affect meiosis of pollen mother cells and release of microspores from the tetrad, but that, at subsequent stages of pollen development when normal microspores complete mitosis and develop into mature binucleate pollen, the transgene causes the microspores to remain uninucleate, and they eventually lose their nuclei and most of the cytoplasmic contents. These results suggest that PRK1 plays an essential role in post-meiotic development of microspores. We have begun to identify the ligand and substrate of PRK1 in order to gain insight into the signal transduction pathway mediated by PRK1.

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Cell Adhesion, Cytoskeleton, and the Recognition Process

A3-003 PLANT CELL RESPONSES TO *RHIZOBIUM* DURING SYMBIOSIS. Sharon R. Long, David W. Ehrhardt, Audrey Southwick, Nina S. Allen[1], Peter Covitz, and Henrik Stotz. Howard Hughes Medical Institute, Gilbert Laboratory, and Department of Biological Sciences, Stanford University, Stanford CA 94305-5020; [1]Wake Forest University, Winston-Salem, N.C.

Legume host plants show distinctive and host specific responses to *Rhizobium* bacteria, including cell growth, cell division and gene expression (1). The bacterial genes that control nodule formation (*nod* genes) have been cloned and sequenced from a number of different *Rhizobium* species, and from *Bradyrhizobium* and *Azorhizobium*. *nod* genes are controlled by factors from the plant, interacting with bacterial gene activator NodD (2). Many other nodulation genes appear to be involved in production of secreted molecules termed Nod factors, which cause plant responses including root hair morphogenetic changes and cell divisions (3). Nod factors are chito-oligosaccharides, modified with an N-acyl group and other substituents on the non-reducing-end GlcNAc residue, and also by certain modifications on the reducing-end GlcNAc residue. Nod factors show plant host specificity, and this correlates with the presence of absence of particular side groups. *Rhizobium meliloti* is a symbiont of alfalfa, and its Nod factor, NodRmIV(S), has a 6-O-sulfate that is required for activity on the alfalfa host (3). We established that the proteins encoded by NodP and NodQ synthesize PAPS, and that the NodH protein transfers this sulfate to a short GlcNAc chain acceptor (4,5). We are using this NodH labelling protocol as a means to create radiolabelled Nod factor for the purpose of testing plant cell fractions for components that bind the bacterial ligand. We are interested in the early reactions of the plant root hair to Nod factor. We have shown that alfalfa root hair membranes depolarize in response to Nod factors of *R. meliloti* (6). We are examining ion fluxes and testing the participation of possible secondary messengers in root hair response (7).

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A3-004 CELL ADHESION AND THE CYTOSKELETON IN CELL POLARITY, Ralph Quatrano[1], Sidney Shaw[1], Brad Goodner[2], Francois-Yves Bouget[1], Susanne Gerttula[1], Crispin Taylor[1], John Fowler[1], and Janice Davis[1], [1]Department of Biology, University of North Carolina, Chapel Hill, NC 27599-3280, and [2]Department of Biology, University of Richmond, Richmond, VA 23173.

Zygotes of the brown alga *Fucus* provide a model system to study the basic mechanisms involved in the generation of cellular asymmetry, as well as subsequent embryo morphogenesis in plants[1,2]. The apolar zygote exhibits a labile polar axis for 8 hours following fertilization and can be oriented with a unilateral light pulse during this period. Stabilization or fixation of the axis occurs while the zygote is still symmetrical (8 to 10hrs), which is then followed by the directed transport of vesicles to the site of polar outgrowth, the rhizoid. The first division of the zygote results in two unequal cells; the smaller rhizoid cell emerges from the shaded portion of the gradient, while the larger thallus cell is on the lighted side. The tip of the rhizoid cell is the initial attachment structure of the developing embryo. A cytoskeleton network and cell wall are both required for polar axis fixation, while adhesion of the embryo to the substratum is required for normal morphogenesis of the developing embryo. This postulated axis stabilizing complex (ASC) at the tip of the emerging rhizoid includes a transmembrane protein (e.g. integrin) which has been hypothesized to link the polar components of the cytoplasm to the extracellular matrix[3]. The ASC would not only immobilize plasma membrane asymmetries established during axis formation, but would also provide the structural basis for the elaboration of the rhizoid attachment structure. Specific assays for polar events (e.g. plasma membrane and mRNA probes and localized vesicle markers) have been monitored after microinjection of antibodies to determine the role of specific proteins (e.g. actin) or protein domains (e.g. cytoplasmic domain of ß-1 integrin) in the fixation and expression of the polar axis. These results will be presented along with those that characterize the localized macromolecules that may play a role in adhesion [4](e.g. the vitronectin-like protein in the cell wall of the rhizoid tip[5]) and the adhesion structure (e.g.the actin collars around the cell plates of the rhizoid filament[6]). Data collected from these experiments will be discussed in relation to mechanisms of cellular differentiation and embryo morphogenesis in higher plants. Supported by research grants from the National Science Foundation (MCB-9318757 to R.Q.; BIR9404025 to J.F.) and the Office of Naval Research (N00014-91-J-4128; N00014-93-1-0888 to R.Q.).

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Plasmodesmata and Nuclear Pores

A3-005 POSTTRANSLATIONAL EVENTS INVOLVED IN NUCLEAR TARGETING OF PLANT VIRAL PROTEINS, James C. Carrington, Ruth Haldeman-Cahill, Xiao Hua Li, and Mary C. Schaad, Department of Biology, Texas A&M University, College Station, TX 77843.

Tobacco etch virus (TEV) is a positive-strand RNA virus that encodes at least 10 proteins through synthesis of a polyprotein. The polyprotein is processed co- and posttranslationally by three TEV-encoded proteinases. The proteinase that catalyzes most cleavage reactions is the NIa proteinase, a multifunctional protein that also functions during initiation of RNA synthesis. NIa originates from a region of the polyprotein that contains the 6-kDa protein, NIa, and NIb (in the order ...6K-NIa-NIb...). NIa functions by autoproteolysis to generate polyprotein intermediates containing these proteins, as well as each of the mature products. Although NIa and NIb, the putative catalytic subunit of the RNA polymerase, function in the cytoplasm during virus replication, both proteins accumulate predominantly in the nucleus of infected cells, suggesting that nuclear translocation of these proteins is a regulated process.

The mechanisms controlling subcellular localization of NIa and NIb were investigated using transient and transgenic expression strategies. Both NIa and NIb were found to translocate efficiently to the nucleus through independent nuclear localization signals (NLSs). However, the 6K-NIa polyprotein failed to translocate, although NIa released by proteolysis from the 6K-NIa polyprotein was transported to the nucleus. Nuclear transport of NIa, therefore, was inhibited *in cis* by the 6K protein. Inhibition of NIa-mediated transport was due to a posttranslational membrane association activity of the 6K protein as revealed by subcellular fractionation and immunogold microscopy. Nuclear translocation of the NIa protein is proposed to be controlled by differential proteolytic removal of the 6K protein from its N-terminus. In contrast, complementation analysis indicates that NIb does not require linkage with 6K-NIa to facilitate localization to sites of RNA replication. The data suggest that multiple mechanisms are involved in control of NIa and NIb subcellular localization.

A3-006 TARGETING OF PROTEINS TO THE PLANT NUCLEUS, Glenn R. Hicks, Stephane Lobreaux, Mark Shieh, Antje Heese-Peck, Harley Smith, and Natasha V. Raikhel, MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824-1312.

The import of proteins into the nucleus is an essential process that has received little attention in plants. In animals, this process involves two steps: nuclear localization signal (NLS) recognition and translocation through the nuclear pore complex (NPC). To characterize NLSs, we have identified targeting signals within the plant transcription factors Opaque2 (O2) and R. To identify factors involved in NLS recognition, radiolabeled peptides to two classes of NLSs, a bipartite signal from O2 and the NLS from the SV40 large T-antigen, were allowed to associate with purified tobacco and maize nuclei. The functional NLSs, which stimulate import in plant cells in vivo, competed for a single low affinity binding site, whereas mutant NLSs, which are inefficient in vivo, were poor competitors. A third class of NLSs, a Mat α-2 like signal found within the R protein, was also shown to compete for the same binding site. Biochemical and localization studies indicate that the binding site is located at the nuclear envelope and NPC. By the use of crosslinking reagents and the radiolabeled O2 NLS peptide under conditions similar to those used for in vitro binding, we have labeled two NLS binding proteins (NBPs) of 50 to 60 kDa and at least two NBPs of 30 to 40 kDa. The biochemistry and affinity of the NBPs indicates that they constitute the NPC binding site. This suggests that some components of NLS recognition reside at the NPC in plants. We are currently identifying NPC proteins in plants in order to purify NBPs and examine the nature of this complex, yet poorly understood structure. Wheat germ aggutinin (WGA), a lectin that specifically binds to *N*-acetylglucosamine (GlcNAc), inhibits the transport of proteins through the NPC in vertebrates suggesting that NPC proteins modified by GlcNAc are involved in import. By EM localization, WGA protein blot analysis and a galactosyltransferase assay, we have shown that plant NPCs also contain proteins modified by GlcNAc. Like vertebrate NPC proteins, the sugars were bound via an O-linkage as demonstrated by β-elimination. Interestingly, unlike vertebrate proteins, which are modified by a single O-linked GlcNAc, plant nuclear proteins contained oligosaccharides that consisted of five or more saccharides. To identify other factors involved in import and functionally test purified NBPs, we developed an in vitro import system using evacuolated protoplasts from cultured cells. In this system, we selectively permeabilize the plasmalemma but not the nuclear envelope. Thus, fluorescent import substrates can be introduced and monitored for nuclear accumulation using fluorescence microscopy. Overall, this combination of approaches will permit us to identify and functionally examine all components of the nuclear import apparatus of plants.

A3-007 *SQUASH LEAF CURL VIRUS* MOVEMENT PROTEIN FUNCTION: NUCLEAR SHUTTLING OF THE VIRAL GENOME AND DIRECT PROTEIN:PROTEIN INTERACTIONS, S.G. Lazarowitz[1], E. Pascal[1], D.J. Ingham[1], A.A. Sanderfoot[1], B.M. Ward[1], R. Turgeon[2], and R. Medville[2], [1]Department of Microbiology, University of Illinois, Urbana, IL 61801, [2]Cornell University, Ithaca.

Using the combined approaches of molecular genetics, biochemistry, and cell biology (model cell systems and transgenic plants), we have begun to understand the functions of the two movement proteins BR1 and BL1 encoded by the bipartite geminivirus SqLCV (squash leaf curl virus). Our primary goal is to define the biochemical functions of these two movement proteins (MPs) and detail their interactions with viral and host cell components to facilitate viral movement. By alanine-scanning mutagenesis, we have constructed a series of partially defective mutants with lesions in either BR1 or BL1. Infectivity assays in different host plants (pumpkin, squash and *N. benthamiana*) demonstrate that both MPs are important in determining viral host range properties as several mutations in both BR1 and BL1 retain infectivity for pumpkin and squash, but completely eliminate infectivity in *N. benthamiana*. However, mutations in only BL1 produce attenuated symptoms, suggesting the importance of this movement protein in the production of disease symptoms. In addition, the coat protein (AR1) specifically masks certain mutations in BR1, thus placing this gene product in the transport pathway. The direct role of BL1 in producing disease symptoms is further demonstrated by our analysis of transgenic *N. benthamiana* plants expressing BL1 or BR1. Although transgenic plants expressing BR1 are phenotypically normal, those expressing only BL1 develop typical viral disease-like symptoms of mosaic and downward leaf curl (epinasty). Concomitant with this abnormal phenotype, BL1-expressing transgenics have growth and developmental defects that mimic auxin-deficiencies. These findings, combined with the host range properties of our BR1 and BL1 mutants, suggest that BL1 may interact with host components to misregulate phloem transport, and that differences in requirements for viral movement in different hosts likely reflects differences in phloem structure among these hosts.

By cell fractionation and immunogold labeling we have localized BR1 to the nuclei of phloem parenchyma cells in infected plants, finding BR1 to be specifically concentrated over nucleoli. We find BL1 in the cell wall and plasma membrane fractions, and are currently doing immunogold cytochemistry to determine whether BL1 is specifically associated with plasmodesmata. *In vitro* DNA binding and gel shift assays demonstrate that BR1 is a single-stranded (ss)DNA binding protein. In contrast, BL1 has very weak affinity for ssDNA, and does not bind dsDNA or RNA. We have also used recombinant baculovirus vectors to express BR1 and BL1 in Sf9 insect cells. By indirect immunofluorescence staining, we find that BR1 localizes to the nucleus and BL1 to the plasma membrane in these cells, thus mimicking their localization in plant cells and providing a powerful model system for analysis of these MPs. We are currently doing transient expression studies in Sf9 cells to define functional domains of each MP important for correct cellular targeting and potential protein:protein interactions. Our studies suggest that BL1 and BR1 have distinct functions, but act in a coordinated manner to facilitate movement of the viral genome. We propose a model whereby BR1 is involved in the shuttling of the viral genome into and out of the nucleus and BL1 facilitates genome movement across cell boundaries.

Workshop: Heat Shock Proteins and Molecular Chaperones

A3-008 STRUCTURE AND MOLECULAR CHAPERONE ACTIVITY OF SMALL HEAT SHOCK PROTEINS, Garrett Lee, Katherine Osteryoung, Teri Suzuki, Nadja Wehmeyer, Denise Krawitz, and Elizabeth Vierling, Department of Biochemistry, University of Arizona, Tucson, Arizona 85721.

Plants respond to high temperature and certain other stresses by synthesizing a discrete set of proteins known as heat shock proteins (HSPs). Our laboratory is interested in understanding the function of the small HSPs (15-30 kDa) (smHSPs) which are unusually diverse and abundant in plants as compared to other eukaryotes. We hypothesize that the smHSPs are required for high temperature tolerance, and that they represent a new type of molecular chaperone. There are four major nuclear gene families of smHSPs which have been conserved through the evolution of higher plants. Two encode cytosolic proteins (class I and II), one encodes an ER-localized protein, and the fourth encodes a protein targeted to the chloroplast. All smHSPs tested show a similar pattern of expression in vegetative tissues. The proteins are undetectable in the absence of heat stress, but are expressed at high levels at elevated temperatures, accumulating up to 1.0% of total cell protein in the case of the class I cytosolic proteins. The cytosolic smHSPs are also expressed during seed maturation in the absence of stress. Biochemical studies indicate that in vivo, both the cytosolic and chloroplast smHSPs assemble into large homo-oligomeric particles. To investigate further the function of these proteins we have initiated both transgenic plant analysis (antisense and over-expression) and in vitro activity assays. Although we have produced transgenic plants which constitutively express the chloroplast-localized smHSP, and plants which show 70% reduced expression of this protein, we have not detected any difference in whole plant thermotolerance associated with the changes in smHSP expression. To test for chaperone activity, two recombinant smHSPs, HSP18.1 (class I) and HSP17.7 (class II) from pea, have been purified to homogeneity and shown to form 240 and 340 kDa complexes, respectively, similar to the authentic proteins from pea plants. The purified complexes appear as homogeneous globular particles of approximately 10 nm in diameter in the electron microscope. Surprisingly, despite the similarity in amino acid sequence and quaternary structure of HSP18.1 and 17.7, these proteins do not co-assemble in vitro. Only homo-oligomeric complexes are reformed when the HSP18.1 and 17.7 complexes are denatured, mixed, and then renatured. These findings indicate that the class I and II smHSPs are structurally distinct, and suggest they have distinct functions or interact with unique substrates. Both HSP18.1 and 17.7 act as molecular chaperones in several in vitro assays. They promote reactivation of chemically denatured citrate synthase (CS) and lactate dehydrogenase (LDH). They prevent thermal aggregation, but not inactivation, of CS at 45 C, and under these conditions HSP18.1 forms a stable complex with the enzyme. HSP18.1 and 17.7 also prevent LDH inactivation during incubation at environmentally relevant stress temperatures (38 C), and promote reactivation of CS which has been inactivated at 38 C. Site directed mutants of HSP18.1 are being prepared to test the structural basis of these activities. An affinity column of HSP18.1 binds a subset of total plant cell proteins, which may represent in vivo substrates of this molecular chaperone.

Workshop: Transport Vesicle: Inside and Outside

A3-009 **ROLE OF SMALL GTP-BINDING PROTEINS AND PHOSPHATIDYLINOSITOL 3-KINASE (PI 3-KINASE) IN VESICULAR TRANSPORT AND MEMBRANE FUSION IN PLANTS.** Desh Pal S. Verma, Zonglie Hong and Xiangju Gu. Department of Molecular Genetics and Biotechnology Center, The Ohio State University, Columbus OH 43210

Following synthesis membrane proteins are folded, shipped, delivered and received at a correct subcellular compartment based on the attached address label on each protein. Membrane vesicles play an essential role in transport of various proteins to their correct location. Many small GTP-binding proteins play a role in vesicle-mediated protein trafficking system although the reactions catalyzed by these proteins are not yet fully understood. We have isolated several plant GTP-binding proteins and have demonstrated, by creating tissue-specific antisense "mutation" that these proteins are vital for correct membrane trafficking. Because root nodule contain an extra subcellular compartment housing the bacteria, the membrane delimiting this compartment provides an interesting system to study the flow of various vesicles and targeting of proteins to this compartment. We have recently cloned and characterized PI 3-kinase from soybean and shown the existence of a novel form of this enzyme that may participate in membrane biogenesis in root nodule. Expression of the root-form of PI 3-kinase is repressed during nodule organogenesis and is re-induced in mature nodules. The polypeptides encoded by soybean PI 3-kinase cDNAs show significant sequence homology (50-60% similarity and 20-40% identity) to both PI 3-kinases and PI 4-kinases from mammalian and yeast cells. *Echerichia coli* expressed soybean PI 3-kinase displayed the activity to phosphorylate phosphatidylinositol specifically at the D-3 position of the inositol ring to generate phosphatidylinositol 3-phosphate. The temporal increase of a specific PI 3-kinase activity during membrane proliferation in young nodules suggests that PI 3-kinase plays a pivotal role in development of the peribacteroid membrane forming a new subcellular compartment. We have also isolated a clone encoding a homolog of Dynamin and will discuss the possible involvement of these proteins in membrane trafficking in plants.

Cell Division

A3-010 REACTIVATION OF MITOTIC ACTIVITY IN ROOT CORTICAL CELLS BY RHIZOBIUM LIPO-OLIGOSACARIDES,
Ton Bisseling[1], Henk Franssen[1], Heribert Hirt[2], Martha Matvienko[1], Katharina Pawlowski[1], Karin Van de Sande[1], Rick Walden[3], Wei-Cai Yang[1], [1]Department of Molecular Biology, Wageningen Agricultural University, Dreijenlaan 3, 6703 HA, Wageningen, The Netherlands, [2]Institute of Microbiology and Genetics, University of Vienna and [3]Max Planck Inst für Züchtungsforschung, Cologne, Germany.

Rhizobium bacteria induce the formation of root nodules on the roots of leguminous plants. In temperate legumes, nodule organogenesis starts with the induction of cell divisions in regions of the root inner cortex opposite proto-xylem poles, resulting in the formation of nodule primordia. By studying the *in situ* expression of the cell cycle genes *cycMs2*, *H4* and *cdc2* in pea and alfalfa root cortical cells after inoculation with *Rhizobium* we showed that the susceptibility of inner cortical cells to *Rhizobium* is not conferred by an arrest at a specific stage of the cell cycle. More likely mitotic reactivation is controlled by two oppositely oriented gradients of morphogens of which one is a specific lipo-oligosaccharide [Nod factors] secreted by Rhizobium. Concomitantly with the formation of nodule primordia, *Rhizobium* forms infection threads and by using cell cycle specific genes we showed that the infection process is derived from the mechanism controling cell division.
ENOD40 is an early nodulin gene that within a few hours is activated by Rhizobium Nod factors in the pericycle of the root. Studies with tobacco protoplast showed that ENOD40 influences plant phytohormone homeostasis or perception. We propose that the activity of this early nodulin is involved in the induction of cortical cell division.

A3-011 CELL CYCLE REGULATION DURING ROOT ORGANOGENESIS, Peter Doerner, Adán Colón Carmona, Jan-Elo Jørgensen, Johannes Steppuhn and Chris Lamb, Plant Biology Laboratory, The Salk Institute, La Jolla, California

Morphogenesis and organ formation occur post-embryonically in meristems. Meristem identity and activity are controlled by developmental pathways responsive to environmental signals such as light or the availability of mineral nutrients. Developmental pathways which control meristem identity determine the type of organs produced as well as the rate at which daughter cells are produced. During lateral root organogenesis, *de novo* meristem formation occurs when quiescent pericycle cells, which are arrested in the G2 phase of the cell cycle, are stimulated by mitogens to undergo cytokinesis within a few hours. The uniform G2-arrest of pericycle cells makes lateral root initiation suitable for the dissection of regulatory pathways that control meristem factivity.
We are interested how regulatory programs control the cell cycle engine at checkpoints in relation to organogenesis. We have previously shown that the functional homolog of cdc2 is expressed at high levels in all meristems in *Arabidopsis* and those tissues developmentally destined to become meristematic, such as the pericycle. Hence, cdc2 expression is not rate-determining for lateral root founder cell division. In contrast, Atcyc1, an *Arabidopsis* mitotic cyclin with homology to both A and B type cyclins, is expressed only in a subset of actively dividing cells within the root apical meristem. It is not expressed in quiescent pericycle cells, but strongly induced prior to the first cytokinetic event in the incipient lateral root meristem. Thus cyclin transcription is a candidate rate-limiting event for post-embryonic root organogenesis. We are currently testing this hypothesis by ectopic expression of cyclin to bypass this checkpoint. Furthermore, we will relate the biochemical activation of the cell cycle engine to the initial events of founder cell cytokinesis.

Membrane Signaling

A3-012 SECONDARY MESSENGER REGULATION OF PLANT K^+ CHANNELS, Sarah M. Assmann, Biology Department, The Pennsylvania State University 208 Mueller Laboratory, University Park, PA 16802

G-proteins are signal-transducing GTPases which link receptors to downstream effectors, including ion channels. We have evidence for G-protein regulation of two types of ion channels in plant cells: an inward-rectifying K^+ channel found in the plasma membrane of *Vicia faba* guard cells (1,2) and an outward-rectifying K^+ channel located in the plasma membrane of *Vicia* mesophyll cells (3). In guard cells, G-protein activation by the non-hydrolysable GTP analog, GTPγS, inhibits the inward K^+ channel. This inhibition is observed in isolated membrane patches, indicating G-protein action via a "membrane-delimited" pathway. Inhibition is also observed in the whole-cell configuration; in this case however, inhibition is eliminated upon buffering cytosolic free Ca^{2+} to low levels, indicating that G-protein regulation of the channel may also proceed via a second, cytosolic pathway, which requires Ca^{2+}. The phytohormone, abscisic acid (ABA), also inhibits these channels (4) in a Ca^{2+}-dependent manner, consistent with the hypothesis that the mechanism by which ABA inhibits stomatal opening includes a G-protein mediated inhibition of K^+ uptake. In mesophyll cells, GTPγS inhibits the outward K^+ channel. Since cAMP is a classic G-protein-regulated effector, we tested whether cAMP likewise inhibited outward K^+ current. This hypothesis was not supported; instead cAMP significantly enhanced outward K^+ current magnitude. This enhancement could be triggered by physiological levels of cAMP, provided that an inhibitor of phosphodiesterase, the enzyme which catalyses cAMP breakdown to AMP, was also provided in the patch pipette solution. These results suggest that cAMP may be a secondary messenger in plant cells (5).

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A3-013 AQUAPORINS FACILITATE WATER TRANSPORT THROUGH THE PLASMA MEMBRANE AND THE TONOPLAST,

Maarten J. Chrispeels[1], Mark J. Daniels[1], and Christophe Maurel[2]. [1]Department of Biology, University of California, San Diego, La Jolla CA 92093-0116 and [2]Institut des Sciences Végétales, CNRS, 91198 Gif-Sur-Yvette, France.

How does water go through a biological membrane? Simply by diffusion through regions where the lipid bilayer is disorganized, or is the passage of water facilitated by specific proteins? It has been known for some time that some membranes are much more permeable than others (about 10-fold) and radiation inactivation studies show that the "water channels" in the permeable membranes have a MW of 30 kDa. The tonoplasts (TP) and plasma membrane (PM) of plant cells and the PMs of mammalian cells contain intrinsic proteins of 27 kDa that are highly hydrophobic, have six membrane spanning domains and cytoplasmic C- and N-termini. These proteins belong to the MIP family of proteins. They are found in many plant species and occur in all organs. In some cases, there are organ-specific isoforms, such as the α-TIP present in the membranes of the protein storage vacuoles in seeds. Several of the plant and animal isoforms have recently been shown to facilitate the passage of water through the Xenopus oocyte PM, and in both animals and plants these proteins are referred to as "aquaporins." We are carrying out a detailed study of three aquaporins: the PM aquaporin known as RD28 and first identified by K. Shinozaki, the tonoplast aquaporins γ-TIP present in roots and shoots, and α-TIP found in the membranes of the protein storage vacuoles of seeds. These aquaporins enhance the water permeability of the membrane by 10 to 12-fold and constitute water-specific channels that exclude ions and metabolites. Different aquaporins appear to have unique features: γ-TIP is sensitive to mercuric chloride, but RD28 is not; α-TIP is phosphorylated and this phosphorylation regulates its activity. The function of aquaporins in the water economy of the plant will be discussed.

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A3-014 CALCIUM GRADIENTS AND CURRENTS IN POLLEN TUBE GROWTH, Peter K. Hepler, Elisabeth S. Pierson, Deborah D. Miller, Bruce A. Rivers and Dale A. Callaham, Department of Biology, University of Massachusetts, Amherst, MA 01003.

Growing pollen tubes of *Lilium*, *Tradescantia*, and *Nicotiana* possess a steep tip-focused gradient of free calcium. In lily pollen tubes that have been loaded with the calcium indicator, fura-2 dextran, the gradient extends from above 3 μM, at the extreme apex, to a basal level of 0.2 μM within a distance of 20-30 μM from the tip. Inhibition of pollen tube growth with caffeine (1.5 μM), elevated extracellular calcium (15 μM), increased osmoticum (from 10 to 17% sucrose), application of a mild temperature shock, or injection of several different BAPTA-type calcium buffers correlates with a dissipation of the tip-focused calcium gradient. Exploiting the BAPTA-type buffers, in which the various analogs differ in their affinity for calcium, we find that 5,5'-dibromo BAPTA (K_d = 1.5 μM), and 4,4'-difluoro BAPTA (K_d = 1.7 μM) are more effective at blocking pollen tube growth than either the higher affinity analogs, 5,5'-dimethyl BAPTA (K_d = 0.15 μM) and BAPTA (K_d = 0.22μM) or lower affinity analog 5-methyl, 5'-nitro BAPTA (K_d = 22 μM). These results affirm the importance of the tip-focused calcium gradient for pollen tube growth, and in addition they independently indicate that the high point of the gradient is greater than 1.7 μM but less than 22 μM. Recovery from growth inhibition typically involves swelling of the pollen tube tip, followed by the cylindrical extension of the tube. The intracellular calcium elevates markedly during tip swelling and subsequently becomes confined to a localized region of the swollen dome; it is from here that the cylindrical tube will extend.

Growing pollen tubes also possess a localized extracellular calcium current, between 1.4-14.0 pM/cm^2/sec, that is directed inward into the pollen tube at its apex. Conditions that inhibit growth and dissipate the tip-focused intracellular calcium gradient also eliminate the inward calcium current. If the tube recovers growth, there is a reemergence of the inward calcium current, with the initial tip swelling being accompanied by a particularly high transient influx; subsequently, the influx returns to normal levels.

Taken together, these studies reveal a strong correlation between pollen tube growth and the presence of both the intracellular calcium gradient and the extracellular calcium influx. These studies also provide direct support for the presence of calcium channels at the tip of the pollen tube. Since both the intracellular gradient and the extracellular influx can be eliminated by culture in high osmoticum, it seem plausible that some of these channels are stretch activated, being opened in response to turgor pressure. Supporting evidence comes from the observations of tubes recovering growth which reveal that the greatest level of intracellular calcium and the highest magnitude of calcium influx occur when the tip initially swells and the plasma membrane stretches. We suggest that the high calcium at the tip facilitates vesicle fusion, which is essential for pollen tube growth.

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A3-015 ABSCISIC ACID-MEDIATED SIGNAL TRANSDUCTION IN GUARD CELLS, Julian, I. Schroeder, Christian Schmidt, and John M. Ward, Department of Biology and Center for Molecular Genetics, University of California, San Diego, La Jolla, CA, 92093-0116

The plant hormone Abscisic acid (ABA) triggers stomatal closing in response to drought stress. Stomatal closing is mediated by efflux of K^+ and anions from guard cells. Recent research has suggested that slow anion channels are a central rate-limiting control mechanism for stomatal closure (1,2). Potent blockers of slow anion channels completely abolish ABA-induced stomatal closing (2). Patch clamp studies will be presented showing that phosphorylation and dephosphorylation of slow anion channels can serve to strongly activate and deactivate slow anion channels. This strong up-and down-regulation of slow anion channels correlates to the proposed rate-limiting control function of slow anion channels for ABA-mediated stomatal closing.

Stomatal closing requires K^+ efflux from guard cell vacuoles. An increase in the cytosolic Ca^{2+} concentration activates vacuolar SV channels and a novel type K^+ selective channel (VK) in the guard cell vacuolar membrane (3). VK channels may provide a mechanism for K^+ efflux from vacuoles during stomatal closing. Furthermore we have found that the broadly distributed slow vacuolar (SV) channels are Ca^{2+} selective in guard cell vacuoles suggesting that SV channels provide a mechanism for Ca^{2+}-induced Ca^{2+} release in higher plants. Data suggest that these SV channels are of central importance for ABA-mediated Ca^{2+} increases in guard cells.

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A3-016 Ca^{+2} STIMULATED PROTEIN KINASES ASSOCIATED WITH THE PLASMA MEMBRANE OF HIGHER PLANTS, Michael R. Sussman, University of Wisconsin, Program in Cellular and Molecular Biology and Department of Horticulture, 1575 Linden Drive, Madison, WI 53706.

The predominant Ca^{+2} sensitive protein kinase in extracts of many higher plants contains a carboxy terminal Mr=20,000 dalton domain with four EF hands resembling calmodulin (1). This C-terminal domain interacts with an adjacent autoinhibitory domain to regulate an amino terminal Mr=30,000 ser/thr protein kinase catalytic domain. This protein kinase is called CDPK for calmodulin domain protein kinase (also known as calcium dependent protein kinase), and has been observed so far in only two organisms besides higher plants, *Paramecium* and *Plasmodium* (malaria parasite). CDPK is unique because it requires only a binary complex for activity (i.e., kinase + calcium), in contrast to the ternary complexes required for the two known calcium sensitive protein kinases in animals and fungi (i.e., calcium+kinase+calmodulin for the Ca^{+2}/calmodulin kinase and calcium+kinase+diacylglycerol or phosphatidyl serine for protein kinase C). In *Arabidopsis thaliana*, CDPK is encoded by a large gene family, most of which contain an additional acidic amino-terminal domain with a myristoylation consensus sequence. My laboratory is engaged in sequencing cDNAs encoding seven different isoforms. The *E. coli* expressed CDPK cDNAs show catalytic properties similar to that observed with enzyme purified from plant extracts (2). Using gene specific *Arabidopsis* CDPK probes we are determining chromosomal map positions and with epitope 'tags' and reporter genes, we are determining the specific tissues and intracellular compartments in which each isoform's gene product is expressed. Biochemical experiments with plasma membrane vesicles purified from higher plant extracts indicate that several hydrophobic polypeptides, in addition to the polytopic proton pump (H^+-ATPase), are substrates for a membrane associated CDPK (3,4). Using *Arabidopsis* CDPK cDNAs expressed and purified to homogeneity from *E. coli*, we have confirmed that at physiological concentrations of ATP (1 mM), the proton pump is a substrate for this protein kinase. The pump is also encoded by a large multigene family in *Arabidopsis*, and conclusions from recent genetic and biochemical experiments on the molecular means of regulating this transporter will be summarized (5).

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The Tonoplast and Plasma Membrane

A3-017 MOLECULAR ANALYSIS OF SUGAR TRANSPORT, Christina Kühn, Brigitte Hirner, Lukas Bürkle, Jörg Riesmeier & Wolf B. Frommer, Institut für Genbiologische Forschung, D-14195 Berlin, Ihnestr. 63, Germany; Fax +49-30-83000736; email frommer@MPIMG-Berlin-Dahlem.MPG.DE

Sucrose is the principal transport form of assimilates in most plants. Depending on the density of plasmodesmata at the interface between the bundle sheath and the *sieve element companion cell complex*, plants have been grouped into symplastic or apoplastic loaders . In the so-called apoplastic species, translocation of assimilates from the mesophyll into the phloem for long distance transport is assumed to be carrier-mediated. Active proton-sucrose symport activities have been found in plasma membrane vesicles from a number of species.

Using a complementation system based on a yeast strain deficient in sucrose uptake, cDNAs encoding sucrose proton-cotransporters were isolated from spinach, potato and tobacco. The biochemical properties of the carriers as analyzed in yeast are similar to those described in plasma membrane vesicles. The transporter gene is mainly expressed in the phloem of mature, exporting leaves. To study the *in vivo* role and function of the protein, plants were transformed with antisense constructs of the sucrose transporter cDNA under control of various promoters. The effects observed in the antisense plants strongly support an apoplastic model for phloem loading, in which the sucrose transporter located at the phloem plasma membrane represents the primary route for sugar uptake into the long distance distribution network. The effects include accumulation of carbohydrates in the leaves, reduced efflux of sugars from the leaves and therefore reduced root development and tuber yield.

The active loading of the phloem is energized by H^+-ATPases. Several genes were cloned from potato and their expression relative to the sucrose transporter was analyzed. Sucrose synthase might play an indirect role in supplying ATP to the H^+-ATPases for active phloem loading. This is supported by its expression in the phloem and its inducibility by anaerobiosis and cold treatment .

The Er

A3-018 THE FAMILY OF ER RESIDENT PROTEINS AND THEIR ROLE IN PROTEIN BIOSYNTHESIS, Jürgen Denecke[1], Lena E. Carlsson[2], Sabina Vidal[3], Anna-Stina Höglund[4], Bo Ek[4], Mieke J. van Zeijl[5] and Karin M.C. Sinjorgo[5], [1]Dept. Biology, University of York, Heslington, York, YO1 5DD, UK, [2]Department of Microbiology, Swedish University of Agricultural Sciences, Box 7934 S-75007 Uppsala, Sweden, [3] Division Biologia Molecular, Instituto de Investigaciones Biológicas Clemente Estable, Av. Italia 3318, 11600 Montevideo, Uruguay. [4] Department of Cell Research, Swedish University of Agricultural Sciences, Box 7055, S-75007 Uppsala, Sweden, [5] Department of Molecular Plant Biotechnology, Center for Phytotechnology, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

The endoplasmic reticulum is responsible for the synthesis and transport of proteins to the Golgi complex. A multitude of regulatoraty mechanisms exist to co-ordinate the action of the vast variety of resident ER proteins in order to maintain its function and identity. To date only few proteins have been identified in plants which play a role in protein biosynthesis by the endoplasmic reticulum. The analysis of protein sorting signals responsible for the retention of reticuloplasmins (RPLs), a group of soluble proteins that reside in the lumen of the endoplasmic reticulum (ER), has revealed a structural similarity between mammalian and plant ER retention signals. We have used monoclonal antibodies which recognize this targeting signal to establish a sensitive procedure to identify ER resident proteins from crude microsomal preparations. We show evidence that the corresponding epitope is conserved in a vast family of soluble ER resident proteins which can thus be identified immunologically. Microsequence analysis and subsequent cDNA cloning have clearly established the presence of C-terminal consensus motifs known to be part of the ER retention signal employed by soluble ER resident proteins. Two abundant members of this reticuloplasmin family, RPL60 and RPL90, show high sequence similarity to mammalian calreticulin (CRP55) and endoplasmin (ERP99/GRP94). RPL60/calreticulin is a very abundant ER protein in cells of tobacco seedlings, and the protein is detected in the nuclear envelope, the cortical ER, in association with the spindle apparatus during cell divisions and close to newly formed cell plates immediately after cell division. RPL60/calreticulin was shown to bind to several polypeptides in a stress- and ATP dependent fashion similar to what has been obeserved for BiP. RPL60/calreticulin transcript levels increase rapidly in abundance during the proliferation of the secretory apparatus and the onset of hydrolase secretion in gibberellic acid treated barley aleurone cells. This induction occurs simultaneously to that of the well characterised ER chaperones BiP and endoplasmin, which points to a common role of these proteins in protein biosynthesis and quality control by the endomembrane system. However, differential responses to treatments with drugs, hormones, heat shock or cell wall degrading enzymes from pathogenic bacteria as well as differences in the tissue specific expression patterns suggest that RPL60/calreticulin does not always act in concert and may have a specialised role. Our work suggests the existence of several signal transduction pathways that control the synthesis of reticuloplasmins.

A3-019 **ASSEMBLY AND INTRACELLULAR TRANSPORT OF WHEAT STORAGE PROTEINS,** Gad Galili, Shirley Giorini-Silfen, Yuval Shimoni, Yoram. Altschuler, Hanna Levanony, Noam Shani, Hagai Karchi and Esra Galun. Department of Plant Genetics, The Weizmann Institute of Science, Rehovot 76100, Israel.

Wheat storage proteins are deposited in protein bodies inside vacuoles. Ultrastructural and molecular analyses were performed to study the assembly and intracellular transport of the storage proteins. Despite of their final deposition in vacuoles, we found that a large part of the storage proteins assemble into protein bodies within the endoplasmic reticulum (ER), the site of their entry into the endomembrane system. We also found that protein bodies that are formed within the ER are subsequently internalized into vacuoles by a process analogous to autophagy. Yet, assembly into protein bodies within the ER was not complete and some part of the storage proteins escaped this assembly process and were transported to the vacuole via the Golgi complex.

To study the mechanism of storage protein assembly and sorting within the ER, we have expressed wild-type and modified gliadin-type storage proteins in the heterologous systems of *Xenopus* oocytes and transgenic tobacco plants. The oocyte study showed that following sequestration into the ER, the gliadins can efficiently diffuse within the organelle. This suggested that the retention and packaging of the gliadins into protein bodies within the ER do not result from protein insolubility. Expression of a wheat gliadin in transgenic tobacco cells possessing various levels of the ER-resident chaperone BiP showed that this chaperone not only interacts with the gliadin, but it is also essential for the assembly of this storage protein into initial complexes of the protein bodies. Based on these results, we suggest that following sequestration into the ER, the gliadins interact with BiP and perhaps also with other ER resident chaperones and that such interactions may dictate the subsequent pathway taken by these storage proteins. On one hand, initial complexes between the gliadins and the ER resident chaperones may serve as basic elements for continuous assembly into protein bodies within the ER. However, some of the gliadins may dissociate from the complexes with the molecular chaperones before the initiation of protein body formation and thus export, apparently as monomers, to the Golgi complex. In addition, our results suggest that the N-terminal repetitive region may play a major role in the assembly of the gliadins within the ER.

A3-020 QUALITY CONTROL IN THE ENDOPLASMIC RETICULUM, Alessandro Vitale[1], Emanuela Pedrazzini[1], Anna Bielli[1], Giovanna Giovinazzo[1], Franco Faoro[2], Roberto Bollini[1] and Aldo Ceriotti[1], [1]Istituto Biosintesi Vegetali, C.N.R., via Bassini 15, [2]Centro Miglioramento Sanitario Colture Agrarie, C.N.R., via Celoria 2, 20133 Milano, Italy.

Secretory proteins start their life in the endoplasmic reticulum (ER). The folding of the newly-synthesized polypeptides and (in the case of multimeric proteins) the assembly of subunits are essential steps in the acquisition of the biological properties of any protein. In most cases this conformational maturation occurs before exit from the ER. Using bean phaseolin (PHSL, a homotrimeric vacuolar storage glycoprotein) as a model system, we are trying to characterize the cellular factors controlling these events and to define the relationships existing between protein maturation and trafficking in plant cells. Our results show that trimer formation is dependent on the rate of PHSL synthesis and is necessary for its intracellular transport out of the ER along the secretory pathway. Trimer formation plays a major role in determining the high resistance of PHSL to proteolytic degradation, and retention of PHSL until assembly has occurred may be essential for its stable accumulation in storage vacuoles. A fraction of newly-synthesized monomeric PHSL is found in ATP-dissociable complexes with the binding protein (BiP), the ER resident member of the heat-shock 70 family. When expressed in transgenic plants, a mutated, assembly-defective PHSL is found in prolonged association with BiP before being degraded. These observations strongly suggest that BiP normally plays a role both in favouring the correct conformational maturation of PHSL and in retaining PHSL monomers in the ER until maturation occurs. However, a consistent pool of trimeric PHSL is present in the ER of bean cotyledons, suggesting that after the release from BiP further control steps act before PHSL is transported out of this compartment. The antibiotic brefeldin A inhibits transport of wild type PHSL to the vacuole, but does not inhibit degradation of the assembly-defective mutant, indicating that the endomembrane system of plant cells is provided with a protein degradation machinery that is devoted to the disposal of structurally defective proteins and does not involve the normal pathway of delivery of soluble proteins to the vacuole.

Plastids

A3-021 MULTIPLE PRECURSOR-SPECIFIC PATHWAYS FOR PROTEIN TRANSPORT INTO THYLAKOIDS, K. Cline[1], R.Henry[1], J. Yuan[1], M. McCaffery[1], C. Li[1]., X. Li[2], and N. E. Hoffman[2]. [1]Horticultural Sciences Department and Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, FL 32611 and [2]Department of Plant Biology, Carnegie Institute of Washington, Stanford, CA 94305

Approximately 50% of thylakoid proteins are nuclear-encoded and cytosolically-synthesized. These polypeptides have the unique requirement of crossing two aqueous compartments and up to three membranes in order to achieve their functional locations. Import of thylakoid precursor proteins across the two plastid envelope membranes is governed by amino-terminal "transit peptides". Transit pepides direct the precursors into a high velocity general protein import apparatus that delivers them into the stroma, where the transit peptides are proteolytically removed. The resulting stromal intermediates then enter one of three thylakoid transport pathways. These pathways were initially distinguished by distinct energy and soluble factor requirements. However, competition studies with saturating concentrations of precursor proteins as well as comparative analyses of thylakoid targeting sequences have confirmed the existance of precursor-specific transport pathways. One pathway, which is responsible for transport of lumenal proteins found in modern day cyanobacteria [plastocyanin (PC) and the 33 kD OEC(OE33)], exhibits characteristics of the general protein export pathway of bacteria. Recent work has identified and purified a chloroplast homolog of the SecA protein, an essential component of bacterial protein export. The chloroplast SecA (CPSecA) is required for transport of PC and OE33, but not for proteins on other pathways. A component involved in the integration of the membrane protein LHCP on a second pathway has now been identified as a homolog of the 54 kD protein of the mammalian signal recognition particle (SRP) and the 48 kD protein of the bacterial SRP. The chloroplast 54K protein (54CP) binds LHCP upon it's import into the stroma and appears to chaperone/target it to the thylakoid membranes. The third pathway, which transports lumenal proteins not found in cyanobacteria, is unique in its reliance only on the trans-thylakoid ΔpH. Both of these latter pathways differ significantly from current day protein transport systems. These data fit a model for organellar routing called "Conservative Sorting" in which nuclear encoded chloroplast proteins are first imported into the stroma by a general import apparatus that evolved after the endosymbiotic event. The imported proteins then enter ancestral pathways that were initially present in the endosymbiont. Differences between transport of thylakoid proteins in plant chloroplasts and protein transport in contemporary prokaryotes may provide insights into the evolution of protein translocation systems.

A3-022 ENVELOPE COMPONENTS OF THE CHLOROPLASTIC PROTEIN TRANSPORT APPARATUS, Kenneth Keegstra, Mitsuru Akita, Jennifer Davila-Aponte, John Froehlich, Arun Goyal, and Pat Tranel, MSU/DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824

The general features of protein transport into chloroplasts have been described in considerable detail during the last decade. However, the molecular details of the transport process are still vague because most of the components of the transport apparatus have not been identified. In an effort to attack this problem we used a cross-linking strategy to identify two polypeptides, one of 75 kDa and one of 86 kDa, that are present in the outer envelope membrane and interact with precursor proteins (1). Current efforts to investigate the structure, function and biogenesis of these two proteins will be presented. Specifically, a cDNA clone for the 75 kDa polypeptide has been isolated. The sequence of the protein has no significant similarity to other proteins in the databases, indicating that it is a novel transport component. Unlike other outer membrane proteins studied to date, the 75 kDa protein is made as a larger precursor that is processed during or after targeting to the outer membrane. Additional details of the biogenesis of this protein will be presented. The deduced amino acid sequence of the 75 kDa protein lacks predicted hydrophobic helices characteristic of most membrane proteins, despite considerable evidence that it is an integral membrane protein. Our current efforts to understand the topology of the 75 kDa protein as well as its functions during the transport process will be presented. Finally, attempts to identify additional components of the transport apparatus are underway and these efforts will be presented.

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A3-023 CONTROL OF THYLAKOID MEMBRANE MORPHOGENESIS, L.Andrew Staehelin, Tanya G. Falbel, and Markus Sigrist, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO 80309-0347.

The light-dependent control of chloroplast development in angiosperms provides a convenient tool for studying the many processes involved in the assembly of functional photosynthetic membranes. When seedlings are grown in the dark, the proplastids of the developing leaves differentiate into etioplasts instead of chloroplasts because of their inability to reduce protochlorophyllide to chlorophyllide, the light-dependent step of chlorophyll (chl) synthesis. Upon illumination, chl accumulates rapidly together with other pigments and is incorporated into newly synthesized proteins to form stable chl-protein complexes, thereby producing functional chloroplasts. All types of photosynthetic activities become measurable within 4h of greening.
We have studied the light-controlled assembly of the different types of chl-protein complexes both by electron microscopical and biochemical techniques in wild type and chl b-deficient mutant plants. Our data show that in wild type barley the assembly of reaction center complexes precedes the development of light harvesting complexes (LHCs) and that the accumulation of type 1, 2 and 3 LHCII polypeptides and LHCI polypeptides occurs simultaneously and at similar rates. Our analysis of chl b-deficient mutants has demonstrated that all such mutants have a partial block in chl synthesis (> 90% at the MG-insertion step!) and a limited chl supply, which results in a pleiotropic mutant phenotype: a reduced chl content, preferential deficiency in chl b, reduced numbers of LHCs and grana-deficient thylakoids. The expression of this multi-faceted phenotype depends upon a combination of light intensity, temperature, the severity of the mutant lesion, and plant age. Light sensitivity of these mutants is most likely directly related to a bottleneck in chl biosynthesis resulting in the accumulation of a larger than normal pool of chl precursors which sensitizes the plants under high light. In support of this idea we have observed that the phenotype of mutants with severe but leaky bottlenecks grown at relatively mild, low-light conditions is similar to the phenotype of mutants with milder bottlenecks grown at relatively severe, high-light conditions. Together the complement of known chl b-deficient mutants generates a spectrum of phenotypes. Based on these and other findings we have developed a hypothesis that explains (1) how these pleiotropic phenotypes arise and (2) why the Mg-chelatase complex is the most likely candidate for the master regulator of chloroplast development.
Supported by NIH grant GM 22912 to LAS.

Keynote Lecture

A3-024 MEMBRANE TRAFFIC EARLY IN THE SECRETORY PATHWAY, Randy Schekman[1], Charles Barlowe[1], Tom Yeung[1], Sebastian Bednarek[1]; Nina Salama[1], Martin Latterich[1], Susan Hamamoto[1], and Lelio Orci[2], [1]Dept. of Molecular and Cell Biology and Howard Hughes Medical Institute, University of California, Berkeley, CA 94720, [2]Department of Morphology, University of Geneva, Switzerland CH-1211.

We have investigated the mechanism of secretory protein traffic by genetic and biochemical means in S. cerevisiae. sec mutations that block one of five stages in the secretory pathway have been isolated and the nature of the affected gene products deduced by molecular cloning. Cytosolic, membrane peripheral, and membrane integral proteins have been found that are required for secretory polypeptide translocation into the endoplasmic reticulum (ER), transport vesicle budding from the ER, transport vesicle targeting and fusion to the Golgi complex, protein transport within the Golgi complex, and targeting and fusion of mature secretory vesicles with the plasma membrane of a daughter cell.

A biochemical assay that reproduces the first half of the secretory pathway has been reconstituted with isolated membranes and cytosolic protein fractions. α-factor precursor (ppαF), a radioactive tracer of the pathway, is introduced by post-translational translocation into the ER in gently lysed yeast spheroplasts. The precursor is core glycosylated (gpαf) in the ER and outer chain carbohydrate is added as gpαF enters the Golgi complex. sec mutations block in vitro transport, and in certain instances the mutant defect is repaired by complementation with wild type cytosolic fractions.

We have purified three cytosolic Sec proteins that are required in vivo and in vitro for the formation of transport vesicles from the endoplasmic reticulum. This process is initiated by the recruitment of Sar1, a small GTP-binding protein, from the cytosol in a reaction that is mediated by the Sec12 protein, an ER membrane glycoprotein that stimulates nucleotide exchange by Sar1. Membrane-bound Sar1p-GTP recruits two heterooligomeric proteins, Sec23/24 and Sec13/31, to the membrane to create a novel coat protein (COPII) that surrounds and presumably shapes a vesicle as it forms from the ER. Upon completion of budding, the Sec23 subunit serves as a GTPase activating protein (GAP) to stimulate GTP hydrolysis by Sar1 which leads to loss of the coat material and the Sec protein subunits from the released vesicles. After release, the vesicles are free to engage and fuse with the Golgi apparatus, thus depositing transported glycoproteins in the next compartment of the secretory pathway. Vesicle formation and release may also be driven by nonhydrolyzable GTP (GMPPNP), but Sec23 GAP is not effective in stimulating Sar1 GTPase, consequently the vesicles retain the coat structure and fail to fuse with the Golgi complex. These properties are strikingly similar to the behavior of the molecularly distinct non-clathrin coat protein (COPI) which mediates vesicle budding in the Golgi complex.

Cytoplasmic Regulatory Mechanisms

A3-025 SUBCELLULAR COMPARTMENTATION OF AMINO ACID BIOSYNTHESIS: USING ARABIDOPSIS MUTANTS TO PROBE *IN VIVO* FUNCTION OF ORGANELLAR ISOENZYMES, Gloria Coruzzi, Karen Coschigano, Ming-Hsiun Hseih, Hon-Ming Lam, Rosana Oliveira, and Carolyn Schultz. New York University, Dept. of Biology, New York, N.Y. 10003

In plants, amino acid biosynthetic enzymes exist as distinct forms or isoenzymes which may be targeted to distinct subcellular compartments. Using biochemical methods, it has been impossible to determine whether the distinct subcellular forms of an enzyme serve overlapping or distinct roles in plant nitrogen metabolism. Molecular studies have shown that genes encoding chloroplastic or cytosolic isoenzymes of glutamine synthetase (GS) are expressed in distinct cell-types and hence serve non-overlapping roles in nitrogen assimilation (1). More recently, we have begun to study the function of isoenzymes involved in nitrogen assimilation into amino acids by isolating Arabidopsis mutants with lesions in a gene for a single isoenzyme. The phenotypic analysis of such mutants should shed light on the function of the "missing" isoenzyme. Our studies have focused on genes encoding isoenzymes involved in the assimilation of primary and "recycled" nitrogen into the amino acids glutamine, glutamate, aspartate, and asparagine. We have been studying the genes encoding chloroplastic and cytosolic forms of GS and those encoding plastid-localized glutamate synthase (ferredoxin-dependent GOGAT and NADH-dependent GOGAT). We have identified two genes for Fd-GOGAT in Arabidopsis and are determining which gene is affected in the previously isolated *gluS* photorespiratory mutants of Arabidopsis specifically deficient in Fd-GOGAT activity (2). We are attempting to define whether the primary role of the mitochondrial-localized glutamate dehydrogenase (GDH) enzyme is to synthesize or catabolize glutamate by characterizing the phenotype of GDH-minus mutants of Arabidopsis which we have isolated. Our molecular-genetic studies also include biosynthetic isoenzymes for the amino acid aspartate, which functions to shuttle nitrogen and carbon skeletons between subcellular compartments. Arabidopsis contains four genes for aspartate aminotransferase (AspAT) which encode plastid, mitochondrial, or cytosolic forms of the enzyme. We have identified Arabidopsis mutants deficient in either the plastid or cytosolic AspAT isoenzymes, and are also characterizing the phenotype of the double mutants. Finally, we are studying the regulation of nitrogen flow into the nitrogen-storage amino acid asparagine. The single gene for asparagine synthetase (Asn1) in Arabidopsis is regulated by light and metabolic control (3). These gene expression studies suggest that the Asn1 gene is regulated by sensing the pools of carbon and nitrogen and suggest that nitrogen is assimilated into asparagine under conditions of nitrogen excess or carbon limitation. The existence of multiple distinct subcellular forms of an enzyme and the cell-specific expression patterns indicate that the process of nitrogen assimilation into amino acids is more complicated in plants than it is in microorganisms. The long term goal of these studies is to construct a "blueprint" of the pathway of nitrogen assimilation into amino acids in a model plant and define the role of subcellular compartmentalization using molecular-genetic tools.

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A3-026 RNA DEGRADATION MACHINERY IN PLANTS, Pamela J. Green, Michael L. Abler, Pauline A. Bariola, Christie J. Howard, Michael L. Sullivan, Pedro Gil, and Ambro van Hoof, MSU-DOE Plant Research Laboratory and Department of Biochemistry, Michigan State University, East Lansing, MI 48824-1312.

The RNA degradation machinery of plant cells facilitates both selective and general RNA degradation pathways. As a first step toward elucidating mechanisms of selective mRNA degradation, we have begun to identify sequences that target transcripts for rapid decay in tobacco. Our studies have shown that DST sequences, highly conserved in unstable *SAUR* transcripts, can markedly destabilize reporter transcripts in tobacco cells. Critical sequences within two regions of the element are required for its instability function. Unlike DST elements which do not resemble well-known instability sequences in other systems, two additional sequences found to target transcripts for rapid decay in tobacco (AUUUA repeats and premature nonsense codons) have similar effects in mammalian cells. AUUUA repeats also appear to be recognized in *Arabidopsis* where there is potential to identify specific components of the degradation machinery through the isolation of mutants. Another aspect of our research focuses on ribonucleases (RNases) because these enzymes are expected to figure prominently in general and selective mRNA degradation pathways. Biochemical characterization of RNases using a substrate-based gel assay showed that *Arabidopsis* contains representatives of most of the major families of RNases classically described in higher plants and therefore should be a good model system to elucidate RNase function. A brute-force screen of M2 *Arabidopsis* plants using the substrate-based gel assay has yielded nine mutants exhibiting altered RNase profiles. These *arp* mutants are being characterized genetically for use in functional studies. We have isolated cDNA clones for three *Arabidopsis* RNases (RNS1, RNS2, and RNS3) that are related to, but distinguishable from, the S-RNases that are involved in gametophytic self-incompatibility in the *Solanaceae*. In *Arabidopsis*, which is self-compatible, the *RNS* gene products are likely to play a more fundamental role in RNA decay. The expression of *RNS1* and *RNS2* is induced during phosphate starvation and all three *RNS* genes are induced during senescence, indicating that the corresponding enzymes may participate in phosphate remobilization under these conditions. Sequence data suggest that all of the *RNS* gene products enter the secretory pathway because each contains a typical N-terminal signal sequence and RNS2 contains a putative C-terminal vacuolar targeting signal. Further analysis of these RNases in wild-type and mutant plants should provide additional insight as to their location and function in *Arabidopsis*.

A3-027 REGULATION OF A PROTEIN WHICH BINDS TO A FUNGAL ELICITOR-DESTABILIZED mRNA ENCODING A BEAN PROLINE-RICH PROTEIN, Shuqun Zhang[1], Yidong Liu[2] & Mona C. Mehdy[1] [1]Department of Botany, [2]Division of Biological Sciences, University of Texas at Austin, Austin, TX 78713

Regulation of mRNA stability constitutes one important determinant of gene expression. The differential turnover rates exhibited by individual mRNAs are generally believed to be due to their differential rates of degradation in the cytoplasmic compartment. One component of the plant defense response to pathogen attack is the rapid reduction in the steady-state levels of certain mRNAs. We have previously shown that the *PvPRP1* mRNA encoding a proline-rich protein is more rapidly degraded in bean cells after fungal elicitor treatment than in unelicited cells (1). A 50-kD protein present in cellular extracts was shown to specifically bind to the U-rich domain in the 3' untranslated region of the *PvPRP1* mRNA (2). On the basis of cell fractionation studies, the 50-kD protein appears to be located in the cytoplasm but not associated with polysomes. We now report the purification of a 41-kD *PvPRP1* mRNA binding protein by DEAE-Trisacryl, heparin-agarose and poly(U)-agarose chromatography. The 41-kD RNA binding protein is apparently a component of the 50-kD protein. Treatment of cellular extracts with 1 mM zinc results in the loss of the 50-kD RNA binding activity concomitant with the appearance of the 41-kD binding activity while subsequent treatment with the metal chelators EDTA or EGTA restores the 50-kD activity. Additional experiments aimed at defining the relationship between the 50-kD and 41-kD proteins will be described. Progress in sequence analysis of the purified 41-kD protein will also be reported. Prior work from our lab has suggested that the signaling pathway linking elicitor recognition and *PvPRP1* mRNA destabilization involves cellular redox alterations. Correlative evidence will be presented suggesting that the activity of the 50-kD PRP-BP may be regulated by an increase in glutathione levels which leads to the destabilization of the *PvPRP1* mRNA.

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Signaling to the Nucleus

A3-028 LIGHT AND INTRACELLULAR SIGNALING PATHWAYS CONTROLLING PHOTOREGULATED GENE EXPRESSION, Joanne Chory, Kim Cook, Richard Dixon[1], Tedd Elich, Hsou-min Li[1], Nobuyoshi Mochizuki, Punita Nagpal, Alan Pepper, Daniel Poole, and Jason Reed. Plant Biology Laboratory, The Salk Institute, La Jolla, CA 92037 and [1]The Samuel Roberts Noble Foundation, Ardmore, OK 73402.

We are taking a combined genetic and molecular biological approach to identify components of the light signal transduction pathways. Our studies have identified a class of Arabidopsis mutants that show many characteristics of light-grown plants even when grown in complete darkness (*de-etiolated*). Because these mutations are both pleiotropic and recessive, we have hypothesized that *DET* genes play a negative regulatory role in photoregulated gene expression and leaf and chloroplast development in Arabidopsis. To determine the molecular mechanisms of light signal transduction, we are cloning the various *DET* genes by the method of chromosome walking. *DET1* encodes a nuclear-localized protein that appears to be ubiquitously expressed during Arabidopsis development. We are testing the idea that DET1 acts to repress the expression of light-regulated promoters by interactions with other nuclear proteins and have identified one candidate protein with which DET1 appears to interact. In a separate set of experiments, we have identified mutations in two of the 5 Arabidopsis phytochrome apoprotein genes. Our results suggest that phytochrome B plays a general role in cell elongation in response to light and also controls flowering time and chlorophyll accumulation. In contrast, phytochrome A appears to play a highly specialized role in Arabidopsis development. The phenotype of *phyA phyB* doubly null mutants, however, indicates that for some processes PHYA and PHYB appear to have complementary functions. We are continuing our studies on phytochrome signaling by identifying and characterizing suppressors of *phyB* mutations. Lastly, we have utilized a genetic approach that focuses on one particular downstram light-regulated response, the transcription of the *CAB3* promoter. Using transgenic lines, we have identified mutations that define at least 5 complementation groups in which the *CAB3* promoter is expressed at aberrant high levels in the dark or low levels in the light. Some of the mutations also affect the levels of *RBCS* mRNAs, while others affect only *CAB*. We used the same transgenic lines to identify mutations in 3 genes defining an intracellular signal transduction pathway from the chloroplast to the nucleus. Presumably, this pathway functions to coordinate the expression of nuclear and plastid-encoded genes involved in photosynthesis. The mutations also impede the early steps in the transition from etioplast to chloroplast. The results from epistasis studies suggest a hierarchical regulatory network among the various genes in the control of light-regulated responses.

A3-029 CYTOSOL TO NUCLEUS SIGNALING IN THE ELICITATION RESPONSE, Richard A. Dixon[1], William P. Lindsay[1,2], Wolfgang Dröge[2], Weiting Ni[1], Nancy L. Paiva[1], Bomao Miao[1], and C.J. Lamb[2]. [1]Samuel Roberts Noble Foundation, Ardmore, OK 73402, [2]The Salk Institute, San Diego, CA 92037.

Plants activate multicomponent defense responses following infection with pathogenic microorganisms *in vivo* or exposure to elicitor macromolecules from the cell walls or culture fluids of these pathogens *in vitro*. These stimuli are perceived at the cell surface and transduced, by as yet poorly understood signal pathways, to the nucleus. This results in rapid transcriptional activation of sets of defense response genes encoding enzymes of antimicrobial phytoalexin biosynthesis, antimicrobial hydrolytic enzymes (chitinase and glucanase) and proteins involved in cell wall strengthening. Transcriptional activation of *PAL* and *CHS* genes, encoding enzymes of the early stages of isoflavonoid phytoalexin biosynthesis, occurs in less than 5 min in elicited bean and alfalfa cells, and is highly co-ordinated. Transcripion of other phytoalexin biosynthetic genes, including isoflavone reductase, is activated after a short lag period, whereas transcription of other defense genes (e.g. glucanase) is delayed by over 30 min. Functional analysis and/or *in vitro* DNA binding studies have identified *cis*-elements potentially involved in the transcriptional regulation of *CHS* and *IFR* genes. The *CHS* promoter contains three copies of a sequence termed the H-box, the TATA proximal copy of which lies 3′ to a G-box sequence and is necessary for transcriptional activation in response to biotic stress. However, the *IFR* promoter contains neither H-box nor G-box elements.

Biochemical and molecular studies have led to the characterization of several putative *trans*-factors which bind the H-box. KAP-1 and KAP-2 are proteins of Mr 97 and 76 kDa respectively, which have similar affinity for each of the three CHS H-boxes. KAP-1 also binds the G-box *cis*-element located 5′ of the TATA-proximal H-box, and a B-Zip factor has been cloned which likewise has affinity for both G-box and H-box in the *CHS* promoter. Biochemical studies indicate that levels of factors binding to the *CHS* and *IFR* promoters increase in nuclei of elicited cells, and that, in the case of KAP-1 and KAP-2, this may involve translocation of pre-existing factors from the cytoplasm, consistent with the rapidity of CHS activation. *De novo* synthesis of *trans*-factors may also be necessary to maintain levels of increased transcription to support the massive accumulation of defense products in elicited cells. Recent results will be discussed in the context of current models for defense gene signal transduction.

A3-030 EARLY AUXIN-REGULATED GENE EXPRESSION IN PLANTS, Athanasios Theologis. Plant Gene Expression Center, 800 Buchanan Street, Albany, CA 94710.

The plant hormone auxin, typified by indoleacetic acid (IAA), transcriptionally activates early genes in pea, soybean, tobacco and *Arabidopsis*. The signal transduction pathways(s) responsible for auxin-induced transcriptional activation and the role of the proteins encoded by the early genes in plant growth and development are unknown. We have been focusing our efforts to elucidate the mechanism of transcriptional activation by auxin using two early auxin-regulated genes *PS-IAA4/5* and *PS-IAA6* in pea. Their induction qualifies as a primary response to the hormone. It is rapid (5 - 7.5 min), specific, and does not require new protein synthesis, suggesting that the hormonal signal is transmitted to the nucleus via preexisting components. Protein synthesis inhibition induces the genes by transcriptional activation and mRNA stabilization. Auxin has no effect on the stability of the induced transcripts. Using a high fidelity transient assay system with protoplasts from the auxin responsive 3rd internode of pea seedling we have defined the auxin-responsive elements (*AuxRE*) of *PS-IAA4/5*. The *AuxRE* contains two domains, A and B. Domain A acts as the auxin switch, whereas domain B has an enhancer-like activity. The A and B domains contain conserved sequences found in various auxin regulated genes. The functionality of the *AuxRE* has been also verified by stable transformation experiments using *PS-IAAA4/5-GUS* fusions in tobacco plants. Putative transcription factors that interact with domains A and B have been isolated using molecular genetic approaches in yeast. Antibodies to the proteins encoded by *PS-IAA4/5* and *PS-IAA6* genes have been produced using proteins expressed in *E. coli*. The antibodies fail to recognize auxin inducible polypeptides on immunoblots from auxin treated tissue and were also unable to immunolocalize the proteins. However, the proteins can be readily detected after immunoprecipitation of *in vivo* labeled Met-S^{35} proteins. Pulse chase experiments have shown that the proteins are short-lived; their half-lives ($t_{½}$) are 8 and 6 min, respectively. Secondary structure analysis predicts that they contain a βαα DNA binding motif found in the prokaryotic repressors of the *arc* family. Biochemical and structural characterization of the plant βαα motif is in progress. Their role in early auxin action will be discussed and a model for the early auxin transcriptional activation will be presented.

Late Abstracts

REGULATION OF THE MAIZE ANTHOCYANIN PATHWAY INVOLVES PROTEIN/PROTEIN INTERACTIONS BETWEEN TRANSCRIPTION FACTORS, Vicki L. Chandler, Manuel B. Sainz, Steve A. Goff, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.

The genes encoding the anthocyanin biosynthetic enzymes are coordinately induced by two classes of transcriptional activators encoded by the *b/r* and *c1/pl* genes. B/R proteins contain a basic-helix-loop-helix motif (bHLH) found in several DNA binding proteins and C1/Pl proteins contain the DNA binding motif found in Myb proteins. Different *b* and *r* alleles activate the pathway in distinct tissues and at different developmental times. Characterization of several alleles has demonstrated that tissue-specific anthocyanin synthesis is regulated at the level of differential expression of the *b* or *r* genes. The important sequences map to the 5' flanking sequences, which are completely different for two *b* alleles (Radicella et al. 1992, Genes & Dev. 6:2152-2164.) Genetic experiments have demonstrated that B and C1 interact to stimulate the transcription of their target genes (Goff et al. 1992, Genes & Dev. 6:864-875.) The functional interaction occurs in both maize and yeast cells demonstrating other maize proteins are not required. The interaction domain within the C1 protein encompasses the Myb motif, whereas the NH_2-terminal interaction domain within B does not include the bHLH motif. The C1 protein has been shown to bind to a subset of the anthocyanin biosynthetic target genes. To begin to address which amino acids in C1 are important for the B interaction and DNA binding activities, mutagenesis of C1 was carried out. Mutations which affect C1 activity in maize cells, yeast, and in *in vitro* DNA binding assays will be described.

MOLECULAR INVESTIGATIONS OF THE CONTROL AND ELICITATION OF SELF-INCOMPATIBILITY IN *PAPAVER RHOEAS* **Noni Franklin-Tong, Jon Ride and Chris Franklin, *Wolfson Laboratory for Plant Molecular Biology, School of Biological Sciences, University of Birmingham, BIRMINGHAM B15 2TT, U.K.***

Self-incompatibility (SI) is the single most important outbreeding device found in angiosperms, operating by regulation of the acceptance or rejection of pollen. SI in *Papaver rhoeas* is gametophytically controlled by a single, multi-allelic S-gene. We are studying SI in this species from a number of angles in order to gain a fuller picture of the recognition, signalling and response mechanisms involved.

We have characterized and cloned the stigmatic S_1 and S_3 alleles of the S-gene from *P. rhoeas*. S-linkage has been demonstrated. The S-gene appears to be single copy and has tissue specific and developmental expression as expected. We have expressed the cloned sequences in *E. coli* and in transgenic *Nicotiana* to demonstrate that the recombinant S_1 and S3 proteins exhibit the S-specific biological activity expected. This S-gene is unrelated to any previously described genes, including other S-genes. Thus, the *Papaver* S-gene represents a distinct class of gametophytic S-gene, which is not a ribonuclease; the SI mechanism is therefore likely to be different. We are currently cloning two further S-alleles (S7 and S8) and using site-directed mutagenesis in order to identify the regions which confer S-specificity.

On the pollen side, we are currently attempting to clone the pollen receptor, using the stigmatic component as a ligand. Events occurring in the pollen during the SI response are also being investigated. The observation that pollen "response" genes appear to be activated during an incompatible reaction led to investigations of the signal transduction pathways involved in SI. We have demonstrated, using pollen microinjected with Ca^{2+}-binding dyes, that the inhibition of incompatible pollen tube growth induced by S-proteins is mediated by a transient rise in $[Ca^{2+}]_i$ acting as a "second messenger". There is preliminary evidence for the involvement of IP3 and for protein kinases and we hope to identify the genes switched on in the pollen as a response to the SI interaction.

CELL-CELL COMMUNICATION IN THE POLLINATION RESPONSES OF FLOWERING PLANTS, June B. Nasrallah, Section of Plant Biology, Division of Biological Sciences, Cornell University.

The interaction between pollen and stigmatic papillar cell in the self-incompatibility response of *Brassica* is based on the operation in the papillar cell of a receptor-like serine/threonine protein kinase encoded at the S locus. The self-incompatibility response is presumably precipitated by activation of the kinase through binding of a pollen-borne ligand to the extracellular domain of the receptor. The genetically defined self-incompatibility system is thus a useful model system for the study of intercellular communication in plants. Because the self-incompatibility reaction is under the control of a single Mendelian locus, all of the cell surface molecules involved in the interaction are expected to be encoded by genes clustered in one chromosomal location and are thus accessible to molecular analysis. Further, recent studies have suggested that the *Brassica* S-locus genes were recruited from genes that function during development of the plant body. Characterization of genes encoded in the S-locus complex and related genes are beginning to define elements of a general mechanism for plant intercellular communication, including a role for cell-wall localized proteins in signaling.

PLANT CELL SURFACE AND CELL INTERACTIONS, Maureen McCann, Fiona Corke, Nicola Stacey, Tim Baldwin, Adrian Turner, Brian Wells and Keith Roberts, Cell Biology Department, John Innes Centre, Colney, Norwich, NR4 7UH. UK.

Plant development is a complex process and at its heart is the behaviour of individual cells with respect to their neighbours. Cells are born in meristems and subsequently undergo an elaborately-controlled process of cell expansion and elongation that needs to be tightly co-ordinated with similar events in neighbouring cells. The molecular basis of cellular shaping, or cell morphogenesis, and the way in which neighbouring cells chat to each other effectively across their boundary wall, is largely unexplored territory. Both events, however, depend crucially on activity at the cell surface and these will be briefly reviewed. The structural rearrangement of wall polymers is a necessary corollary of cell shaping and expansion. There are certain prerequisites for expansion which include adequate turgor, wall loosening, wall synthesis and vesicle deposition. Disruption of any of these will affect expansion. Much less understood is the way that cells stop expanding and the role of wall "tightening" and the way that this may feed back on wall synthesis will be discussed. A major feature of our recent work is the recognition that cell walls from adjacent cells may have chemically and structurally distinct compositions and furthermore, within a single cell wall, there may exist domains with similar heterogeneity. Two aspects of this work will be discussed in more detail. First, arabinogalactan proteins (AGPs) are found associated with the plasma membrane and also as cell wall components, and they are regulated developmentally in a complex and cell-specific manner during a variety of developmental events. Work on the molecular characterisation of the major secreted carrot AGP will be discussed and their potential function as extracellular matrix attachment proteins explored. Second, local modifications of the wall, directly involved in local cell-cell interactions, are the plasmodesmata. We have recently made preparations from maize root tips enriched in plasmodesmata and have begun to characterise some of the proteins that may be associated with plasmodesmatal structure. A structural analysis using partial extraction methods, has revealed some new features of plasmodesmatal ultrastructure, and monoclonal antibodies to these preparations have identified a group of proteins that are candidates for plasmodesmatal components.

REGULATION OF INTRACELLULAR LIPID MOVEMENT IN HIGHER PLANTS, C.R. Somerville, M. Nikoloff, J. Ogas, Carnegie Institution, Department of Plant Biology, 290 Panama Street, Stanford, CA 94305

In many cells of higher plants, lipids are synthesized in plastids, mitochondria and the endoplasmic reticulum and may move from one compartment to another or may be secreted from the cell. The mechanisms involved in intracellular lipid transport are poorly defined in plant, animal or fungal cells. Lipid transfer proteins have been proposed to play a role in some aspects of lipid transfer. We have examined the role of one of the non-specific lipid transfer proteins in Arabidopsis by determining the tissue and cellular localization of the protein and by the production of transgenic plants in which the accumulation of the protein is strongly reduced. reduced levels of the protein had not apparent effect on the composition or amount of intracellular or extracellular lipid, although the transgenic plants did exhibit phenotypes that could not be directly related to lipids. Therefore, at present we have no direct evidence for the mechanism by which lipid move between intracellular membranes in plants.

In order to investigate the possible role of exocytotic vesicle transport in secretion of lipids, we have cloned two synaptobrevin-like cDNA clones from Arabidopsis. These clones are being used to develop appropriate probes for an investigation of where and when these proteins accumulate and the properties of plants in which their expression is reduced by antisense expression of the genes.

LEUCINE AMINOPEPTIDASE: A COMPLEX ARRAY OF PROTEINS INDUCED DURING THE PLANT DEFENSE RESPONSE, Linda L. Walling[1], Veronique Pautot[2], Yongqiang Gu[1], Wun S. Chao[1] and Frances M. Holzer[1], [1]Department of Botany and Plant Sciences, University of California, Riverside, California 92521 USA and [2]Laboratoire de Biologie Cellulaire, INRA, 78026 Versailles, France

An exoprotease with a high degree of similarity to the bovine leucine aminopeptidase and the *E. coli* XerB was identified as part of the tomato defense response. Leucine aminopeptidase (*lap*) mRNAs are induced in response to mechanical wounding, bacterial infection and insect infestation. After wounding, *lap* mRNAs are induced at the site of injury and systemically in the plant. 2D-PAGE immunoblot analyses using LAP polyclonal and affinity-purified antibodies indicate that tomato plants have four classes of LAP or LAP-like proteins. The LAP-like proteins are 77- and 66-kD in size and present in healthy and wounded leaves. The antibodies also detect two classes of 55-kD proteins with different pIs. The neutral LAP isoforms accumulate in both healthy and wounded leaves, whereas the acidic LAP isoforms are wound- and infection-induced. Low levels of two acidic LAP isoforms are detected in healthy leaves and five isoforms are abundant after wounding. Activity gels, immunoblot analyses and partial purification of native LAP from native PAGE gels reveals that the wound-induced LAP is a multimeric enzyme consisting of acidic 55-kD subunits; LAP activity is also induced after wounding. Two cDNA clones encoded by two *lap* genes have been isolated. Comparison of the sizes of mature LAP and protein translated *in vitro* indicates that LAP is synthesized as a pro-protein and is post-translationally processed. Immediately upstream from the N-terminal ends of the mature LAP subunits, there is a consensus commonly found in animal propeptide hormones. Transgenic tomato plants expressing a *lap* antisense gene have been used to test for the role of LAP in the plant defense response. Several roles for LAP in plant defense response will be discussed.

Cell Surface/Extracellular Matrix; Cell Adhesion, Cytoskeleton; Recognition Process; Exocytosis; Golgi

A3-100 IDENTIFICATION OF MICROFILAMENT ASSOCIATED PROTEINS IN COTTON

John M. Andersland and Barbara A. Triplett, ARS, USDA, Southern Regional Research Center, New Orleans, LA 70179

Actin filaments (microfilaments) carry out a variety of functions in plant cells, presumably in conjunction with other, less abundant proteins that may serve regulatory, structural or motor functions.

One approach for identifying these microfilament associated proteins is to selectively remove microfilaments from cytoskeletons and monitor the concomitant loss of other proteins. Any protein that is less abundant in cytoskeletons when actin filaments are removed may be specifically associated with microfilaments.

Cotton ovular trichomes (fiber cells) are an excellent source material for anucleate cytoskeletons. Cytoskeletons are isolated from trichomes in two steps. First, plasmolysed trichomes are enzymatically digested to produce anucleate cytoplasts. Next, cytoplasts are detergent extracted and centrifuged on a sucrose gradient to yield cytoskeletons.

Cytoskeletons produced by extracting cytoplasts with detergent plus millimolar Ca^{2+}, taxol (to stabilize microtubules), and phalloidin (to stabilize microfilaments) contained actin and tubulin as major proteins, and many minor proteins as judged by SDS-PAGE. When phalloidin was omitted from the extraction mixture, actin and a ~120 kD protein were lost from the isolated cytoskeleton preparations. The ~120 kD protein was not a contaminant of the phalloidin. The selective removal of the ~120 kD protein with actin suggests that this protein may be specifically associated with microfilaments. Progress on the characterization of this protein will be reported.

A3-101 ASSESSMENT OF ANNEXINS AS REGULATORS OF EXOCYTOSIS IN PLANT CELLS,

Nicholas Battey, Andrew Carroll, Pim van Kesteren, Alison McClung and Andrew Greenland[+], School of Plant Sciences, The University of Reading, Reading RG6 2AS, U.K., and [+]Zeneca Seeds plc, Jealott's Hill Research Station, Bracknell RG12 6EY, U.K.

Exocytosis is a key event in plant cell biology, because it delivers polysaccharide precursors to the cell wall, and membrane material and proteins to the plasma membrane[1]. The annexins are a family of proteins that bind to membranes in the presence of Ca^{2+}, and have potential importance as regulators of exocytosis[3]. Consistent with this, we have previously shown that maize annexins p35/p33 cause aggregation of secretory vesicles[2]. However, exocytosis is a complex process, involving vesicle targetting, docking and fusion, and much more information is needed to clarify the role that annexins play. We are currently using a combination of approaches to achieve this.

* cDNA sequence analysis: maize annexin p33 has fewer predicted Ca^{2+}-binding sites than animal annexins, consistent with a higher Ca^{2+} requirement for membrane association.
* Binding studies: there is no evidence of cooperativity, implying that a mechanism other than polymerisation is responsible for the vesicle aggregation induced by maize annexins.
* Cell-free fusion assays: suggest that maize annexins are not directly fusigenic .

This work is funded by the BBSRC, Leverhulme Trust, Royal Society, University of Reading Research Endowment Trust Fund, and Zeneca Seeds plc.

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A3-102 ECTOPIC CONTROL OF VASCULAR DIFFERENTIATION BY A PARASITIC NEMATODE, David M. Bird, Mark A. Wilson and Jennifer S. Becker, Department of Nematology, University of California, Riverside, CA 92521.

Root-knot nematodes (*Meloidogyne* spp.) induce an apparently novel cell type (called a *giant cell*) in the roots of infected plants. Morphologically, giant cells share some features with developing xylem cells, including increased cytoplasmic density, high metabolic activity, multiple nuclei and extensively remodeled cell walls. Giant cells serve as the obligate nutritive source for the developing nematode, which ultimately becomes sedentary.

To understand the transcriptional basis for the parasitic interaction, a subtracted cDNA library was generated from a small number of hand-dissected tomato giant cells. This bank contains 287 clones encoding the 3'-ends of tomato genes whose expression is up-regulated (compared to healthy root tissue) during feeding by *M. incognita*. We have analyzed ~125 of these clones by sequencing, and their expression in a range of tomato tissues has been determined. Nearly all the clones define unique or low copy number genes. A global picture is emerging of elevated expression in giant cells of transcripts that also are expressed in young and expanding tissues, including meristematic and vascular precursor cells. Function can be assigned to many of these transcripts, which can be grouped into categories (including signal reception/transduction; differentiated giant cell function; anti-sense transcripts; general metabolic up-regulation).

We have begun to localize giant cell transcripts in infected and healthy plants by *in situ* analysis. Our most extensive data is for the *LeUbc4* gene which encodes a ubiquitin-conjugating enzyme abundantly expressed in giant cells and also in vascular parenchyma cells. This is consistent with a model in which giant cell formation shares features with, and perhaps is a modified form of, metaxylem differentiation.

A3-103 THE CYTOSKELETON IN MORPHOGENESIS OF ENDOSPERM, R. C. Brown, B. E. Lemmon and O.-A. Olsen, Department of Biology, University of Southwestern Louisiana, Lafayette, LA 70504 and Plant Molecular Biology Laboratory, Agricultural University of Norway, P.O. Box 5051, 1430 Aas, Norway

The endosperm is emerging as a model system for investigating the cellular and molecular processes of plant morphogenesis. Endosperm develops rapidly, is structurally simple, and mutants affecting its development are known. The microtubular cytoskeleton undergoes dynamic reorganization in association with events of endosperm development in barley. Not until the final stages of aleurone differentiation is the microtubule cycle typical of plant histogenesis. In the syncytium, where both components of the cytokinetic apparatus (preprophase bands of microtubules and phragmoplasts) are absent, nuclear-based radial microtubule systems organize the cytoplasm into units of nuclear cytoplasmic domains (NCDs), and control placement of the initial walls during cellularization. Walls deposited at the boundaries of NCDs compartmentalize the peripheral syncytium in an alveolate pattern. Microtubules, which emanate from nuclei in the peripheral compartments, extend into the canopy of cytoplasm that advances into the central vacuole. The onset of phragmoplasts occurs soon after the initial walls are deposited. At this time, phragmoplasts develop adventitiously wherever opposing microtubule systems interact in the cytoplasm as well as in the interzones after mitosis. Since variations in the microtubular cycle reflect underlying mechanisms that control distinct phases of endosperm morphogenesis, the system offers potential for using genetic mutants to further our understanding of the fundamentals of plant histogenesis.

A3-104 A NOVEL *IN VITRO* ASSAY OF EXOCYTOSIS IN PLANT CELLS.

Andrew Carroll, Pim van Kesteren, Nicholas Battey, School of Plant Sciences, University of Reading, Whiteknights, Reading RG6 2AS UK.

Exocytosis is a pivotal step in many aspects of plant development and cell physiology, but the mechanisms of control are largely unknown. Understanding the nature and function of putative regulators is dependent upon reliable methods for the measurement of exocytosis.

In this poster we present a novel method for assaying exocytosis *in vitro* using membranes prepared from a highly secretory system, the maize root tip. The method is based upon the detection of transfer of a radioactive marker from a donor fraction to an acceptor fraction and the subsequent isolation of the acceptor fraction and analysis of the amount of radioactivity transferred. Donor membranes consist of a radioactively labelled membrane fraction enriched in secretory vesicles and prepared by gel filtration of a Golgi apparatus-enriched root tip microsomal membrane fraction. The acceptor membranes consist of plasma membrane vesicles prepared by phase partitioning from maize coleoptile microsomal membranes. Repeated cycles of flash freezing and thawing of the plasma membrane vesicles enables us to produce a population of vesicles which are predominantly of the 'cytoplasmic side out' orientation. Acceptor membranes, carrying radioactivity transferred by fusion of the donor membranes, are subsequently separated using biotin/avidin affinity beads. Here we present data confirming the validity of this method as a reliable *in vitro* assay of exocytosis. This work was funded by the BBSRC and the Leverhume Trust.

A3-105 PEA LECTIN ASSOCIATES WITH LIPID MONOLAYERS,

Sylvia de Pater[1], Pettie Booij[2], Rudy Demel[3] and Jan Kijne[1,2], [1]Center for Phytotechnology and [2]Institute of Molecular Plant Sciences, Leiden University, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands, [3]Centre for Biomembranes and Lipid Enzymology, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands.

Pea lectin (PSL) is a secretory sugar-binding protein, abundantly present in pea seeds in which it is targeted to protein storage vacuoles. Much less PSL is produced in roots, where it has an important function in determination of the Rhizobium host range for formation of nitrogen-fixing root nodules. PSL is secreted in the rhizosphere, but is also present on the outer surface of the plasma membrane at the tip of growing root hairs and in root hair-forming cells. This membrane association is not abolished in the presence of haptenic sugars, which suggests that the sugar binding sites of PSL are not involved in this interaction. We tested PSL-lipid interactions by means of the monolayer technique (Wilhelmy plate method). PSL was found to be able to insert in lipid monolayers. The rate of insertion was dependent on the pH of the buffer. Tests with a sugar-binding mutant of PSL or with wild-type PSL in the presence of haptenic sugars showed that the PSL-lipid association is independent of the sugar binding ability of the protein. This result opens the possibility that PSL is directly involved in membrane-mediated root hair infection after binding of rhizobial ligands.

A3-106 MOLECULAR CHARACTERISATION OF THE GOLGI APPARATUS OF *ARABIDOPSIS THALIANA*.

Paul Dupree, Department of Plant Sciences, Cambridge University, Downing Street, Cambridge CB2 3EA, United Kingdom

Many of the biosynthetic and sorting capabilites of the plant Golgi complex are essential functions specific to plant cells. However, the compartmental organisation and mechanisms of operation of this organelle remain unclear. Studies of these aspects of Golgi function have been hindered by the paucity of molecular marker proteins for Golgi subcompartments. I have begun a project with the aim of identifying and characterising such proteins. Individual proteins are followed by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). The approach is based on a comparison of protein components between cellular fractions at successive stages of compartment enrichment. Since each subcellular compartment has a unique 'Rab signature', the presence of small GTP-binding proteins of the Rab family has also been investigated using the GTP-overlay technique after 2D-PAGE. Images of 2D gels showing the protein compositions of various *Arabidopsis thaliana* membranes and compartments will be presented.

A3-107 EXPRESSION OF PECTATE LYASE IN TOBACCO PISTILS RESULTS IN FEMALE STERILITY, Tina Grater, Jeffrey Stein, Trang Le, Stacy Harper, Linda Mercer Dawson and Lyle Crossland, Ciba-Geigy Agricultural Biotechnology, Research Triangle Park, NC 27709

Production of pectate lyases by plant pathogenic bacteria in susceptible hosts results in the maceration and soft-rotting of tissue. In this study, we evaluated whether production of pectate lyase in the pistil tissue of tobacco would interfere with its ability to be pollinated and produce viable seed. The *pelE* gene from *Erwinia chrysanthemi* was fused to the 260bp SLG promoter from Brassica (Dzelkalns et al. 1993, Plant Cell 5:855-863), previously demonstrated to be active exclusively in the stigma and style of transgenic tobacco. This construct was cotransformed into tobacco leaf tissue (Xanthi) with a second plasmid harboring a plant-active NPTII gene, using a helium-powered particle gun. Following selection and regeneration on kanamycin, plants were transferred to the greenhouse. Nine of these 26 primary transformants failed to either self pollinate or produce pods when backcrossed with wild-type pollen. Pollen from six of these lines was outcrossed to obtain T1 seed, and the female-sterile phenotype was found to be stably inherited. Northern analysis of southern positive plants detected an mRNA of the expected size for *pelE*, and the presence of the *pelE* protein was confirmed by western analysis. Microscopic observation revealed that the transmitting tissue of pistils from *pelE*-transformed plants appeared watersoaked and tended to oxidize quickly following dissection. Pollen germination on these pistils was substantially reduced, and germ tubes that did form were short and deformed compared to controls.

A3-108 REGULATION BY ETHYLENE OF CELL WALL BOUND INVERTASE EXPRESSION IN AUTOTROPHIC *CHENOPODIUM RUBRUM* CELL CULTURES. James C. Linden*, Thomas Roitsch and Rainer Ehneß. Lehrstuhl für Zellbiologie und Pflanzenphysiologie der Universität Regensburg, 93040 Regensburg, Germany and *Colorado State University, Fort Collins, CO 80523

Physiological concentrations of ethylene dissolved in the growth medium of autotrophic *Chenopodium rubrum* repressed specific activity and messenger RNA synthesis for cell wall bound invertase. Reductions in cell wall bound invertase activity by 30 to 50 percent, compared to controls, were measured following incubation with 0.1 ppm ethylene in the growth medium for 12 to 36 hours. Isolation of RNA from cells handled in a similar manner for 24 and 36 hours with and without 0.1 ppm ethylene were subjected to northern blot analysis using homologous probes for cell wall bound invertase and sucrose synthase. In each case reductions of approximately 50 percent in probe binding were observed to RNA isolated from ethylene treated materials, compared to controls.

The cytoplasmic invertases are of acidic and basic forms and have been shown to be distinct genes from the cell wall invertase. These invertases are thought to be involved in compartmentalization of sucrose in internal pools, such as in the vacuole. The specific activities of the intracellular invertases following ethylene treatment were very similar to the controls. Because these activities do not appear to be regulated by ethylene (homologous probes for hybridization studies are not available), an effect of ethylene may hypothetically be in regulation of uptake and compartmentalization of carbon nutrients in the plant cell.

A3-109 A NEW CLASS OF PROTEINS SYNTHESISED IN THE TAPETUM AND TARGETED TO THE POLLEN EXTRACELLULAR MATRIX IN THE *BRASSICACEAE*, Denis J. Murphy and Joanne H.E. Ross, Department of Brassica and Oilseeds Research, John Innes Centre, Norwich, NR4 7UH, United Kingdom.

Oleosins are abundant amphipathic proteins found in most seeds where they encapsulate the intracellular lipid storage bodies [1]. We recently described the purification and partial sequencing of an oleosin-like protein from intracellular pollen lipid-body preparations and the isolation of homologous cDNA and genomic clones [2,3]. Further studies have now revealed a third class of oleosin-like protein which is one of the most abundant components of the extracellular matrix of pollen grains in several species of the *Brassicaceae*, including rapeseed and *Arabidopsis* [4,5,6, D. Preuss, pers. commun.]. The extracellular pollen oleosins share many sequence similarities with their intracellular homologs but their function is unknown. They may bind the extracellular lipids found in the pollen tryphine and may also interact with components of the sculpted pollen wall. Interestingly, the extracellular oleosins contain a glycine-rich domain which is similar to regions found in several cell-wall proteins [4-7]. In this report, the structures and possible evolutionary relationships between all three classes of oleosins are compared and the putative role of the pollen extracellular oleosins in signalling events between pollen and stigma cell surfaces is discussed.

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A3-110 ANALYSIS OF THE MECHANISM OF POLYSACCHARIDES BIOSYNTHESIS IN GOLGI VESICLES FROM PEA STEMS, Ariel Orellana, Lorena Norambuena and Patricia Muñoz, Departamento de Biologia, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile.

The primary cell wall is made out of 90% polysaccharides. Cellulose, hemicellulose and pectin are the more abundant polysaccharides present in the primary cell wall. Biosynthesis of hemicellulose and pectin occur in the Golgi apparatus and then they are secreted in vesicles. There is evidence that biosynthesis of these elements is a luminal process within the Golgi apparatus. However, nucleotides sugar which are the substrates for biosynthesis are present in the cytoplasm. Therefore there is a question regarding the topology and mechanism of polysaccharides biosynthesis, and what other components besides the glicosyltransferases or syntheses are involved in the biosynthetic machinery. To address this question we have attempted to reproduce the biosynthetic process "*in vitro*" using sealed right-side-out Golgi vesicles derived from pea stems. Vesicles incubated at 25 C in 1 uM UDP-[3H]glucose and then separated from the medium by filtration, incorporated the substrate in a temperature dependent manner. At least 50% of the label associated to vesicles is insoluble in 70% ethanol. Linear sucrose gradient analysis showed that the incorporation is specific for Golgi apparatus. The time course of this incorporation has biphasic behavior. The use of DIDS, an anionic blocking agent, impermeable to vesicles produces a very strong inhibition of the incorporation. Permeabilization of the membranes with detergent produce an effect that is dependent upon the concentration of UDP-glucose. Preliminary analysis using [3H]UDP-glucose suggest that UDP-glucose it would be completely incorporated into the lumen of the Golgi vesicles. These results suggest the presence of an UDP-glucose transporter in the membrane of the Golgi apparatus, and a mechanism of biosynthesis of polysaccharides in the Golgi apparatus from plant cells similar to the mechanism proposed for biosynthesis of proteoglycans and modifications of glycoproteins in Golgi from mammals cells. Supported by: Grant Fondecyt 1940571 and PEW Latin-American Postdoctoral Fellowship.

A3-111 CELL SIGNALING DURING FERTILIZATION : INTERACTIONS BETWEEN THE POLLEN COATING AND THE STIGMA, Daphne Preuss and Ron W. Davis, Stanford University, Department of Biochemistry, Beckman Center, B400, Stanford, CA 94305

To ensure that their eggs are fertilized by sperm cells from their own species, plants have evolved the means to recognize and inhibit the germination of foreign pollen. This is accomplished through a series of cell-cell interactions that begin at the uppermost portion of the pistil. As a first step toward understanding these interactions at the molecular level, we have identified hundreds of sterile mutations in *Arabidopsis*. Many of these mutant plants are defective in pollen-pistil communication.

We have characterized in detail a male-sterile mutation (*cer6-2*) that affects fertilization at an early step. Stigma cells respond to the mutant pollen as if it were foreign and prevent its germination by blocking pollen hydration. The mutant pollen lacks an extracellular coating, which in wild-type plants consists of long-chain lipids and nine lipophilic proteins. We have identified two of the major pollen coat proteins and are presently investigating their role in fertilization.

We have shown that *cer6-2* male-sterility can be reversed by hydrating the mutant pollen in a humid chamber. The conditional nature of this mutation has allowed us to obtain suppressing mutations. Some of these restore fertility to the pollen, while others affect the stigma's ability to discriminate among pollen grains. We are using these mutants to identify molecules that play a role in pollen-stigma signaling. Finally, because addition of wild-type pollen coat extract to *cer6-2* pollen rescues fertility, the critical signaling component(s) can be purified. These studies ultimately will provide insight into the mechanisms that allow plants to discriminate among pollen grains.

A3-112 **SPECIFIC PERCEPTION OF TWO OLIGOSACCHARIDE SIGNALS WHICH INDUCE DEFENSE RESOPONSES IN SUSPENSION-CULTURED RICE CELLS AT nM RANGE**

Naoto Shibuya, Akira Yamada, Noriaki Ebisu#, Yoshiaki Kamada*, Hanae Kaku, Kazuyuki Kuchitsu and Yuki Ito. ***Dept. Cell. Biol., Natl. Inst. Agrobiol. Resources, Tsukuba, Ibaraki, 305 Japan. Present addresses; #Okinawa Agric. Exp. St.; *Johns Hopkins Univ.***

Oligosaccharide elicitors derived from the cell surface of pathogenic microbes as well as host plants are known to induce various defense reactions in plants. However, molecular basis of the specific perception and transduction of these signals largely remain unsolved.

We recently found that two types of oligosaccharides, both potentially derived from the cell walls of pathogenic fungi, could act as potent elicitor in suspension-cultured rice cells. *N*-acetylchitooligosaccharides of specific size induced phytoalexin (momilactones) formation in the rice cells even at nM range, while the deacetylated forms did not[1]). Specific fragments obtained by the partial acid or enzymatic hydrolysis of β-glucan from rice blast fungus, *Pyricularia oryzae* , also showed potent elicitor activity at nM range. Comparative studies with soybean cotyledon cells indicated that the rice cells recognize quite different oligosaccharides as the elicitor signal compared to the soybean cells.

Binding experiments with an ^{125}I-labeled tyramine derivative of *N*-acetylchitooctaose showed the presence of a specific, high-affinity binding site for this elicitor on the microsomal[2]) as well as plasma membrane of the cultured rice cells. Affinity constant obtained from these experiments (nM-10nM range) well corresponded to the concentration with which this oligosaccharide induced responses in the cultured cells. Inhibition studies with various oligosaccharides also showed the binding specificity of this site corresponded well with that observed for the cultured cells. These results support the possibility that this binding site represents a true, functional receptor for this elicitor.

Various cellular responses, including early membrane responses such as the changes in membrane potential[3]) and ion flux, oxidative burst, protein phosphorylation, induction of jasmonic acid, expression of genes relating to defense reactions, are also induced by the *N*-acetylchitooligosaccharide elicitor and have been the subject of collaborative studies.

1) A. Yamada *et al., Biosci. Biotech. Biochem.*, **57**, 405 ('93); 2) N. Shibuya *et al., FEBS Lett.*, **32**, 75 ('93); 3)K. Kuchitsu *et al., Protoplasma*, **174**, 79 ('93)

A3-113 POTATO TUBER LECTIN SHARES SEQUENCE HOMOLOGY TO EXTENSINS, THE HEVEIN LECTIN FAMILY, AND SNAKE VENOM DISINTEGRINS (PLATELET AGGREGATION INHIBITORS)

Allan M. Showalter[1], Marcia J. Kieliszewski[2], Joseph F. Leykam[3], Jinsong Sheng[1] and Zhixiong Yuan[1], [1]Dept. of Environmental and Plant Biology, Molecular and Cellular Biology Program, Ohio University, Athens, OH 45701; [2]Complex Carbohydrate Research Center, University of Georgia, Athens, GA 30602; [3]Biochemistry Dept., Michigan State University, East Lansing, MI 48824

Potato tuber lectin (PTL) is a chimeric protein composed of a chitin-binding lectin domain fused to a hydroxyproline-rich protein domain. Amino acid sequence analysis of seven peptides derived from chymotryptic digestion of reduced S-pyridylethylated PTL yielded sequences with homology to certain "P3" type extensins (i.e., LPSOOOOOO(H)OSOOOOSOOSOOOOSOO-) and to the chitin-binding domains of wheat germ agglutinin and other members of the hevein lectin family (i.e., CGTTSDY, CSPGY, and TGECCSI). Additionally, all plant chitin-binding domains examined shared remarkable sequence similarity to snake venom disintegrins (platelet aggregation inhibitors), indicating that a common archetypal binding module may have given rise to both the chitin-binding domains of plants and the reptile disintegrins. Efforts to clone PTL have been unsuccessful to date, but have resulted in the isolation and characterization of several potato extensin genomic clones. One such potato extensin clone, Pot 1, is described here. Pot 1 resides on a 1.5 kb Hind III-Eco RI fragment and encodes a sequence consisting of several SSPPPPPP and SPPPPTYYY repeats and is highly homologous to one of our previously characterized tomato genomic extensin clones, Tom L4.

A3-114 FUNCTIONAL STUDIES OF ACTIN-DEPOLYMERIZING FACTORS FROM *ARABIDOPSIS THALIANA*..

Chris Staiger and Sharon Ashworth, Dept. of Biological Sciences, Purdue University, West Lafayette, IN 47907

The actin cytoskeleton of plant cells participates in diverse intracellular processes including cytokinesis, cytoplasmic streaming, tip growth and pollen tube extension. The dynamic nature of the actin cytoskeleton is regulated by actin-associated proteins, very few of which have been identified in plants. We have characterized two *Arabidopsis thaliana* cDNA clones that show a high degree of sequence similiarity to published non-plant actin depolymerizing factors (ADF). Members of this family of G-actin binding proteins include: cofilins from mammals and yeast, and actophorin from *Acanthamoeba*.. The two *Arabidopsis* clones, ADF1 and ADF2, encode putative polypeptides of 139 and 137 amino acids, respectively, both with calculated molecular weights of ~16 kDa. Their deduced amino acid sequences are 80% identical to each other and 70% identical to ADF-like clones from *Lilium* and *Brassica*. *Arabidopsis* ADF1 shares 48% identity with actophorin and 40% identity with yeast cofilin. Both ADF1 and ADF2 contain a stretch of 25 amino acids in their c-terminal region with 50-60% identity to the postulated actin-binding site of pig cofilin. To study the functional properties of ADF1 and ADF2 we have over-expressed them in *E coli* as translational fusions with glutathione S-transferase. Binding studies with purified, recombinant ADF and maize pollen extracts demonstrate a functional interaction between these proteins and actin. In addition, the recombinant proteins show a functional interaction with vertebrate skeletal muscle actin that is dependent upon pH. ADF1 and ADF2 bind and co-sediment with rabbit muscle F-actin at neutral pH. At higher pH, the two ADFs cause depolymerization of F-actin, resulting in an increased concentration of monomeric actin in the supernatant. Rabbit polyclonal antisera are being raised against these recombinant proteins for further biochemical and immunocytochemical studies. The combination of sequence data and *in vitro* binding studies suggest that plant ADFs are indeed functional actin depolymerizing factors. The role of ADFs in signal-induced cytoskeletal reorganization will be the subject of future investigations.

A3-115 CLONING OF A FUNGAL HYDROPHOBIN EXPRESSED EARLY IN EUCALYPT MYCORRHIZA DEVELOPMENT, Denis Tagu and Francis Martin., Microbiologie Forestière, INRA, 54280 Seichamps, France.

Ectomycorrhizal symbiosis between trees and soil-borne ectomycorrhizal fungi yields an intimate relationship between the plant and its symbiotic partner. The development of ectomycorrhiza involves the differentiation of structurally specialised fungal tissues and interfaces between the symbionts and a highly co-ordinated metabolic interplay. Morphogenesis of ectomycorrhiza results from the expression of plant and fungal developmental programmes and a complex set of signals triggers morphogenetic and physiological changes.

In order to characterized genes involved in the differenciation of the fungal mycorrhizal tissues, a differential screening was performed on a cDNA library from eucalypt ectomycorrhiza. Fungal mRNA populations were characterized and about 50 % of these populations were affected by the formation of the symbiotic organ. One of the clone shared identities with hydrophobins; these are small fungal polypetides involved in multilayer hyphae differentiation and in pathogenicity. The amino acid sequence is characterized by the presence of conserved 8 and by a high hydrophobicity. Our cDNA clone encoded a protein which harboured these characteristics. In ectomycorrhiza, we demonstrated that over accumulation of hydrophobin transcripts took place very early in the colonization process. This could serve as a molecular marker of mycorrhiza differentation and could be a tool for analysing putative root signals triggering symbiotic development.

The role of these hydrophobic proteins on the mycorrhiza formation will be discuss.

A3-116 DO FIBRONECTIN ANALOGUES EXIST IN HIGHER PLANTS? ANOMALOUS RECOGNITION OF A COTTON FIBER PROTEIN BY ANTI-FIBRONECTIN ANTIBODIES,
Barbara A. Triplett, USDA, ARS, Southern Regional Research Center, New Orleans, Louisiana 70179

Recent reports suggest that extracellular matrix components from animal cells may have homologues in plants[1,2,3,4]. The identification of cotton fiber cell wall components is necessary for establishing how fiber physical properties are determined. Cotton fibers are single, very long (>2.5 cm) ovule epidermal cells that, at maturity, have a highly crystalline, cellulosic secondary cell wall. At early stages of development, there are sufficient numbers of immature fiber cells in a single carpel to permit isolation of fiber primary cell wall components. Cotton fiber proteins from 1D gels were transferred to membranes and incubated with either a polyclonal (Calbiochem, anti-human) or a monoclonal (NIH-DSHB, B3/D6) antibody to fibronectin. A single cotton protein (48 kDa) was recognized by the antibodies, however pre-incubation of the antibody with a 10-fold molar excess of human fibronectin (BRL) did not prevent binding of the antibody to the 48 kDa cotton fiber protein on Western blots. The molecular mass of the cotton fiber protein was similar to a protein recognized by an antibody to repetitive-proline-rich protein (RPRP). Consequently, 2D gels of cotton fiber proteins were blotted to membranes and probed with both the polyclonal anti-fibronectin and the anti-RPRP. Both antibodies recognized the same cluster of highly basic proteins at 48 kDa. Conversely, anti-RPRP did not recognize human fibronectin on Western blots. Control experiments in which pre-immune serum or other antibodies to cell-wall-associated-proteins were used failed to detect the 48 kDa protein. Taken together, these results suggest that cotton repetitive-proline-rich proteins may have some epitope(s) similar to fibronectin epitope(s). Additional characterization of repetitive-proline-rich-protein interaction with anti-fibronectins will be presented.

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A3-117 **XYLOGLUCAN BIOSYNTHESIS IN PEA GOLGI MEMBRANES**, Alan R. White, Vida Pezeshk, and Zhiyong Yang, Department of Botany, North Dakota State University, Fargo, ND 58105-5517 USA

Xyloglucan is a major hemicellulose polysaccharide in plant cell walls that is closely linked to plant cell growth and development. It consists of a β-1,4 glucan backbone with sidechains of xylose, galactose, and fucose. Cellular membranes from etiolated pea seedlings, *Pisum sativum* L. cv Caprice, were prepared by ultracentrifugation of homogenates on linear sucrose gradients, fractionated, and subcellular fractions were assayed for membrane markers and xyloglucan glucosyltransferase (XGT) activities. Glycosyl linkages of ^{14}C-labeled sugars incorporated from UDP-glucose and UDP-xylose into ethanol-precipitated polysaccharides were determined by a combined GC-radiogas proportional counting(RPC) / GC-MS assay (AR White *et al.* 1993 *Biochemical Journal* **294**:231-238). This assay showed that XGT reaction products from Golgi fractions had ^{14}C-glucose incorporated into 4- & 4,6-linked polymer. Digitonin was used to solubilize proteins from Golgi membrane fractions. Membrane preparations were subjected to differential centrifugation at 100,000 *g* for 1 hr, to separate solubilized proteins (or micelles) from unsolubilized membranes. Supernatant fractions were assayed for XGT activity and active fractions were used in protein purification regimes that included anion exchange chromatography (Sepharose Q), affinity chromatography (AffiGel and UDP-hexanolamine), and gel filtration chromatography. The structures of reaction products from partially purified XGT proteins were determined by methylation linkage analysis (GC-RPC/GC-MS), HPAE-HPLC (Dionex CarboPak PA-1), and enzyme digestion (endo-1,4-β-glucanase). Protein components of column fractions were analyzed by SDS-PAGE.

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A3-118 MODE OF ACTION OF FLAVONOLS DURING POLLEN TUBE GROWTH OF PETUNIA HYBRIDA, Bauke Ylstra, Jacqueline Busscher and Arjen J. van Tunen, Department of Developmental Biology, DLO-Centre for Plant Breeding and Reproduction Research (CPRO-DLO), PO Box 16, 6700 AA Wageningen, The Netherlands. FAX: +31 8370 18094

Flavonols form an important class of flavonoids which serve an essential function during plant reproduction. Flavonoid biosynthesis is initiated by the enzyme chalcone synthase (CHS). Transformants were generated with the use of the *chs* promoter fused to *chs* cDNA in the anti-sense orientation. With this construct it was possible to block flavonoid production in all reproductive tissues of transgenic plants. The complete absence of flavonols by this dominant mutation renders transgenic plants self-sterile. This inability of the *chs* anti-sense petunia plants to produce seed after self-pollination could be defined as a form of sporophytic self-incompatibility (*Ylstra et al.*, Plant J. **6** 1994: 201-212).

Mutant pollen without flavonols in their exine mature and germinate normally. However after a short period of *in vitro* tube growth, the cell wall and membrane breaks acentral of the tip and the protoplasm disloads leading to the death of the pollen grain. Addition of flavonol aglycones could complement these aberrations *in vitro* and *in vivo*. Confocal Laser Scanning Microscopy (CLSM) in combination with a flavonol-specific fluorescent stain enabled us to detect unglycosylated flavonols within the cytoplasm of wild-type pollen tubes. From this we conclude that flavonols, which are synthesized in the sporophytic tissues, are translocated to the male gametophyte during germination enabling the pollen tube to grow.

We hypothesize that the flavonols either have a structural role and interact with cell membranes or alternatively interact with known tube growth factors such as calcium, potassium or boric acid. Currently we compare intracellular structures of wild-type and flavonol depleted pollen using Transmitting Electron Microscopy (TEM) and CLSM.

A3-119 **INTERSPECIFIC SOMATIC CYBRIDS IN *SOLANUM* AS A MODEL FOR STUDYING NUCLEAR-ORGANELLE INTERACTION**
Dmitry P. Yevtushenko, Anatoly M. Shakhovsky, Vladimir A. Sidorov, Institute of Cell Biology & Genetic Engineering, Zabolotnogo 148, Kiev-DSP-22, 252022, Ukraine.

Interspecific potato cybrids were obtained using protoplast fusion to study nuclear-organelle compatibility and plastome-encoded traits. The production of the cybrid plants resulted in novel nucleus-organelle combinations. The modified polyethylene glycol fusion protocol with high fusion frequency was applied. Mesophyll protoplasts from the chlorophyll deficient plastome mutant of *Solanum tuberosum* L. cv."Zarevo" served as recipients. *S. stoloniferum* was used as organelle donor after irradiation of its mesophyll protoplasts with the lethal γ-ray dose (1000 Gy). This irradiation dose minimized the transfer of donor nuclear genome into recipient one. The cells were immobilized in calcium-alginate and cultivated in liquid medium. This procedure significantly increased cell survival and yield of putative cybrids. Selection of the cybrids was based on their ability to form green calli on the solid medium with low level of carbon sources. Plant regeneration from the selected green calli was obtained on the modified Shepard's medium containing IAA, GA_3 and zeatin as growth regulators. The cybrid plants obtained were morphologically similar to *S.tuberosum* L. Isozyme analyses for esterase and peroxidase showed that these plants contained potato nuclei. Restriction endonuclease analysis of ctDNA revealed the presence of *S. stoloniferum* chloroplasts in the fusion-derived *Solanum* cybrids. These results demonstrate the compatibility between the nuclear genome of potato and organelles from *S. stoloniferum* and provide potential for further studies.

Plant Cell Biology: Mechanisms, Molecular Machinery, Signals and Pathways

Plasmodesmata and Nuclear Pores; Cell Division; Transport Vesicles; Chaperones; Targeting to Organelles

A3-200 ISOLATION OF AN *ARABIDOPSIS THALIANA* cDNA ENCODING A SYNTAXIN HOMOLOGUE. Diane C. Bassham, Susannah Gal[1], Natasha V. Raikhel; MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824-1312.

Proteins destined for the plant vacuole traverse the secretory pathway via a series of vesicle budding and fusion events between organelles. Transport of soluble proteins to the vacuole requires positive sorting information, and absence of a sorting signal results in secretion. While these *cis* elements have been relatively well characterized, little is known about the elements involved in the targeting of transport vesicles to the vacuolar membrane. In mammalian cells, a number of proteins have been identified which are involved in the fusion of transport vesicles with their target membranes. One family of proteins, the syntaxins, are anchored on the cytosolic face of the target membrane and are thought to be receptors for transport vesicles. Pep12 is a yeast syntaxin homologue implicated in the fusion of vesicles with the vacuolar membrane, and the *pep12* mutant fails to correctly deliver several proteins to the vacuole. We have isolated an *Arabidopsis thaliana* cDNA by functional complementation of the yeast *pep12* mutant, and sequence analysis indicates that it is a syntaxin homologue. Characterization and functional analysis is now in progress.

[1]Current address; Dept of Biological Sciences, State University of New York, Binghamton, NY 13902-6000.

A3-201 **Formation of Endoplasmic Reticulum-Derived Protein Transport Vesicles,** Sebastian Bednarek[1], Randy Schekman[1], and Lelio Orci[2]. [1]Department of Molecular and Cell Biology, Howard Hughes Medical Institute, University of California, Berkeley CA 94720, and [2] Department of Morphology, University of Geneva Medical School, 1211 Geneva 4, Switzerland.

A combined genetic and biochemical approach has led to the identification and characterization of many of the proteins required for vesicle mediated transport of secretory proteins from the endoplasmic reticulum (ER) to the Golgi apparatus in the yeast *Saccharomyces cerevisiae* . *In vitro* , ER vesicle formation requires several components: Crude microsomal membranes containing yeast ER, two protein complexes (Sec13/31p and Sec23/24p), the small GTPase Sar1p, GTP, and an ATP regenerating system. Incubation at physiological temperature results in the formation of ER-derived vesicles. Purified vesicles display a distinct electron dense protein coat (COPII) containing the proteins used for their synthesis (Sar1p, Sec13/31p and Sec23/24p). To define the molecular mechanisms of vesicle biogenesis, we used morphological and biochemical studies to examine the assembly of the COPII coat and its role in driving vesicle budding from the ER. To facilitate electron microscopic analysis of vesicle budding and of COP II assembly, purified yeast nuclei were used as a homogeneous source of ER membranes in the *in vitro* assay. Thin section electron microscopy of nuclei incubated in the presence of COPII components, the non-hydrolyzable GTP analog GMP-PNP, and an ATP regenerating system at 20°C, showed the presence of both coated vesicle buds (whose membrane bilayer is continuous with the outer envelope) and fully formed vesicles attached to the outer nuclear envelope. Neither vesicle buds nor vesicles were detected on the outer nuclear membrane when any one of the COPII components (Sar1p, Sec13/31p, Sec23/24p) was left out of the *in vitro* assay. The small GTP-binding protein Sar1p, is likely to be responsible for initiation of coat formation: Binding studies indicate that activated Sar1p (e.g. the GTP bound form) binds preferentially to nuclei membranes and stimulates binding of the Sec23/24p complex. Recruitment of the Sec13/31 complex into the COPII coat requires the presence of both activated Sar1p and Sec23/24p. By immunoelectron microscopy, the recruited Sar1p, Sec13p and Sec23p are localized to the COPII protein coat that surrounds both buds and vesicles on the outer nuclear envelope. Additional data on the roles of the individual subunits of the Sec13/31p and Sec23/24p complexes in vesicle coat formation will be presented.

A3-202 COORDINATE INDUCTION OF THREE ER-LUMINAL STRESS PROTEINS IN MAIZE ENDOSPERM MUTANTS. Rebecca S. Boston, Jeffrey W. Gillikin and Russell L. Wrobel, Dept. of Botany, North Carolina State University, Raleigh, NC 27695

Synthesis, folding, assembly and sequestration of storage proteins are major functions of endosperm in developing kernels of maize. We have previously shown that three kernel mutations cause quantitative and qualitative changes in the synthesis and assembly of storage proteins and induction of the molecular chaperone, BiP. Recent work from this laboratory revealed a defect in the signal sequence processing site of a storage protein in the mutant, *floury-2*. Accumulation of such an aberrant protein provides a likely explanation for the chaperone induction. We have now characterized a variety of ER-molecular chaperones in endosperm mutants. Using RNA gel blot and immunoblot analyses we have found that levels of PDI, GRP94, calnexin and BiP are elevated in the mutants *fl2*, *De*-B30* and *Mc*. At least two of these, BiP and GRP94, are preferentially associated with protein bodies. We propose that the coordinate induction of these proteins in the three mutants is a cellular stress response to the presence of abnormally folded or packaged storage proteins. Experiments are in progress to further characterize the regulation and role of these in protein body formation in maize.

A3-203 CONTROL OF NUCLEAR MOVEMENT IN SUBSIDIARY MOTHER CELLS OF *TRADESCANTIA*, Ann L. Cleary and Janelle L. Kennard, Plant Cell Biology, Research School of Biological Sciences, Australian National University, Canberra, ACT 2601, Australia.

In *Tradescantia*, functional stomatal complexes composed of a pair of guard cells surrounded by four subsidiary cells, are formed by a complex series of divisions. Divisions in subsidiary mother cells (SMCs) are asymmetrical and are preceded by the migration of nuclei from their random locations to positions adjacent to the guard mother cells (GMCs) (a process we refer to as nuclear polarisation). We have investigated two aspects of nuclear polarisation: (1) mechanism of nuclear movement and (2) putative signals between GMCs and SMCs controlling nuclear movement. Labelling with bromodeoxyuridine (a thymidine analogue that incorporates into DNA in S phase and is detectable by antibodies) shows that nuclei polarise in G1 of the cell cycle and remain polarised for a minimum of 22 h before entering mitosis. Thus the signal to polarise is issued very early in the ontogeny of the cells and it is sustained for a prolonged period (>24 h). Polarised nuclei can be displaced by centrifuging epidermal peels at 1320g for 15 min but return to their pre-centrifuged position within 60 min. The return of the nuclei is unaffected by the addition of 10μM oryzalin (a microtubule depolymerising drug). However, nuclei are unable to reposition in the presence of the actin disrupting drug cytochalasin B (5 μg/ml). Carboxyfluorescein injected into SMCs passes into GMCs, indicating that the plasmodesmata are open and that a symplastic pathway for signalling molecules is available. An alternative mechanism for signalling could involve ions passing through channels. Local application of ionophore A-23187 in a high $[Ca^{2+}]$ buffer to the surface of a non-polarised epidermal cell induces rapid movement of the nucleus towards the application site suggesting nuclei can respond to a Ca^{2+} gradient. Experiments are underway to elucidate further the mechanism of signalling from GMCs to SMCs, a factor critical to the asymmetrical division of SMCs and ultimately to the formation of the stomatal complex.

A3-204 MOLECULAR CHARACTERISATION OF PLANT ENDOPLASMIC RETICULUM. Sean J. Coughlan, Craig Hastings and Ron Winfrey Jr. Trait & Technology Development Department, Pioneer Hi-Bred International, PO1004, Johnston, IA 50131-1004.

Purified endoplasmic reticulum devoid of contaminating endomembranes was prepared from germinating castor bean endosperm by a modified two step centrifugation procedure. The resulting membranes were characterised for enzymatic and processing activities, protein and lipid composition, and a start has been made on cloning the major protein constituents.
A major lumenal polypeptide [c55kDa] has been cloned in the following manner. Antisera raised against a total ER lumenal fraction was reacted against the excised polypeptide [bound to PVDF after separation by SDS-PAGE and electrophoretic transfer]. The membrane was rinsed to remove contaminants, and the bound sera eluted by acid shock [1% propionate]. The affinity purifed antisera was used to screen a germinating castor bean endosperm cDNA library. A full length clone encoding a major ER lumenal chaperonin, protein disulphide isomerase was obtained. This was confirmed by functional expression of a recombinant protein in Pet 15b E.coli system.

A3-205 TAGGING WITH HDEL CAUSES EFFICIENT RETENTION OF SPORAMIN IN THE PLANT ENDOPLASMIC RETICULUM. Loïc Faye and Véronique Gomord. LTI-CNRS URA 203, UFR des Sciences, Université de Rouen, BP 118, 76 821 Mt St AIGNAN, FRANCE - Tel: 35 14 66 92 / Fax: 35 14 67 87

The formation and maintenance of subcellular compartments in plant cell require the delivery of newly synthesized proteins to their correct cellular location. A signal peptide-mediated translocation across the endoplasmic reticulum (ER) is the first step on the route common to vacuolar, ER or Golgi apparatus-resident proteins and those transported to the plasma membrane and cell wall. The molecular signals that are necessary for the correct targeting of plant proteins in the secretory pathway have been described recently. In addition, both HDEL and KDEL ER retention signals used in yeast and animal cells have been identified at the C-terminal end of plant reticuloplasmins. The addition of a KDEL extension to vacuolar proteins is sufficient for their retention in the ER of transgenic plant cells. However recent results have illustrated that HDEL does not contain sufficient information for phaseolin retention in the ER (Herman et al, 1994, Plant Physiol. *105*,94.)

In the present study, HDEL C-terminal extension was fused to the vacuolar (Spo) and extracellular (Δpro) forms of sporamin. We have obtained transgenic tobacco cells (*Nicotiana tabacum*) expressing high levels of recombinant Spo, Δpro, SpoHDEL and ΔproHDEL. The intracellular localization of these different forms of recombinant sporamin was studied by subcellular fractionation. Our results clearly indicate that the addition of an HDEL extension to either Spo or Δpro induces the accumulation of both recombinant sporamin forms in a compartment that copurify with the ER markers NADH cyt. C reductase, BiP and calnexin. In addition, we have shown that a minor fraction of SpoHDEL and ΔproHDEL that still escape the ER retention machinery, are transported to the vacuole more probably by a signal-independent pathway.

This study was supported by a grant from SEITA, France. We are indebted to Prof. K. Nakamura (University of Nagoya, Japan) for his gift of Sporamin cDNA

A3-206 AN IN VIVO STUDY OF TARGETING AND IMPORT OF ISOCITRATE LYASE IN PLANT CELLS. Xiaoping Gao and Alison Baker, Department of Biochemistry, University of Cambridge, Cambridge CB2 1QW, U.K.

Studies using an *in vitro* system established in our laboratory showed that the carboxyl terminus of castor bean isocitrate lyase (ICL) was not essential for the import of the protein into glyoxysomes[1]. However, contradictory results were obtained from studies on the oilseed rape ICL expressed in transgenic plants[2]. In order to understand and attempt to reconcile these results the present work has investigated the sequence requirements for the targeting and import of castor bean ICL in transgenic plants. A C-terminally truncated version of ICL lacking the last 19 amino acids (ICL-19) and a chimeric construct consisting of the last 19 amino acids of ICL fused at the C-terminus of a reporter protein, mouse dihydrofolate reductase (DHFR), have been constructed. These constructs were placed under the control of the RUBISCO small subunit promoter and introduced into tobacco plants by *Agrobacterium* mediated transformation. Transformed plantlets were screened by western blotting for expression of the ICL-19 and DHFR+19 constructs. The localisation of ICL-19 has been examined by immunoelectron microscopy and is clearly present in peroxisomes, confirming the conclusions of the *in vitro* experiments. The immunolocalisation study of the DHFR+19 protein and the subcellular fractionation study of both proteins are in progress.

1. Behari, R. and Baker, A (1993) J. Biol. Chem. 268. 7315-7322.
2. Olsen, L.J. *et al.*, (1993) Plant Cell 5. 941-952.

A3-207 THE SEMI-DOMINANT MUTATION, *floury*-2, IS ASSOCIATED WITH AN UNPROCESSED α-ZEIN Jeffrey W. Gillikin, Fan Zhang, and Rebecca S. Boston, Department of Botany, North Carolina State University, Raleigh, NC 27695

The semi-dominant mutation, *floury-2* (*fl2*), is associated with an elevated level of molecular chaperones, irregularly shaped protein bodies and both qualitative and quantitative differences in the major storage proteins of maize seeds. We are currently studying the molecular mechanism by which this mutation causes such a pleiotropic phenotype. Two-dimensional gel electrophoresis of protein body proteins isolated from normal and *fl2* kernels revealed a 24 kDa polypeptide specific to the *fl2* mutant. We have purified this protein and identified it as a 22 kDa α-zein with an N-terminal signal peptide. *In vitro* translation assays programmed with normal or *fl2* mRNA revealed identical patterns of proteins. Co-translational processing with maize microsomes, however, showed a shift in product size expected of signal sequence cleavage for zeins from normal mRNA, but incomplete processing was observed for the large zeins in the *fl2* sample. Taken together, these data suggest that the *fl2* mutant encodes an aberrant zein which may be anchored to the ER membrane. Consequently, the normal segregation of this protein in the central region of the protein body would be disrupted. In turn, improper folding, aggregation or packaging of zeins may be sufficient to elicit an ER stress response and explain the abnormal protein bodies found in this mutant. Experiments to dissect the protein-protein interactions occurring in *fl2* protein bodies are ongoing.

A3-208 PLANT NUCLEAR PORES CONTAIN PROTEINS MODIFIED BY *N*-ACETYLGLUCOSAMINE

Antje Heese-Peck[1], Robert N. Cole[2], Olga N. Borkhsenious[1], Gerald W. Hart[2], Natasha V. Raikhel[1]; [1]MSU-DOE Plant Research Laboratory, Michigan State Univ., East Lansing, MI 48824-1312; [2]Dept. of Biochemistry and Molecular Genetics, Univ. of Alabama, Birmingham, AL 35294-0005

Nucleocytoplasmic transport of macromolecules occurs across the nuclear envelope through the nuclear pore complex (NPC), a supramolecular structure that is composed of at least 100 distinct proteins. The addition of wheat germ agglutinin (WGA), a lectin that specifically binds to *N*-acetylglucosamine (GlcNAc), inhibits the transport of proteins through the NPC in vertebrates. This suggests that some vertebrate NPC-proteins modified by single O-linked GlcNAc are involved in this transport. Very little is known about plant NPC-proteins and their function in protein import into the nucleus. In these studies, we show that plant NPCs also contain proteins that are modified by GlcNAc. By EM immunocytochemistry, specific binding was detected with WGA-conjugated gold particles at the NPC of nuclei from tobacco suspension cultures. Using protein blot analysis of isolated tobacco nuclei, several proteins of low- and high-molecular weight were specifically detected with a WGA probe. Some proteins of the same sizes were also labeled with [^{3}H]-galactose by galactosyltransferase, an enzyme that specifically adds galactose to terminal GlcNAc. The radiolabeled sugars were released from the nuclear proteins by ß-elimination indicating that these sugars are bound to the proteins via an O-linkage. Interestingly, the ß-eliminated sugar chains of the plant nuclear proteins were different from those of vertebrate NPC-proteins in that the oligosaccharides of plant origin consisted of five or more saccharides whereas those of vertebrate origin are single GlcNAc. Currently, we are purifying the proteins that contain terminal GlcNAc by WGA-affinity chromatography.

A3-209 IDENTIFICATION OF PLANT NUCLEAR LOCALIZATION SEQUENCE BINDING PROTEINS,

Glenn R. Hicks and Natasha V Raikhel, DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824-1312

The import of proteins into the nucleus is a vital process that is mediated by proteins that specifically recognize nuclear localization sequences (NLSs). Essentially nothing is known of these factors in plants. Previously, we have used an in vitro nuclear binding assay (Hicks, G.R. and Raikhel, N.V. (1993) *Plant Cell*, 5, 983-994) to demonstrate that dicot and monocot species possess a low affinity site at the nuclear pore that specifically binds to two of the three known classes of functional NLSs. Recently, the third class of NLSs was shown to compete for binding to this site (see poster abstract of Smith, Hicks and Raikhel, this meeting). We report here, the use of crosslinking reagents and a radiolabeled peptide to the bipartite NLS from the maize transcription factor, Opaque2, to identfy two NLS binding proteins (NBPs) of 50 to 60 kDa and at least two NBPs of 30 to 40 kDa. As observed for nuclear binding in vitro, competition studies indicated that labeling was specific for the functional NLS but not a mutant NLS (shown to be impaired in vivo) or a peptide unrelated to NLSs. Also, the apparent dissociation constant (100 to 300 µM) for labeling was similar to that of the binding site. Proteins of similar mass were labeled using two different crosslinking reagents, and crosslinker-concentration and reaction-time studies indicated that the NBPs were distinct proteins and not aggregates. Treatment with salt, detergent or urea prior to or during NLS binding demonstrated that the properties of the binding site and the NBPs were essentially identical. This tight correlation demonstrates that some or all of the NBPs constitute the nuclear pore binding site. Overall, our results indicate that at some components of NLS recognition are located at the nuclear pores in higher plant cells.

A3-210 CLONING, EXPRESSION ANALYSIS & *IN VITRO* PRENYLATION OF PUTATIVE *YPT1* HOMOLOGS FROM TOMATO. Ann E. Loraine, Shaul Yalofsky and Wilhelm Gruissem. Department of Plant Biology, University of California, Berkeley, CA 94720.

We have isolated three putative tomato homologs of *YPT1*, an essential gene in *Saccharomyces cerevisiae* encoding a small guanine nucleotide binding protein of the rab family required for the ER to Golgi step in the yeast secretory pathway. The tomato genes (TRAB1A, B and C) are differentially expressed during fruit ripening and are all strongly expressed in tomato roots and immature leaves. Trab1A, but not Trab1B or C, is up-regulated in seedlings exposed to ethylene gas and is also most highly expressed in ripening fruit. The ability of TRAB1B and C to substitute for the *YPT1* gene in yeast was tested; it was found that both complement *ypt1*. Trab1B and C tagged at the amino terminus with the amino acid sequence EYMPME, which forms an epitope recognized by a monoclonal antibody, were also able to complement *ypt1*, demonstrating the potential usefulness of this epitope in immunolocalization and/or biochemical studies with transgenic plants expressing "tagged" rab proteins. Proper localization of rab proteins to membranes in yeast and mammalian cells requires that they be prenylated; this post-translational modification is performed by the enzyme geranylgeranyl transferase type II, which attaches the sterol intermediate geranylgeranyl to conserved carboxy terminal cysteine residues via a thio-ether linkage. Rab proteins which lack this consensus motif fail to become prenylated and are unable to participate in the secretory pathway. The ability of a tomato extract to prenylate bacterially produced Trab protein in an *in vitro* assay was tested and it was found that tomato contains a geranylgeranyl transferase activity similar to that present in yeast and mammalian cells. Immunogold EM studies are planned that will determine the intracellular localization of the tomato rab proteins in order to identify which step or steps in the tomato secretory pathway these proteins are likely to mediate.

A3-211 EFFECTS OF WORTMANNIN ON THE VACUOLAR TARGETING OF PROTEINS MEDIATED BY N-TERMINAL AND C-TERMINAL PROPEPTIDES

Ken Matsuoka*, Natasha V. Raikhel# and Kenzo Nakamura*, *Laboratory of Biochemistry, School of Agriculture, Nagoya University, Nagoya 464-01, Japan, #MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, USA

Vacuolar-matrix proteins are sorted from the secretory pathway to the vacuoles at the Golgi apparatus. Previously, we reported that the N-terminal propeptide (NTPP) of the precursor to sporamin (NTPP-SPO) and the C-terminal propeptide (CTPP) of the precursor to barley lectin (BL-CTPP) contain information for vacuolar sorting. To analyze whether these propeptides can be interchangeable, we expressed constructs NTPP with the mature part of BL (NTPP-BL) and mature sporamin with CTPP (SPO-CTPP) in tobacco BY-2 cells. The vacuolar localization of these constructs indicated that the signals were interchangeable. We next analyzed the effect of wortmannin (WM), a specific inhibitor for mammalian phosphatidylinositol 3-kinase (PI3-kinase), on vacuolar delivery by NTPP and CTPP in tobacco cells. Pulse-chase analysis indicated that 3.3×10^{-5} M WM caused almost complete secretion of BL-CTPP and SPO-CTPP. In contrast, only a portion of NTPP-SPO or NTPP-BL was missorted, even in the presence of 10^{-4} M WM. Thus, there are at least two different mechanisms for the vacuolar sorting in tobacco cells, and the CTPP-mediated one is sensitive to WM. We compared the dose-dependencies of WM on the missorting of BL-CTPP and SPO-CTPP and on the inhibition of the synthesis of phospholipids (PLs) in tobacco cells, to determine whether sorting by CTPP to the vacuoles is related to the activity of PI3-kinase. The dose-dependency of WM for the missorting is almost identical to that of inhibition of synthesis of PLs, but not for the formation of PI3-phosphate. Thus, the synthesis of PLs could be involved in CTPP-mediated vacuolar transport.

A3-212 EXPRESSION AND CORRECT LOCALIZATION OF ANIMAL SECRETORY PROTEINS IN PLANT CELLS
Jean-Marc Neuhaus, Maciej Pietrzak, Ernst Freydl and Thomas Boller, Botanisches Institut, Hebelstrasse 1, CH-4056 Basel, Switzerland
Plant soluble proteins entering the secretory pathway in the endoplasmic reticulum are transported to the Golgi apparatus and then secreted out of the cell by default. However, if their sequence contains a specific motif recognized by a sorting system, they are transported to their proper location. For several vacuolar proteins the targeting information has been identified as a small contiguous peptide sequence either within an N-terminal propeptide, within a C-terminal propeptide, or within the mature polypeptide chain. In animal cells the lysosomal proteins are sorted by specific receptors that recognize a terminal mannose-6-phosphate on the sugar side chains of glycoproteins. We have expressed two animal proteins, the secreted hen egg white lysozyme (HEWL) and the lysosomal rat ß-glucuronidase (RGUS) in plant cells. We found that in *Nicotiana plumbaginifolia* protoplasts HEWL was secreted into the medium. Addition of the 7 amino acid long C-terminal vacuolar targeting peptide (VTP) from the tobacco chitinase A was sufficient to redirect it to the vacuole. In animal cells, ß-glucuronidases are synthetized with a C-terminal propeptide that improves the folding efficiency and the phosphorylation of terminal mannose groups. In the plant protoplasts, intact RGUS was sorted to the vacuole. Deletion of the 19 amino acid long propeptide caused secretion into the medium and a reduced production of RGUS activity. Deletion of 15 amino acids also caused secretion into the medium without affecting the level of expression. Addition of the chitinase VTP redirected both of these deletion mutants to the vacuole. As in animal cells, tunicamycin dramatically reduced the expression of active RGUS in plant protoplasts, and brefeldin A blocked the transport of both secreted and vacuolar RGUS to their final location. RGUS is, thus, a very convenient new reporter protein to study intracellular protein transport.

A3-213 TARGETING AND IMPORT OF PROTEINS INTO HIGHER PLANT PEROXISOMES
Laura J. Olsen, Dept. of Biology, Univ. of Michigan, Ann Arbor, MI 48109-1048
All peroxisomal proteins are encoded by nuclear genes and synthesized on free polyribosomes in the cytoplasm. The proteins are then post-translationally transported across the single organellar membrane. Most peroxisomal proteins possess a carboxyl-terminal targeting determinant, though a few proteins may be targeted by an amino-terminal presequence, and some peroxisomal proteins contain neither signal type. Recent results using genetic approaches in yeast suggest that peroxisomal proteins with carboxyl-terminal signals are imported via a different pathway than proteins with amino-terminal presequences. We have developed an in vitro assay to study the import of proteins into glyoxysomes (a class of peroxisomes prevalent in germinating seedlings). Radiolabelled peroxisomal proteins are incubated with isolated pumpkin cotyledon glyoxysomes under conditions designed to favor protein transport. Imported proteins are assessed primarily by protease resistance. We have shown that the import of the peroxisomal proteins isocitrate lyase and glycolate oxidase (both possessing carboxyl-terminal targeting signals) is time-, temperature-, and energy-dependent; both proteins appear to compete for the same sites of transport across the membrane. The peroxisomal protein thiolase, however, is synthesized with an amino-terminal prepiece that may direct its import into the organelle at transport sites distinct from those utilized by proteins possessing carboxyl-terminal targeting signals. Results describing several physiological aspects of the in vitro import assays and a comparison of the pathways of import of thiolase and glycolate oxidase, peroxisomal proteins with different types of targeting determinants, will be presented. The existence of multiple pathways for protein import into peroxisomes may have implications for the mechanisms of protein translocation across membranes as well as providing insight into possible evolutionary origins of the organelle.

A3-214 TARGETING AND TRANSPORT OF THE SUCROSE BINDING PROTEIN TO THE SOYBEAN PLASMA MEMBRANE,
Paul J. Overvoorde and Howard D. Grimes, Department of Botany, Washington State University, Pullman, WA 99164-4238
Despite the importance of the plasma membrane in the biology of a plant cell there is a paucity of information regarding the proteins that form this selective barrier and the molecular cues which target these proteins to this organelle. A sucrose binding protein (SBP) was identified by labeling soybean cotyledon membranes with a photoaffinity- labeled sucrose analog and this protein has been purified, cloned, and shown to be a plasma membrane protein by both immunocytochemical and biochemical methods. The SBP is very tightly associated with the extracellular leaflet of the plasma membrane as evidenced by the fact that only treatment with 0.1M Na_2CO_3 (pH 11.5) or detergents above their critical micelle concentration can disassociate significant amounts of the SBP from the membrane. Sequence analysis of the SBP cDNA indicates that the N-terminal 29 amino acids form the most hydrophobic region of the protein and might function as a signal sequence. However, when the SBP is *in vitro* transcribed and translated in the presence of canine pancreatic or maize microsomes no shift in molecular weight is detected regardless of the concentration of membranes used. Furthermore, when Proteinase K is added after translation in the presence of microsomes, the SBP is not protected from digestion regardless of the concentrations of membranes used or the temperature of incubation. In experiments where translation is carried out in the presence of microsomes and the synthesis reaction is fractionated across a sucrose step gradient, the SBP becomes associated with the membrane fraction and treatment with 0.1M Na_2CO_3 (pH 11.5) can release the SBP from the membrane fraction. These results indicate that the N-terminal hydrophobic domain is not sufficient to allow translocation of the SBP into the interior of these membrane vesicles which is atypical for a protein entering the secretory pathway. Purification of poly (A^+) RNA from free and membrane-associated ribosomes followed by probing with labeled SBP cDNA indicates that the SBP is translated *in vivo* by free ribosomes. Also, the SBP is not a glycoprotein, even though it contains a predicted glycosylation site. Collectively, these results suggest that the SBP is not processed through the secretory pathway but rather reaches its extracellular destination via an as yet undefined pathway. Additional studies are underway at the *in vivo* level, (i.e. pulse-chase, transient expression) to corroborate or refute the *in vitro* results.

A3-215 THE MAIZE RNA-BINDING PROTEIN, MA16, IS A NUCLEOLAR PROTEIN LOCATED IN THE DENSE FIBRILLAR COMPONENT.
Montserrat Pagès, M. Mar Albà, Francisco A. Culiáñez-Macià and Adela Goday. Departament de Genética Molecular. Centre d'Investigació i Desenvolupament C.S.I.C. Jorge Girona 18-26. 08034 Barcelona. Spain.
We have previously described a developmentally and environmentally regulated gene in maize, *MA16*, encoding an RNA-binding protein that binds preferentially to uridine and guanosine-rich RNAs. To gain some insight into the function of *MA16* we investigated the distribution of *MA16* mRNA and protein during maize development by using *in situ* hybridization, RNA and protein gel blot analysis and immunocytochemistry. Our results show that MA16 is expressed throughout development of the embryo and seedling in different tissues and at different levels. The level of *MA16* mRNA is higher in elongating and expanding structures such as the root elongation zone and young leaves. After stress treatment *MA16* mRNA increases in total and polysomal RNA, but no significant change in the level of the protein was detected. The MA16 is a non-ribosomal nucleolar protein. By immunoelectron microscopy the MA16 protein has been located in the dense fibrillar component and to a lesser extent in the granular component of the nucleolus. We found that MA16 contains the conserved sequence motifs $R(G)_aY(G)_aR$ and $RR(E/D)(G)_aY(G)_a$ repeated in the C-terminal of the molecule that conforms imperfectly to the GAR motif proposed for nucleolar proteins. In light of these results the stress regulation of *MA16* and a likely role for this protein in pre-rRNA processing and/or ribosome assembly is discussed.

A3-216 CHARACTERIZATION OF A PLANT VACUOLAR TARGETING RECEPTOR, Nadine Paris, Thomas Kirsch*, Leonard Beevers* and John C. Rogers, Biochemistry Department, University of Missouri, Columbia MO 65211, and *Department of Botany and Microbiology, University of Oklahoma, Norman, OK 73019.

We have purified an 80 kDa integral membrane protein from pea clathrin coated vesicles that has a binding affinity of 30 nM for the vacuolar targeting determinants on the vacuolar protease, pro-aleurain. The protein binds poorly or not at all to peptide sequences that do not mediate vacuolar targeting. The amino-terminus of this protein is intralumenal and a carboxy-terminal tail of ~5 kDa projects outside the vesicles on the cytoplasmic surface. Optimal binding of the proaleurain ligand occurs at neutral pH, and the ligand is released when the pH is lowered to 5. Its binding specificity, its presence in an appropriate intracellular compartment, and the pH effects on binding all argue that the 80 kDa protein functions as a vacuolar targeting receptor. We have obtained three protein sequences from the N-terminus and tryptic peptides; none are represented on other proteins in the sequence data bases. Antibodies directed towards 18 amino acids at the N-terminus precipitate a single 80 kDa protein from [35]S-labeled developing pea cotyledons and identify a single 80 kDa protein on western blots. This anti-"receptor' antiserum decorates numerous bead-like organelles when used in immunofluorescence microscopy on pea root tip cells. These organelles are distinct from the endoplasmic reticulum and from the vacuolar compartment. The vacuolar compartment in root tip cells is identified by anti-αTIP antibodies and by antibodies to plant complex Asn-linked oligosaccharides; it forms a worm-like interconnected tubular network throughout the cell periphery. We hypothesize that organelles identified by the anti-"receptor" antibodies represent Golgi complexes. Immunogold electron microscopy is in progress to test this hypothesis. Molecular cloning results using approaches based on expression screening with antibodies and on synthetic oligonucleotides will be presented.

A3-217 CHARACTERIZATION OF THE TWO MOVEMENT PROTEINS OF SQUASH LEAF CURL VIRUS, E. Pascal[1], R. Medville[2], R. Turgeon[2], and S.G. Lazarowitz[1], [1]Department of Microbiology, University of Illinois, Urbana, IL 61801, [2] Section of Plant Biology, Cornell University, Ithaca, NY 14853

The spread of plant viruses cell-to-cell and systemically through the host plant requires viral- encoded movement proteins (MPs). In the single-stranded DNA-containing bipartite geminiviruses such as squash leaf curl virus (SqLCV) two MPs are required for infection of cucurbit and *N. benthamiana* hosts. Our current work has focused on the mechanisms by which these two MPs, BL1 and BR1, participate in viral movement. We have constructed transgenic tobacco which express BL1 and/or BR1. Expression of BL1 alone is sufficient to produce a phenotype which resembles virus-infected plants, namely downward leaf curl and mosaic leaves. In contrast, plants expressing BR1 are phenotypically indistinguishable from wild type tobacco plants. The role of BL1 in symptom formation is further demonstrated by the effects of alanine scanning mutations in BL1 on SqLCV infection. BL1 mutants display a broad range of effects, from no infectivity to mild and attenuated symptoms to near wildtype BL1 symptoms. In addition, some of the BL1 mutations alter the host range of SqLCV, permitting infection of cucurbits but not *N. benthamiana*. Construction of transgenic tobacco lines expressing these BL1 mutants indicates that some of the mutations affect the ability of BL1 to produce the disease-like phenotype. In both transgenic and virus-infected plants wild type BL1 localizes to the plasma membrane fraction in a phase partition membrane fractionation. This localization suggests that BL1 acts at the cell periphery, similar to other well-characterized plant virus MPs, to facilitate cell-to-cell movement of the virus, perhaps at plasmodesmata. However, we have shown that BL1 is distinct from these other MPs in its inability to directly binding nucleic acids. Using immunogold electron microscopy we have localized BR1 to the cell nucleus in both virus-infected and transgenic plants. In addition we have demonstrated that BR1 binds nucleic acids with a preference for single-stranded DNA over RNA and double-stranded DNA. This data suggests that BR1 is involved in the transport of the SqLCV single-stranded DNA genome into and/or out of the nucleus. The requirement for nuclear transport is unique to DNA viruses such as SqLCV which replicate in the nucleus and must then find their way to the cell periphery to move to the next cell. We propose a model whereby BR1 is involved in the shuttling of the virus into and/or out of the nucleus and BL1 facilitates viral movement across cell boundaries.

A3-218 STRUCTURE AND LIGHT REGULATION OF A NUCLEOSIDE TRIPHOSPHATASE ASSOCIATED WITH THE ENVELOPE OF PEA NUCLEI, Stanley J. Roux, Hsu-Liang Hsieh, Sonal Blumenthal and Chii-Gong Tong, Botany Department, University of Texas, Austin, TX 78713

A cDNA encoding a 47 kD NTPase that is associated with the envelopes of pea nuclei has been isolated and sequenced. The translated sequence of the cDNA includes five motifs characteristic of the ATP binding domain of many proteins. It also has a predicted calmodulin-binding sequence, consistent with the finding that the purified NTPase is activated by calmodulin *in vitro*, and several potential casein kinase II phosphorylation sites, consistent with results showing that NTPase is phosphorylated by casein kinase II *in vitro*. Earlier findings indicated that nuclear NTPase activity could be stimulated by phytochrome both *in vivo* and *in vitro*. Expression of the NTPase mRNA in roots, leaves and stems of both light-grown and etiolated pea seedlings was assayed by Northern blot analysis. The NTPase mRNA is strongly expressed in etiolated plumules, but poorly or not at all in the leaf and stem tissues of light-grown plants. Its abundance in etiolated plumules is up-regulated by brief treatments with both red and far-red light, as is characteristic of very low fluence phytochrome responses. A genomic Southern analysis using 4 restriction enzymes suggests that there is only one gene encoding the NTPase. An immunofluorescent assay of pea plumule cells by confocal laser microscopy indicates that the enzyme is localized primarily along the periphery of nuclei.

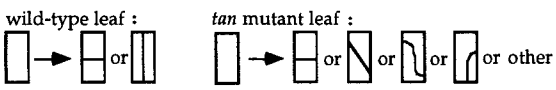
A3-219 SPECIFIC BINDING OF THREE CLASSES OF NUCLEAR LOCALIZATION SEQUENCES IN PLANTS, Harley Smith, Glenn R. Hicks and Natasha V. Raikhel, DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824-1312

The nuclear import of proteins in plants probably involves two steps; specific recognition of a nuclear localization sequence (NLS) followed by translocation through the nuclear pore complex. We are examining the recognition component of import by using an in vitro binding assay. Using this system, we previously reported (Hicks and Raikhel *Plant Cell* 5: 983, 1993) that radiolabeled peptides to two classes of NLSs, the SV40 large T-antigen NLS and the bipartite NLS from the Opaque2 protein of maize, compete for binding to a single class of low affinity sites (k_d about 200 μM) in purified tobacco and maize nuclei. The binding site is specific for functional NLSs because mutant NLSs, which are functionally impaired in plant cells in vivo, as well as reverse wild-type and non-NLS peptides are poor competitors. Confocal and immunofluorescence microscopy indicate that the binding site is probably located at the nuclear pore. Here, we report that a third class of NLSs competes for binding to this nuclear site. This signal, known as NLS C, is one of three NLSs found within the maize R protein that have been shown to function in vivo. NLS C is similar to the NLS in the Mat α-2 protein of yeast in having a high content of hydrophobic as well basic residues. Interestingly, the Mat α-2 NLS does not function in mammalian cells. Our results indicate that all three of the known classes of NLSs are recognized in plants by a single site at the nuclear pore. The specific association of NLS C provides added confidence that the in vitro binding is biologically relevant and will aid in our effort to identify the binding site biochemically.

A3-220 THE ROLE OF THE MAIZE GENE, *TANGLED*, IN THE SPATIAL CONTROL OF CELL DIVISION

Laurie G. Smith, Sarah C. Hake and Anne W. Sylvester*, U.S.D.A./U.C. Berkeley Plant Gene Expression Center, 800 Buchanan St., Albany, CA 94710, and *Department of Biological Sciences, University of Idaho, Moscow, ID 83843

The phenotype of recessive *tangled* (*tan*) mutations suggest that the wild-type *Tan* gene is involved in the spatial control of cell division in maize. In all tissue layers of wild-type maize leaves, almost all elongated cells divide either transversely (new wall perpendicular to the long axis of the cell) or longitudinally (new wall parallel to the long axis of the cell). In *tan* mutant leaves, elongated leaf cells undergo normal, transverse divisions, but longitudinal divisions are largely substituted by abnormal divisions that are rare or absent in normal leaves: divisions that join the two long sides of an elongated cell in a crooked or curved path, divisions that join the end of the cell to one of the two long sides, and some other divisions that don't fall into these categories. The cytoskeletal basis of this mutant phenotype is currently under investigation. Examination of the microtubule cytoskeleton in *tan* leaf primordia has shown that interphase arrays, preprophase bands, spindles and phragmoplasts are all present, and do not have obvious structural abnormalities. Preliminary results suggest that mutant leaf cells are deficient in longitudinal preprophase band formation, suggesting that the *Tan* gene is required for the establishment of normal, longitudinal division planes in elongated leaf cells.



A3-221 NUCLEAR IMPORT OF MAIZE ANTHOCYANIN REGULATORY PROTEINS,

Marguerite J. Varagona, Batoulli Said-Salim and Vicki Chandler*, Department of Biology 3AF, New Mexico State University, Las Cruces, NM 88003, *Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.

The process of targeting proteins into the nucleus is essential to gene expression, and thus, essential to proper growth and development of eukaryotic organisms. Nuclear import of proteins larger than 50 kD requires energy and a portion of the imported protein which functions as a nuclear localization sequence (NLS). Our work focuses on the import of the transcriptional regulatory proteins, C1 and B, which, together, activate the genes for anthocyanin biosynthesis in maize. The anthocyanin gene cascade has been extensively studied at the genetic, biochemical and molecular level. In addition, evidence is accumulating that indicates the conservation of this pathway and its regulatory proteins in all higher plants. However, little is known about the cell biology of the pathway. In 1992, Shieh et al. (Plant Physiol. 101: 353-361.) demonstrate that the R protein, a functional homologue to the B protein, contains NLSs. Our study focuses on the nuclear import of the C1 regulatory protein. C1 is a 29 kD protein that is in the myb family of transcriptional activators. Due to its small size, C1 may enter the nucleus by three possible mechanisms. First, like 14 kD histone proteins, C1 may import using NLSs. Second, C1 may co-import with B or R through protein-protein interactions. Finally, C1 may simply diffuse into the nucleus, since it is smaller than the exclusion of the nuclear pore (50kD). Data presented will address the first two possible mechanisms. Translational fusion constructs were made to encode a fusion proteins containing the C1 protein, or regions of the C1 protein, fused to the GUS reporter protein. These fusion proteins are being examined in transgenic onion epidermal cells to determine if the amino acids in C1 will confer nuclear localization on the GUS protein. In addition, experiments are designed to determine the effect of the presence of the B protein on import of the C1::GUS fusion protein.

A3-222 CHARACTERIZATION OF HSP-70 COGNATE PROTEIN ISOLATED FROM GLYOXYSOMES OF CUCUMBER COTYLEDONS,

Micha Volokita and Gila Granot, Ben-Gurion University of the Negev, The J. Blaustein Desert Research Institute, Sede-Boker campus 84990, Israel

By analogy with the import of proteins into other organelles, it has been speculated that molecular chaperones may be involved in the assembly of proteins into microbodies, either in the cytosol for maintaining the protein in an unfolded state and/or inside the microbodies for assisting in the translocatrion, folding, and the oligomerization of the imported protein. However, there is yet no experimental evidence to suggest the involvement of molecular chaperones in microbodies assembly. As part of our effort to elucidate the mechanism of the assembly of plant microbodies (leaf-peroxisomes and glyoxysomes), we undertake to examine whether hsp-related protein(s) are present in glyoxysomes.

Utilizing antibodies raised against HSP-70 cognate proteins from wheat as well as antibodies raised against the *Drosophila* HSP-70, we have identified and characterized a family of closely related proteins in cucumber. One protein band of ca. 79 kDa was localized in the glyoxysomes while other three protein bands were localized in the endoplasmic reticulum. The glyoxysomal 79 kDa protein is present in nonheat-shocked seedlings and its synthesis is not responsive to heat shock treatment, thus it can be assign as a cognate member of the HSP-70 family. This protein was further confirmed as HSP-70 cognates by its ability to bind to ATP.

A3-223 LIGHT INACTIVATION OF ARABIDOPSIS PHOTOMOPHOGENIC REPRESSOR COP1 INVOLVES A CELL-SPECIFIC REGULATION OF ITS NUCLEOCYTOPLASMIC PARTITIONING,

Albrecht G. von Arnim and Xing-Wang Deng, Dept. of Biology, Yale University, P.O.Box 208104, New Haven, CT 06520-8104

Arabidopsis COP1 (constitutively photomorphogenic 1) acts as a repressor of photomorphogenesis in darkness, and light stimuli abrogate this suppressive action. COP1, when fused to β-glucuronidase (GUS), is enriched in the nucleus in darkness, but not in the light, in hypocotyl cells of Arabidopsis seedlings and epidermal cells of onion bulbs. In Arabidopsis hypocotyl cells the nuclear GUS-COP1 level changes in response to dark-light transitions and quantitatively correlates with the extent of repression of photomorphogenic development. In root cells GUS-COP1 is constitutively nuclear, consistent with an established role of COP1 in suppressing root chloroplast development in both light and darkness. We conclude that COP1 acts inside the nucleus to suppress photomorphogenesis, and that light inactivation of COP1 involves a cell type-specific control of its nucleocytoplasmic partitioning. To confirm the working hypothesis we are currently analyzing the light control of subcellular localization of the endogenous COP1 protein in various cell types of Arabidopsis seedlings by immunocytochemistry. The progress in this analysis will also be presented.

A3-224 TOBACCO MOSAIC VIRUS MOVEMENT PROTEIN MEDIATED MOVEMENT OF MACROMOLECULES THROUGH TRICHOME PLASMODESMATA E.Waigmann and P.Zambryski. Dept. of Plant Biology, 111 Koshland Hall, University of California, Berkeley, 94720 California, USA.

To establish systemic infection, plant viruses spread from an initially infected cell to adjacent cells via cytoplasmic bridges, the plasmodesmata (PD). Generally, in wild-type plants PD only permit passage of small molecules up to 1 kDa. To achieve passage of a large structure like tobacco mosaic virus (TMV) genome, a virally encoded movement protein (TMV-MP) a) binds to TMV-RNA, thereby unfolding it to a thin thread and b) increases the size exlusion limit (SEL) of PD. The combined effects of these two functions probably allow transfer of viral RNA through PD.

Here,we monitor TMV-MP function in vivo by direct microinjection of biochemically pure TMV-MP into leaf trichomes together with fluorescently labeled dextrans. Dextran movement is thought to be solely dependant on the hydrodynamic radius of the dextran molecule and thus indicates PD channel size. Results suggest, that trichome PD have an unusually high basal SEL of ~7 kDa for dextrans, that cannot be further increased upon co-injection with TMV-MP. This contrasts with mesophyll PD, whose basal SEL of 1 kDa for dextrans can be dramaticllay increased to 20 kDa upon co-injection of TMV-MP (1). Correlating with the high basal SEL of trichome PD, EM studies show clearly larger overall dimensions of trichome PD than of mesopyll PD. Obviously, functional and structural differences between PD's exist within a plant, depending on cell and tissue type. Despite the inability to increase SEL for dextrans in trichomes, TMV-MP itself moves through trichome PD and can also specifically mediate movement of a GUS:TMV-MP fusion protein, while GUS alone cannot move. This indicates that TMV-MP mediated protein movement through trichomes is -unlike dextran movement - not driven by molecular size; instead, we propose that a plasmodesmal transport signal resides in TMV-MP that is essential for movement.

1. E. Waigmann, W.L. Lucas, V. Citovsky and P. Zambryski, *PNAS* **91:** 1433-1437, 1994

A3-225 RAB PRENYL-TRANSFERASE IN TOMATO,Shaul Yalovsky, Ann E. Loraine and Wilhelm Gruissem The Department of Plant Biology, University of California - Berkeley, Berkeley, CA 94720

The Rab Geranylgeranyl-transferase (Rab GGTase) is a member of the prenyl transferase enzyme family. These enzymes attach prenyl groups [either, the 15 carbon - farnesyl-PP (FPP), or the 20 carbon - geranylgernyl-PP (GGPP)] to cystein residues which are part of consensus sequence motifs located at the C-terminus of certain proteins involved in signal transduction, growth regulation, and secretion. It is assumed that prenylation increases the affinity of proteins to membranes. Rab GGTase attaches GGPP groups to two cystein residues which are part of cys-cys , or cys-x-cys sequence motifs located in the C-terminus of Rab proteins. The Rab protein family are small GTP-binding proteins which control membrane fusion and exocytosis. Geranylgeranylation of exogenous TRab proteins was detected in tomato protein extracts. Immunoprecipitation of Rab proteins showed that it was the Rab protein which was specifically prenylated and not a protein in the tomato extract. No labeling of TRab with FPP was detected. Moreover, unlabeled GGPP but not FPP was efficient as competitor for the reaction. A mutant Rab protein in which the two C-terminal cysteine residues were substituted was not geranylgeranylated. The mutant protein did, however, inhibit the geranylgeranylation of wt TRab protein. These data show that, similar to the mammalian and yeast systems, the cys-cys motif is the probable geranylgeranylation site but the binding of the GGPP transferase to the Rab occurs via a domain/s which are located elsewhere in the protein.

Membrane Signaling; The Tonoplast and Plasma Membrane, Channels, Pumps, Plastids

A3-300 IDENTIFICATION OF PROTEIN IMPORT MACHINERY IN CHLOROPLAST BY CHEMICAL CROSS-LINKING, Mitsuru Akita and Kenneth Keegstra, DOE-Plant Research Laboratory, Michigan State University, East Lansing, MI 48824

Protein targeting is one of the major topics in cell biology. For chloroplasts, most chloroplastic proteins are imported after synthesis in the cytoplasm. Protein translocation into chloroplasts is thought to occur at contact sites where the outer and inner envelope membranes are in close proximity. Recently two envelope proteins of 75kDa and 86kDa have been reported to be involved in protein translocation. But there remains uncertainty whether or not other proteins are involved. To address this problem, we are trying to identify other components via chemical cross-linking. When chloroplasts, with precursor proteins bound at the surface, are treated with cross-linker, larger cross-linked products are observed. They are specific to precursor proteins and contain the two envelope proteins previously identified. One large complex of 600kDa is currently beeing purified. Our efforts to identify the proteins present in this complex will be presented.

A3-301 DEVELOPMENTAL AND STRESS REGULATION OF *aERD2*, GENE IN *ARABIDOPSIS* PLANTS

Maor Bar-Peled, Lorenzo Frigerio, Alexandre Conceicao, and Natasha V. Raikhel
MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing MI 48824 USA

Different lumenal endoplasmic reticulum (ER)-resident proteins contain *cis*-acting signals that act as retention signals. Genetic and biochemical studies in yeast and mammalian cells identified the *ERD2* gene product (Erd2) that involved in ER protein retention and in membrane sorting, and showed that it is an essential gene in yeast.

We have recently isolated a cDNA clone from *Arabidopsis thaliana* (*aERD2*) that encodes a 26 kDa protein homologous to yeast and mammalian counterparts. The *aERD2* gene functionally complements the yeast mutant lacking *ERD2*, whereas the human homologue does not. We have detected significant differences in the *aERD2* message levels among various tissues. In addition, a single gene for this message has been observed indicating that there may be a fundamental difference in the mechanisms directing ER protein retention and membrane flow within different cell types in plants.

Since it is known that under certain developmental and stress conditions ER protein gene expression can be induced in plants, we are analyzing regulation of *aERD2* gene expression under these conditions. The results of these experiments will be discussed.

A3-302 EFFECT OF SYNTHETIC AND NATURAL TYROSINE KINASE INHIBITORS ON AUXIN EFFLUX IN ZUCCHINI (*Cucurbita pepo*) HYPOCOTYLS, Paul Bernasconi, Sandoz Agro Inc., Research Division, 975 California Ave, Palo Alto, CA 94304

Auxin transport along the Zucchini hypocotyl requires the cell to cell translocation of this hormone. There is good evidence that, whereas no influx carrier is needed, an efflux carrier, located on the plasmalemma (PM) must be present. The nature of this protein is still obscure but sensitivity of auxin transport towards inhibitors of the sucrose and hexose carriers may suggest that it belongs to the same family. Several authors have demonstrated that naphthylphthalamic acid (NPA) block auxin efflux. It is believed that NPA interacts with a regulatory protein that modulates the activity of the carrier rather than inhibiting the carrier itself. Indeed, a saturable NPA binding site is present on the PM. A few regulatory mechanisms have been suggested but all lack strong supporting experimental evidence. In the present paper, four types of tyrosine kinase inhibitors have been used to investigate the nature of the NPA perception pathway. First, six synthetic tyrphostins, inhibitors of the epidermal growth factor receptor (EGF-R), were shown to specifically compete with NPA binding on PM fractions. The most potent compound was tyrphostin A47. As a control, the inactive tyrphostin A1 was unable to displace NPA. Tyrphostins did not inhibit auxin efflux but were antagonists of the auxin efflux inhibition by NPA. Again, tyrphostin A47 was the most effective and tyrphostin A1 was ineffective. Second, the flavonoid genistein, also a EGF-R inhibitor, showed an effect on NPA binding and NPA antagonism similar to the one for the tyrphostins. In this case, an inactive flavonoid, daidzein, demonstrated the specificity of genistein action. Third, another inhibitor, curcumin, was shown to effectively displace NPA and antagonize NPA inhibition of auxin efflux. Curcumin also induces morphological changes related to auxin action in *Arabidopsis* roots. Finally, lavendustin A, another EGF-R inhibitor also displaces NPA but is weaker than the other compounds tested. Taken together these results suggest that the NPA binding protein is related to mammalian tyrosine receptor kinases and regulates the auxin efflux carrier by phosphorylation.

A3-303 MULTIPLE REGULATORY ELEMENTS CONTROL ION CHANNEL ACTIVITY IN THE TONOPLAST OF BARLEY ALEURONE STORAGE PROTEIN VACUOLES, Paul C. Bethke and Russell L. Jones, Department of Plant Biology, University of California, Berkeley, CA 94720

Storage protein vacuoles (SPVs) within barley aleurone cells contain reserves of nitrogen, potassium and phosphate. We are interested in the activity of the SV channel, an abundant cation channel in the tonoplast which we postulate is involved in mobilization of SPV-stored K. Using the patch clamp technique, we have begun to identify regulatory elements important for control of SV channel activity. Three of these components have been found: membrane potential, Ca/CaM and phosphorylation. Increasing membrane potential (E_m=$E_{cytoplasm}$-$E_{vacuole\ lumen}$) in the range of E_m=20-80 mV increases the open probability of the channel. Little activity is observed at negative potentials. Channel activity is strongly reduced by 10 μM of the calmodulin (CaM) inhibitors TFP or W7 (92 and 94% inhibition at E_m=80 mV) and is stimulated by exogenous CaM at free calcium concentrations of 2.5-25 μM, suggesting that SPV associated Ca/CaM promotes channel opening. Two protein phosphatase inhibitors, okadaic acid and calyculin A, are highly effective at reducing SV channel activity. IC_{50} for whole vacuolar (WV) currents (E_m=80 mV) is ~100 pM for both compounds, indicating that activity of a protein phosphatase is important for channel gating. Removal of ATP from the bath solution by addition of glucose and hexokinase also significantly reduces WV currents, indicating that more than one regulatory element may be phosphorylated. This work is continuing, as we try to identify additional components participating in the regulation of SV channel activity, and as we seek to understand how these components are integrated into a cellular signal which modifies channel activity.

A3-304 KINETIC ANALYSIS OF H^+-COUPLED PLANT NUTRIENT TRANSPORTERS EXPRESSED IN *XENOPUS* OOCYTES. K.J. Boorer, D.D.F. Loo and E.M. Wright. Department of Physiology, UCLA School of Medicine, Los Angeles, Ca 90024

Amino acids and sugars are transported into plant cells via H^+-dependent cotransporters. Many of these nutrient transporters have been cloned and sequenced including a H^+/hexose cotransporter from *Arabidopsis* (STP1), a H^+/sucrose cotransporter from spinach (SoSUT1) and a H^+/amino acid cotransporter from *Arabidopsis* (NAT2/AAP1). We are investigating the mechanism of transport of these transporters by expressing them in *Xenopus* oocytes and measuring their transport kinetics using the 2-electrode voltage-clamp method. Steady-state substrate-dependent currents were recorded between -150 and 50 mV as a function of $[substrate]_o$ and $[H^+]_o$. For STP1, SoSUT1 and NAT2/AAP1, the maximal current (i_{max}) for 3OMG, sucrose and L-alanine respectively is voltage-dependent and the apparent affinity of the transporters for their respective substrates ($K_{0.5}$) is dependent upon $[H^+]_o$. The $K_{0.5}$ obtained at pH 5.5 is 15-60 μM for STP1, 3-5 mM for SoSUT1 and 0.8-1 mM for NAT2: these values are in a similar range to those determined from substrate uptake into yeast cells expressing the transporters. For STP1, presteady-state or transient currents were recorded after rapid steps in membrane potential which relaxed with time constants τ between 3 and 14 ms. At saturating [3OMG] and at low external $[H^+]$ (pH 7.5) the transient STP1 currents were eliminated. The charge movement Q (integral of the transient currents) fitted to a Boltzmann relation with maximal charge Q_{max} of 3.4 nC and apparent valence $z \approx 1$ corresponding to a transporter density of 2 x 10^{10}/oocyte. In summary, all three transporters are electrogenic, and by expressing them in *Xenopus* oocytes and using an electrophysiological approach their transport kinetics can be studied with very high voltage and temporal resolution.

A3-305 SPINACH CARBONIC ANHYDRASE: INVESTIGATION OF THE ZINC-BINDING LIGANDS BY SITE-DIRECTED MUTAGENESIS, ELEMENTAL ANALYSIS, AND EXAFS, Michael H. Bracey, Jason Christiansen, Pilar Tovar, Stephen P. Cramer, and Sue G. Bartlett, Department of Biochemistry, Louisiana State University, Baton Rouge, LA 70803

The enzyme carbonic anhydrase has been well characterized in mammalian systems, but the structural properties of the plant isozymes remain elusive. To investigate the nature of the zinc binding site in spinach carbonic anhydrase, we targeted potential zinc ligands for mutagenesis and examined the resulting enzymes for catalytic activity and stoichiometric zinc binding. In addition, we examined the wild-type protein using extended X-ray absorption fine structure analysis. Our results suggest that spinach carbonic anhydrase utilizes a Cys-His-Cys-H_2O ligand scheme to bind the zinc ion at the active site.

A3-306 EXPRESSION OF THE THREE MAJOR VACUOLAR H^+-ATPASE GENES IS DIFFERENTIALLY REGULATED IN RAPIDLY EXPANDING TISSUES. Marie-Paule Hasenfratz, Ching-Yi Wan and Thea A. Wilkins, Department of Agronomy and Range Science, University of California, Davis, CA 95616-8515.

The vacuolar H^+-ATPase (V-ATPase) holoenzyme is a large complex of 450-750 kD comprised of 8-10 different subunits that resides in most of the endomembrane compartments of a cell. The hydrophilic catalytic sector is composed mainly of a nucleotide-binding catalytic subunit (subunit A) and a regulatory subunit (subunit B) of about 69 and 55 kD, respectively. The integral membrane moiety is represented mostly by the 16 kD proteolipid subunit (subunit c). In order to enhance our understanding of the compartmentalization and regulation of the V-ATPase in different cell types and tissues, we have studied the expression of the three major subunit genes. The peripheral V-ATPase A and B subunits and the proteolipid subunit are present in the holoenzyme in a stoichiometry of $A_3B_3c_6$, but interestingly proteolipid RNA transcripts are approximately two-fold more abundant than the 69 kD catalytic subunit message and four-fold more abundant than the 55 kD subunit B transcript. These data suggest that the three major V-ATPase subunits are coordinately regulated, in part, at the transcriptional level. Northern blot analysis also shows that all three mRNA transcripts increase several-fold specifically in tissues undergoing rapid cell expansion (petals, roots and ovules). This induction is presumably related to vacuolation during cell expansion. Moreover, we have demonstrated that two proteolipid genes, belonging to a small gene family, are also differentially expressed in rapidly expanding tissues. RT-PCR experiments revealed that the relative level of both proteolipid mRNAs increases concomitantly, although one of the transcript is four-fold more abundant than the other. The two genes may encode functionally significant isoforms present in the same cells, organelles or even within the same holoenzyme.

A3-307 **Laser microsurgery of the cell wall facilitates patch clamp access to the guard cell plasma membrane.**

Gordon H. Henriksen and Sarah M. Assmann, Department of Biology, 208 Mueller Laboratory, The Pennsylvania State University, University Park, PA 16802

Plasma membranes of guard cells in epidermal peels of *Vicia faba* can be made accessible to a patch clamp pipet by removing a small portion (1-3 μm dia.) of the guard cell wall using a microbeam of ultraviolet light generated by a nitrogen laser. The protoplast bleb (that portion of the protoplast that protrudes through the laser hole) maintains its connection to the remainder of the cell within the cell wall compartment in all but a few instances. The longevity of the protoplast blebs is of sufficient duration to permit the execution of standard patch clamp protocols. Major advantages of the laser microsurgical technique are: 1) the ability to target a specific region of the plasma membrane from which to make patch clamp measurements; 2) avoidance of enzymatic protoplast isolation which may influence membrane properties, and; 3) the ability to study cell types which have been previously difficult to isolate as protoplasts (e.g. subsidiary cells). The region of the protoplast from which the bleb is produced may be manipulated by directing the laser beam to a specific site on the cell wall. This enables the study of ion channel distribution and cell polarity by preserving the original orientation of the cell, a feature which is lost when protoplasts are isolated enzymatically. Comparisons of patch clamp measurements made using laser-produced protoplast blebs and enzymatically isolated guard cell protoplasts are currently underway.

A3-308 A CHLOROPLAST HOMOLOGUE OF THE 54 K SUBUNIT OF SIGNAL RECOGNITION PARTICLE CHAPERONES LHCP TO THYLAKOID MEMBRANES FOLLOWING IMPORT INTO CHLOROPLASTS, Neil E. Hoffman*, Xingxiang Li*, Ralph Henry†, Jianguo Yuan†, Kenneth Cline†.

*Department of Plant Biology, Carnegie Institution of Washington, 290 Panama Street, Stanford, CA 94305.
†Plant Molecular and Cellular Biology Program and Horticultural Sciences Department, University of Florida, Gainesville, Florida 32611

The light harvesting chlorophyll a/b protein (LHCP) is synthesized as a precursor in the cytosol and post-translationally imported into chloroplasts. Upon translocation across the chloroplast envelope membranes, the N-terminal transit peptide is cleaved and the apoprotein integrated into the thylakoid membrane. Despite its three transmembrane domains, LHCP can exist in a soluble "transit complex" prior to its integration into the thylakoid membranes, presumably through an interaction with a protein factor present in the chloroplast stroma. Antibodies raised against 54CP, a chloroplast homologue of the 54 K subunit of the mammalian signal recognition particle (SRP54), immunoprecipitated the transit complex. Furthermore, addition of crosslinker resulted in the formation of a major 82 kD crosslinked product between LHCP (27 kD) and an ~ 55 kD stromal protein. This product could be efficiently immunoprecipitated with antibodies against 54CP or LHCP. Immunodepletion of 54CP from the stroma abolished the ability of stroma to support transit complex formation and LHCP integration into thylakoid membranes. These data indicate that 54CP is present in the transit complex, essential for complex formation, and required for LHCP integration into the thylakoid membrane. Our results show that 54CP functions as a molecular chaperone during LHCP trafficking. Furthermore, 54CP can operate post-translationally to facilitate intra-organellar protein trafficking of thylakoid membrane proteins.

A3-309 BIOSYNTHESIS OF VERY LONG CHAIN FATTY ACIDS IN PLANTS: SUBSTRATE SPECIFICTY, Jan G. Jaworski[1], Alenka Hlousek-Radojcic[1], Hiroyuki Imai[1], Kim Evenson[2], and, Dusty Post-Beittenmiller[2] 1) Chemistry Dept., Miami University, Oxford, OH 45056, 2) Plant Biology Division, The S.R. Noble Foundation, P.O. Box 2180, Ardmore, OK 73402

Very long chain fatty acids (C≥20) are required for the synthesis of waxes in epidermal tissue and very long chain fatty acids, such erucic acid, 22:1, are components of triacylglycerols in some oil seeds. These syntheses are catalyzed by a membrane-bound elongase system localized within the endoplasmic reticulum and other membranes outside the plastid. Thus, their synthesis is compartmentally separated from the ACP-based fatty acid synthesis. It is widely accepted that the acyl moiety that is extended is delivered to the elongase as an acyl-CoA. We will provide evidence from *in vitro* studies of elongases from developing seeds of *Brassica napus* and leek epidermis which indicate that the immediate precursor to very long chain fatty acids is not an acyl-CoA. Kinetic evidence from both systems is inconsistent with an acyl-CoA precursor. Moreover, the specific activities of 14C in very long chain fatty acids synthesized from an exogenous [14C]acyl-CoA were analyzed by GC/MS. This analysis provided evidence that the [14C]acyl group passed through an intermediate acyl pool during the elongation process. These data suggest that an acyl-lipid such as phosphatidyl choline may be the substrate for the membrane bound elongases.

A3-310 IAP75 - A CANDIDATE FOR A PROTEIN CONDUCTING CHANNEL OF THE CHLOROPLAST OUTER MEMBRANE

Felix Kessler and Günter Blobel
Laboratory of Cell Biology, The Rockefeller University, Howard Hughes Medical Institute
New York, NY, 10021

Our goal is to understand how nuclear encoded precursor proteins enter the chloroplast. A previously proposed model involves gated protein conducting channels in both inner and outer chloroplast envelope membranes.
By it's association with a partially translocated precursor protein we have identified a candidate for such a protein in the outer membrane. IAP75 (translocation intermediate associated protein of 75 kD) is a major integral protein of the outer membrane. Protease protection data reveal that IAP75 is largely embedded in the outer membrane.
We cloned and sequenced IAP75 using degenerate oligonucleotides. The cDNA deduced primary structure doesn't predict any Kyte-Doolittle type alpha helical transmembrane segments. However, using the beta turn identification paradigm of Rosenbusch, IAP75 could have as many as 26 transmembrane segments resembling those found in the porins.

A3-311 THE IDENTIFICATION OF SUBSTRATES AND EFFECTORS OF THE PLANT VACUOLAR H^+-PUMPING INORGANIC PYROPHOSPHATASE, Roger A. Leigh, Ruth Gordon-Weeks, Victor D. Koren'kov and Susan H. Steele, Biochemistry and Physiology Department, IACR, Rothamsted Experimental Station, Harpenden, Hertfordshire AL5 2JQ, UK

The plant vacuole possesses two H^+-pumps: a V-type ATPase similar to that in all eukaryotes and an inorganic pyrophosphatase (H^+-PPase) that appears to be unique to plants. The H^+-PPase has been cloned and sequenced and attempts are now being made to determine its functional domains. This requires knowledge of the identity of the substrates and effectors of the enzyme so that the number of functional sites is known. The H^+-PPase requires PPi, Mg^{2+} and K^+ for complete activity but shows complex kinetics when PPi and Mg^{2+} concentrations are varied. Using reaction kinetic modelling these responses have been explained with a model requiring four separate binding sites for (1) Mg_2PPi as the substrate, (2) Mg_2PPi as a non-competitive inhibitor, (3) Mg^{2+} as an activator and (4) K^+ as an activator. We are useing residue-specific reagents to investigate this model. Mixtures of Mg^{2+} and PPi protect the H^+-PPase from inhibition by N-ethylmaleimide (NEM) and detailed analysis has shown that the protecting complex is Mg_2PPi which binds with an affinity predicted by the model. Similarly, Mg^{2+} protects from inhibition by N-(3-dimethylaminopropyl)-N-ethylcarbodiimide (EDAC) with a Km of about 25 μM, again in good agreement with the model. In related work, we have shown that Tris buffer, which is commonly used in studies of the H^+-PPase, is a competitive inhibitor of K^+ stimulation (Ki = 12 mM). When this effect is taken in to account, the Km for K^+ is 1-2 mM, much lower than suggested in several studies where Tris has been used as a buffer. We now hope to use these observations to help identify the functional domains in the major subunit of the H^+-PPase.

A3-312 POTASSIUM HOMEOSTASIS IN BARLEY ROOT CELLS: MEASUREMENTS WITH TRIPLE-BARRELLED ION SELECTIVE MICROELECTRODES, Anthony J. Miller, David J. Walker and Roger A. Leigh, Biochemistry and Physiology Department, IACR Rothamsted Experimental Station, Harpenden, Hertfordshire AL5 2JQ, UK

When K^+ supply to plant tissues is varied from excess to deficiency, cytosolic K^+ activity is thought to be maintained at 100-150 mM to maintain K^+-dependent processes such as protein synthesis, while that in the vacuole varies with K^+ supply. We have used triple-barrelled microelectrodes which measure membrane potential, K^+ activity and pH to investigate changes in cytosolic and vacuolar K^+ activities in a quantitative way. The pH-selective barrel allows the location of the electrode to be determined unequivocally from the compartment pH of about 5.5 for the vacuole and about 7.5 for the cytosol. Measurements were made on cells of barley roots grown with a range of K^+ supplies. For 7 d old plants growing with a K^+ supply ranging from excess to mild deficiency the cytosolic K^+ activity in both epidermal and cortical cells remained almost constant at around 70 mM, while that in the vacuole K^+ changed in parallel with K^+ supply. With more severe K^+ starvation, imposed by growing in 2 μM K^+, the cytosolic K^+ activity in epidermal cells decreased significantly to 46 mM after 14 d and 24 mM after 21 d. In these cells, there was also a decrease in cytosolic pH from 7.2 initially to 6.9 and 6.7 after 14 and 21 d, respectively. In contrast, under the same conditions, cortical cells maintained the cytosolic K^+ activity and pH constant at around 70 mM and 7.3, respectively. These differences between the cortical and epidermal cells may be related to the restriction of expression of a high affinity H^+/K^+ uptake mechanism to cortical cells of cereal roots (Schachtman & Schroeder, Nature 1994 370: 655). The measurements enable the calculation of the electrochemical gradients for K^+ and indicate that under replete conditions there must be active transport into the vacuole while during starvation this changes to active transport out of the vacuole.

A3-313 TWO POLYPEPTIDES OF THE CHLOROPLAST PROTEIN IMPORT APPARATUS,
Eva Muckel, Matthias Seedorf, Stephan Hirsch, Frank Heemeyer, Karin Waegemann and Jürgen Soll, Botanisches Institut, Universität Kiel, Olshausenstr. 40, 24098 Kiel, Germany
Most of the chloroplast localized proteins are nuclear encoded and imported into the organelle from the cytosol in a posttranslational process. A new crosslink-approach was used to identify two proteins of the outer envelope membrane that directly interact with the precursor of the small subunit of RUBISCO (Perry, S.E. and Keegstra, K. (1994), *Plant Cell* 6, 93-105); a protease sensitive 86 kD protein (OEP 86) and a protease resistant 75 kD protein (OEP 75). A functional active membrane protein complex was isolated from chloroplasts outer envelopes (Soll, J. and Waegemann, K. (1992), *Plant Journal* 2, 252-256). This import complex contained OEP 75 the translocation pore, OEP 86 and OEP 34 as constituents. We identified OEP 86 as the general import receptor from import inhibition studies with antibodies against OEP 86. OEP 34 that represents a new type of GTP-binding protein is associated with OEP 75 *in situ* and may be involved in regulation of the import process. OEP 86 and OEP 34 are highly phosphorylated under ATP conditions that allow binding of precursor proteins to the chloroplastic surface. OEP 34 and OEP 86 were purified and cloned. Further sequence analysis revealed homology between the two proteins in their N-terminal cytoplasmic domain, where they contain a conserved phosphate-binding motif (P-loop). Both proteins are anchored to the membrane by their C-terminus and are inserted in the outer envelope of intact chloroplasts in an ATP dependent manner requiring protease sensitive surface components. OEP 86 contains a N-terminal cleavable presequence different from stromal targeting sequences suggesting the existence of a new type of processing peptidase.

A3-314 ABUNDANT CHANNELS SPECIFIC FOR EXCHANGE OF WATER ACROSS THE PLASMA MEMBRANE.
Anton R. Schäffner, Werner Kammerloher and Katja I. Warkalla, Institut für Biochemie, University of München, D-81375 MÜNCHEN, Germany.

A novel approach to access plant genes encoding plasma membrane proteins was developed. Anti-plasma membrane antibodies were used for immunoselection and cloning of genes from an *Arabidopsis thaliana* cDNA library expressed in mammalian COS cells. Five related genes were identified that encode members of the evolutionary conserved major intrinsic protein (MIP) family. They comprise two subfamilies named PIP1 and PIP2 for plasma membrane intrinsic proteins. Their subcellular localization was confirmed by cosegregation with plasma membrane marker enzymes in purified fractions generated by aqueous two-phase partitioning. PIP proteins are highly abundant, they account for at least 1% of total plasma membrane proteins in *Arabidopsis* leaf and root tissue. PIP mRNAs and protein levels are not subject to significant changes by water or heat stresses. Interestingly however, after injection into *Xenopus laevis* oocytes all five PIP mRNAs lead to the expression of functional channels allowing the osmotic entry of water. This is simply shown by an accelerated swelling of the oocytes after transfer to a hypotonic solution. These activities are reversibly inhibited by $HgCl_2$, the only pharmaceutical agent known to block a water channel present e.g. in highly specialized resorptive cells in renal proximal tubules. Furthermore, except water other small molecules like urea and glycerol but also a variety of ions are shown not to pass the channels.
Thus, we conclude that there exists a family of constitutively and abundantly expressed specific water channels, or aquaporins, in the plasma membrane of plants. This surprising activity has not been postulated by plant physiologists due to the high diffusional water conductance of lipid bilayers. Nevertheless, due to their high expression levels these water channels may be generally involved in water transport across the plasma membrane possibly in concert with the corresponding activities in the tonoplast via γ- and α–TIP (Maurel *et al.*, 1993, *EMBO J.* **12,** 2241-2247) during rapid cell expansion or all other processes known or not yet known to rely on or to profit from rapid exchange of water.

A3-315 MOLECULAR INTERACTIONS AMONG COMPONENTS OF THE CHLOROPLAST ENVELOPE PROTEIN IMPORT APPARATUS,
Danny J. Schnell, Fang Tian and Yongkang Ma, Department of Biological Sciences, Rutgers, The State University of New Jersey, Newark, NJ 07102
Because of its dual genetic origin, a majority of chloroplast proteins are synthesized in the cytoplasm and posttranslationally imported into the organelle across the double membrane of the chloroplast envelope. We have reported the isolation of a set of four chloroplast envelope proteins that associate with an early import intermediate representing an envelope bound precursor protein. We refer to these proteins as early import intermediate associated proteins (IAPs). All four of the IAPs are localized in the outer membrane and are candidates for components of the outer membrane import machinery. The early IAPs include an hsp70 homologue, two GTP binding proteins, and a unique 75 kD protein. It is not known whether these IAPs act independently during import or whether they function as a complex to mediate precursor recognition and translocation. We have used a combination of immunoprecipitation and Western blotting with IAP-specific antibodies to identify IAP complexes from envelope membranes solubilized under nondenaturing conditions. We will present our results on mapping the molecular interactions of the early IAPs in the presence and absence of an import substrate.

A3-316 A PUTATIVE CAM AND CYCLIC-NUCLEOTIDE-REGULATED ION CHANNEL FROM BARLEY
Robert C. Schuurink and Russell L. Jones. Department of Plant Biology, University of California, Berkeley, CA 94720-3102
Abscisic acid (ABA) and gibberellic acid (GA) regulate the synthesis and secretion of hydrolytic enzymes in the barley aleurone cell. At least one receptor for these hormones is localized at the plasma membrane of the aleurone cell. An early event following exposure of aleurone cells to GA is a rise in cytoplasmic [Ca^{2+}] to about 200-300 nM, a response that is dependent on an external supply of millimolar amounts of Ca^{2+}. ABA, which antagonizes the effects of GA on enzymes synthesis and secretion, resets cytoplasmic Ca^{2+} to resting levels of about 50-100 nM. Cytosolic Ca^{2+} interacts with Ca^{2+} binding proteins such as calmodulin (CaM) to regulate the activity of target proteins in the aleurone cell. Biochemical and biophysical approaches have identified a Ca^{2+}-ATPase on the endoplasmic reticulum and a K^+ channel in the protein body vacuole whose activities are regulated by Ca^{2+}-CaM. We have used a molecular approach to search for other putative Ca^{2+}-CaM regulated proteins in aleurone cells. Expression cDNA libraries (made from barley aleurone mRNA) were screened with CaM conjugated to horse radish peroxidase. Seven positive clones were isolated that bound CaM in a Ca^{2+}-dependent manner. Clone CBP-13 (CaM binding protein C13) shows high homology at the amino acid level to a rat olfactory channel (49% similarity, 26% identity) and the *Arabidopsis* KAT1 K^+ channel (46% similarity, 20% identity). The partial cDNA clone (2.1 kbp) has five of the six characteristic transmembrane spanning domains in which homology with other ion channel sequences is even higher. The S4 domain of CBP-13 contains 4 arginines, indicating that it is more closely related to cyclic nucleotide-gated channels than to voltage gated channels that have 5-7 positive charges in this domain. The carboxy terminus of CBP-13 has all of the conserved amino acids present in cyclic nucleotide binding (cNMP) domains. Moreover, there is an amphiphilic helix downstream of the cNMP binding site, which has features of a CaM binding site. The molecular characteristics of CBP-C13 make it a good candidate for involvement in the ABA/GA signal transduction cascade in barley aleurone cells.

A3-317 THE SPIROSTANOL-INDUCED CALLOSE SYNTHESIS DOES NOT DEPEND ON MEMBRANE POTENTIAL OR CYTOSOLIC CALCIUM AND pH.

Pierre Van Cutsem, Johan Messiaen and Frédéric Nérinckx, Unité de Biotechnologie théorique, Facultés Universitaires de Namur, B-5000 Namur, Belgium.

Carrot cells and protoplasts treated with spirostanol elicitor from *Yucca* accumulate callose after a 1h lag phase. The PAL enzyme is not activated by the treatment. Fluorescence imaging of spirostanol-treated protoplasts was performed using DiBAC4(3), SNARF-1/AM and Indo1 for assaying $\Delta\Psi$, cytosolic pH and calcium activity, respectively. No significant modification of any of these three parameters has been recorded.

Several modulators affecting directly or indirectly the cytosolic free calcium concentration have been used to test their effect on callose synthesis. High concentrations of verapamil killed protoplasts and cells. Nifedipine, an other calcium channel blocker, was much less toxic, but it did not inhibit callose synthesis. EGTA blocked completely callose production, but killed all cells and protoplasts. A calcium ionophore, Br-A23187, was unable to induce callose deposition.

Vanadate, an inhibitor of the plasmalemma proton pump, induced membrane depolarization and cytosolic acidification, but no callose synthesis, even in presence of the calcium ionophore.

We conclude that spirostanol induces callose formation without affecting PAL activity. No clear correlation could be found with changes in membrane potential, cytosolic free calcium or cytosolic pH.

A3-318 NUCLEAR MUTANTS IN MAIZE WITH DEFECTS IN INTRACHLOROPLAST PROTEIN TARGETING,

Rodger Voelker and Alice Barkan, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.

We have obtained evidence for nuclear genes in maize, *tha1* and *hcf106*, that function in two different pathways by which proteins are translocated across the thylakoid membrane. Recessive mutations in these genes cause a decrease in the levels of the PSI, PSII, and cyt f/b6 complexes. Since each affected complex contains at least one subunit with a lumenal targeting signal sequence, we reasoned that these mutations might interfere with the translocation of proteins to the thylakoid lumen. Results of pulse labelling experiments supported this hypothesis in that the rate of processing of a subset of lumenal proteins was reduced in each mutant: OEE33, plastocyanin (PC), and cytochrome f processing is defective in *tha1* ; OEE23 and OEE17 processing is defective in *hcf106*. Precursors accumulated outside the thylakoid, indicating that the processing defects are due to targeting defects and not to defects in the processing protease itself. These *in vivo* results support models based upon *in vitro* experiments from other laboratories that have suggested that OEE33/PC and OEE23/OEE17 use distinct import pathways. Further, these results suggest that the chloroplast-encoded protein, cytochrome f, uses the same targeting machinery as two nuclear-encoded proteins (OEE33 and PC). Little is known about the components that mediate targeting via these two pathways. The *hcf106* gene has been cloned by transposon tagging (Martienssen et al, 1989) and the *tha1* gene is tagged by a transposon. Thus, these and other similar mutations should facilitate the identification and characterization of the protein machinery involved in targeting proteins to the thylakoid lumen.

A3-319 VACUOLAR H^+-ATPASE GENES ARE TEMPORALLY REGULATED IN DEVELOPING COTTON TRICHOMES,

Ching-Yi Wan, Dhammika Gunasekera, Marie-Paule Hasenfratz and Thea A. Wilkins, Department of Agronomy and Range Science, University of California, Davis, CA 95616-8515

Plant vacuoles are dynamic multifunctional organelles that perform important cellular functions, including maintenance of cell turgor. The dramatic morphological and biochemical changes that occurred in the vacuolar compartment during rapid expansion of developing cotton seed trichomes provides a unique opportunity to investigate vacuole biogenesis. Vacuolar H^+-ATPase and inorganic H^+-pyrophosphatase are tonoplast-residing proton-pumps responsible for generating the electrogenic gradient that facilitates water and solute transport across tonoplast. The histochemical localization of significant ATPase activity only on the tonoplast of elongating fibers (Joshi et al., 1988) suggests that the V-ATPases are regulated during rapid cell expansion. The expression of genes encoding peripheral subunits A and B, and integral subunit c of the V-ATPase in developing cotton ovules was examined at the mRNA, protein and enzymatic activity levels. V-ATPase subunit A, B, and c mRNAs are induced specifically during trichome elongation to significant levels in two successive waves. The first minor peak precedes the formation of primordial trichomes. The second and most dramatic increase in V-ATPase mRNAs is correlated with the period of rapid unidirectional cell expansion. As elongation ceases, mRNA levels markedly decrease to basal levels. Preliminary results also show that the accumulation of V-ATPase subunits A and B, and V-ATPase enzymatic activity exhibit developmental patterns similar to that observed at the RNA level in developing cotton trichomes.

A3-320 ONTOGENY AND COMPOSITION OF MEMBRANES INVOLVED IN INITIATION AND DEVELOPMENT OF CALCIUM OXALATE CRYSTALS IN *VITIS* (GRAPE),

Mary Alice Webb, John Cavaletto, and Jennifer Bell, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907

In leaves and seeds of *Vitis* (grape) crystals of calcium oxalate develop in a needle-like morphology that is unique to plants. Ultrastructural studies have shown that development of these crystals involves a proliferation of membrane systems within the vacuole of the crystal-forming cells, including membrane chambers that enclose each developing crystal, reticulate membranes associated with bundles of crystals, and additional membranes extending between crystals. We are using a variety of approaches to investigate the ontogeny and composition of these membranes. Antibodies against the 70-kDa subunit of vacuolar-type proton ATPase react with Western blots of crystal chamber extracts and with the crystal chambers *in situ*, suggesting that the crystal chamber membranes are ontogenetically related to the tonoplast. The ATPase may also provide a mechanism for removal of protons released within the crystal chambers by precipitation of calcium and oxalic acid. The nature of the ATPase and its expression in grape leaves are being investigated further. In a complementary approach we have also produced complex polyclonal antibodies against components of the crystal chamber membranes. These antibodies recognize polypeptides of 60 and 70 kDa in Western blots of crystal chamber extracts, and they also label the crystal chambers *in situ*. We have used these antibodies to purify the corresponding antigens by immunoaffinity chromatography for characterization and sequence analysis. We presently are screening a cDNA expression library to identify and isolate genes encoding the membrane proteins.

A3-321 BIOGENESIS OF DISTINCT VACUOLE-TYPES DURING CELL DIFFERENTIATION OF SEED TRICHOMES, Thea A. Wilkins and Suresh C. Tiwari, Dept. of Agronomy & Range Science, University of California, Davis, CA 95616-8515

Several novel aspects of vacuole dynamics and biogenesis have been newly discovered in differentiating cotton seed trichoblasts using rapid-freeze fixation and freeze-substitution techniques in electron microscopy. Cotton seed trichomes are single cells in excess of 28 mm in length that differentiate from the outer epidermis of the ovule. Initially, epidermal cells in immature ovules exhibit a homogeneous population of spherical, electron-lucent vacuoles characteristic of most plant cells. During the period of trichome differentiation in the days preceding anthesis, however, a second type of morphologically distinct vacuole (type 2) becomes predominant in epidermal cells. Highly pleiomorphic, type 2 vacuoles consist of a complex, anastomosed reticulate network filled with unidentified electron-dense material. Cytoplasmic microtubules are found in close association with only type 2 vacuoles. Interestingly, the tonoplast of type 2 vacuoles are stained intensely for polysaccharides, a feature reflective of glycoprotein-rich lysosomal membranes. In the hours preceding anthesis and the onset of cell expansion, the electron-dense material of type 2 vacuoles begins to dissipate in primordial trichomes. Concomitantly, existing vacuoles of both types coalesce and fuse to form a single, homogeneous large central vacuole within 24-26 hours. The existence of two morphologically and biochemically distinct vacuole types is suggestive of a functional compartmentalization to maintain physical separation of constituents that may potentially trigger the onset of cell expansion in developing cotton seed trichomes.

A3-322 CHLOROPLAST IMPORT AND REGULATION OF THE TRYPTOPHAN BIOSYNTHETIC ENZYMES OF THE FLOWERING PLANT *ARABIDOPSIS THALIANA* Jianmin Zhao and Robert L. Last, Boyce Thompson Institute for Plant Research and Section of Genetics and Development, Cornell University, Tower Road, Ithaca, NY 14853

The plant tryptophan biosynthetic pathway leads to the biosynthesis of auxin and many other secondary metabolites in addition to the essential amino acid tryptophan. In order to study the Arabidopsis tryptophan biosynthetic enzymes at the protein level, polyclonal antibodies were raised against five proteins of the Trp biosynthetic pathway from Arabidopsis: anthranilate synthase α subunit, phosphoribosylanthranilate transferase, phosphoribosylanthranilate isomerase (PAI), tryptophan synthase α subunit (TSA), and tryptophan synthase ß subunit. These antibodies are being used in the study of the regulation of the Trp biosynthetic pathway and in the isolation of new mutants in this pathway.

All available evidence suggests that the Trp biosynthetic pathway is localized to the chloroplasts of plants. For example, all cloned genes of the Trp biosynthetic pathway encode proteins that have sequences that resemble chloroplast target peptides at their NH_2-termini. To test whether these putative transit peptides are functional chloroplast-targeting sequences, we synthesized radioactive labeled PAI and TSA protein precursors *in vitro*. These precursors were efficiently imported and processed into their mature forms by isolated spinach chloroplasts. These results provide direct evidence that the Trp biosynthetic enzymes from Arabidopsis are synthesized as larger molecular weight precursors and then imported into chloroplasts and processed into their mature forms.

Cytoplasmic Regulatory Mechanisms; Signaling to the Nucleus; Processing, Plant Defense

A3-400 DEVELOPMENT OF A HELA-PLANT *IN VITRO* COMPLEMENTATION SYSTEM Clair E. Baynton and Mary A. Schuler, Department of Plant Biology, University of Illinois, Urbana, IL61801

Although it may be logical to assume that all eukaryotes share a common mechanism for pre-mRNA splicing, *in vivo* analysis of the *cis*-acting factors required for intron recognition in plant nuclei has indicated that AU-rich elements spread throughout the length of plant introns play a critical role in intron recognition and splice site selection in plant nuclei.

In an effort directed at identifying factors critical for the recognition of plant introns we have developed an *in vitro* Hela-plant complementation system. Plant introns, which lack strong 5' and 3' splice site and branchpoint consensus motifs, are often poorly spliced in Hela splicing extracts, which rely heavily on these consensus sequences.

We have now identified pea nuclear fractions, which when added to Hela splicing extracts, enhance recognition and splicing of plant introns. The protein factor which enhances splicing of the *b*-conglycinin intron 4 is partially stable at 65°C and fractionates away from the bulk of the small nuclear spliceosomal snRNPs.

Western blot analysis suggests this factor is not one of the serine arginine-rich (SR) proteins, which have been shown to play essential roles in mammalian intron splicing. Native gel electrophoresis indicates that this intron enhancing factor(s) acts at an early stage in intron recognition prior to association of the U2snRNP with the branchpoint.

A3-401 TiC: A DNA-BINDING COMPLEX REGULATED BY THE CIRCADIAN CLOCK, Isabelle A. Carré and Steve A. Kay, NSF Center for Biological Timing, University of Virginia, Charlottesville, VA 22903.

The *CAB* gene family from higher plants encodes the chlorophyll *a/b*-binding proteins. The transcription of the Arabidopsis *CAB2* gene is under the control of an endogenous circadian oscillator, so that expression of the *CAB2* transcript is "gated" to the day phase of the circadian cycle. A 78 bp promoter fragment corresponding to -111/-33 is sufficient to confer circadian regulation to a luciferase reporter gene. In order to identify elements of the signalling pathway for the control of *CAB2* expression by the clock, complexes that bind this promoter element were characterized in gel shift assays. Three activities were identified in Arabidopsis whole cell extracts. CUF2 and CUF3 (*CAB* Upstream Factors 2 and 3) bind the 5' boundary of the -111/-74 region, and are not regulated by the circadian oscillator. A third complex, TiC (Timing of *CAB*), exhibits circadian changes in its DNA-binding activity in gel shift assays. In extracts from transgenic plants bearing a *cab2*-luciferase gene fusion, peaks of TiC correlated with troughs of luciferase activity, suggesting that TiC may act as a repressor of *CAB2* transcription. The TiC binding site was mapped to the -94/-65 region, which contains a CCAAT-box that is conserved in many *CAB* genes from several species. Redundant TiC binding sites were also identified within -321/-111. We wish to determine how TiC binding is modulated by the circadian clock.

A3-402 CHARACTERIZATION AND REGULATION OF A DIHYDROBENZOPHENANTHRIDINE OXIDASE FROM ELICITED *SANGUINARIA CANADENSIS* CELL CULTURES, C.J.Coscia*, W.G.Clark*, H.Arakawa*, M.Psenak* and R.J.Krueger+, *E.A. Doisy Dept. of Biochemistry and Molecular Biology, St. Louis University School of Medicine, St. Louis, MO 63104, +Dept. of Pharmacognosy, Ferris State University, Big Rapids, MI 49307, Upon treatment of *Papaveracea* cell cultures with fungal elicitors, the activity of dihydrobenzophenanthridine (DHBP) oxidase, which catalyzes one of the last steps in the biogenesis of the benzo[c]phenanthridine alkaloids, is induced. The oxidase (Arakawa et al. Arch. Biochem. Biophys. **299**, 1-7 1992) has now been purified to near homogeneity by a sequence of ion exchange, hydrophobic interaction, and gel exclusion FPLC. SDS-PAGE of the purified oxidase revealed a single band with an estimated molecular weight of 65 kDa. Evidence was also gained to suggest that the oxidase is a copper-protein. Addition of carbohydrate-rich fungal cell wall preparations or putative plant defense signal molecules (methyl jasmonate, acetylsalicylic acid) to *S. canadensis* cultures enhanced the activity of DHBP oxidase. Both methyl jasmonate and acetylsalicylic acid induced oxidase activity to a greater extent than fungal elicitors. In some experiments a 2-fold induction of specific activity was observed, without change in total enzyme activity in the cultured cells. The results suggest that DHBP oxidase, jasmonate and salicylate may play roles in *S. canadensis* defense against pathogens. Supported in part by NIH grants GM41421 and 5T32 HL 0705040.

A3-403 ISOLATION AND CHARACTERIZATION OF TWO PLANT CYTOCHROME P450S IN THE PHENYLPROPANOID BIOSYNTHETIC PATHWAY. MICHAEL R. FRANK*, JOY M. DEYNEKA, and MARY A. SCHULER. Department of Plant Biology. University of Illinois, Urbana IL 61801

Cytochrome P450 monooxygenases (P450s) mediate a wide range of oxidative reactions including the degradation of drugs, insecticides, and plant defense compounds. While P450s have been implicated in the production of many of these defensive compounds and other secondary metabolites, few plant P450s have been cloned and characterized at a regulatory level.

To investigate the regulation of these P450s in the phenylpropanoid biosynthetic pathway, we have used a reverse transcriptase-PCR (RT-PCR) strategy to isolate pea P450 cDNAs. Utilizing degenerate primers encoding conserved plant P450 sequences, we have isolated PCR clones encoding four distinct cytochrome P450s and obtained full-length copies for two of these. Sequence analysis indicates that one of these, cDNA26, encodes trans-cinnamic acid hydroxylase (tCAH), the P450 which catalyzes a core reaction in the phenylpropanoid pathway. The second, cDNA50, is moderately homologous to several flavonoid hydroxylases.

Northern analysis of poly (A)+ mRNA suggests that cDNA26 mRNA is relatively abundant in root and stem tissue of light-grown seedlings and less abundant in leaf tissue. cDNA26 message is also induced over twenty-fold in four-hour wounded stem sections, preceding observed maximal wound induced increases in tCAH activity at ten hours. The cDNAs are currently being used to investigate the mechanisms regulating gene expression in response to wounding as well as protein activity analysis.

A3-404 PROCESSESING ENZYMES OF PROBARLEY LECTIN FROM TOBACCO AND *ARABIDOPSIS*, Susannah Gal, Department of Biological Sciences, The State University of New York, Binghamton, New York, 13902-6000

Many proteins are synthesized with propeptides whose removal by processing enzymes is essential, in some cases, for correct functioning of the protein. In plants, a variety of protein processing enzymes have been isolated and characterized *in vitro*, but relatively little is known about the function of these enzymes *in vivo*. I am interested in characterizing these processing enzymes in plants and will initiate my work by studying the processing enzyme(s) of probarley lectin (proBL). Barley lectin (BL) is synthesized with a carboxy-terminal propeptide (CTPP) of 15 amino acids which is removed concommitant with or just prior to delivery to the plant vacuole in barley seeds and in transgenic tobacco and *Arabidopsis thaliana*. I have recently identified a barley aspartic proteinase (HvAP) in collaboration with Pia Runeberg-Roos in Finland which is able to process proBL *in vitro*. We were also able to co-localize BL and HvAP in barley plants.

The question I wish to address now is whether HvAP is involved in processing proBL *in vivo* in the transgenic systems. I intend to approach this question in three ways. 1) Purify vacuoles from tobacco and *Arabidopsis* plants and assay for proBL processing activity and determine whether this activity has properties similar to those of HvAP. 2) Transform *Arabidopsis* plants expressing proBL with the *HvAP* gene in an antisense orientation to determine the effect of lower levels of HvAP protein on proBL processing. 3) Isolate mutants of *Arabidopsis* in proBL processing and determine if the lost activity corresponds to an HvAP-homolog. These different approaches will not only determine whether the HvAP is involved in the processing of proBL *in vivo*, but will also define the processing enzyme(s) in tobacco and *Arabidopsis*.

A3-405 THE *axr4* AUXIN-RESISTANT MUTANTS OF *ARABIDOPSIS*: CHARACTERIZATION AND GENETIC INTERACTIONS WITH OTHER AUXIN-RESISTANT MUTANTS. Lawrence Hobbie and Mark Estelle, Department of Biology, Indiana University, Bloomington, IN 47405

To understand the molecular mechanism of auxin action, we have identified and characterized mutants of *Arabidopsis thaliana* with altered responses to auxin. We have isolated two auxin-resistant mutants that define a new locus involved in auxin response, named *AXR4*. The *axr4* mutations are recessive and map near the *chl* mutation on chromosome 1. Mutant plants are 3-5-fold resistant to auxin and are not resistant to other plant hormones tested. The *axr4* mutants are also defective in root gravitropism and reduced in lateral root formation. Double mutants between *axr4* and the recessive auxin-resistant mutants *axr1* and *aux1* were constructed and characterized to test for possible genetic interactions between the mutations. The roots of the *axr4 axr1* double mutant plants are less sensitive to auxin, respond more slowly to gravity, and form fewer lateral roots than either parental single mutant. These results suggest that the two mutations have additive or even synergistic effects. The AXR1 and AXR4 gene products may act in independent pathways of auxin response or perhaps perform partially redundant functions in a single pathway. The *axr4 aux1* double mutant forms far fewer lateral roots than either parental single mutant but has the same sensitivity to auxin as the *aux1* mutant. The *aux1* mutation thus appears to be epistatic to *axr4* with respect to auxin-resistant root elongation, suggesting that the AXR4 and AUX1 gene products may act in the same pathway. In lateral root formation, however, the effect of the two mutations appears to be additive. The complexity of the genetic interactions indicated by these results suggests that there is more than one pathway of auxin response. Based on these results, the AXR4 gene product has an important role in auxin sensitivity, lateral root formation, and root gravitropic response.

A3-406 NETWORKING OF CYTOKININ, ETHYLENE AND LIGHT RESPONSES DURING SEEDLING DEVELOPMENT IN ARABIDOPSIS, Stephen H. Howell, Andrew Cary, Wennuan Liu, Wenpei Su and Christina Smart, Boyce Thompson Institute, Cornell Univ., Ithaca, NY, 14853

The action of light and plant hormones are closely intermeshed in their effects on developmental events in seedlings, such as root and hypocotyl elongation, apical hook formation, cotyledon opening and expansion. Using physiological and genetic approaches in Arabidopsis, we have demonstrated that cytokinin and ethylene responses are coupled in the inhibition of root and hypocotyl elongation, whereas the action of the hormones counteract each other in their effects on cotyledon expansion. On the other hand, light and cytokinin act in parallel to inhibit hypocotyl elongation. Benzyladenine (BA), a cytokinin agonist, inhibits root and hypocotyl elongation in dark-grown seedlings. It was found that the inhibitory effects of BA on root and hypocotyl elongation were largely mediated by ethylene, and the action of BA could be blocked by ethylene mutants (*ein1-1* and *ein2-1*) or ethylene inhibitors. In addition, we found that BA stimulated ethylene production sufficient to account, in part, for the inhibition of root and hypocotyl elongation. The interaction of cytokinin and ethylene are further supported by the discovery that cytokinin resistant mutant, *ckr1*, is resistant to ethylene and is allelic to the ethylene resistant mutant *ein2*. BA mimics the effects of light on the inhibition of hypocotyl elongation. However, under standard growth conditions, light is saturating for the inhibition response, and BA has no effect on hypocotyl elongation. These effects can be explained if cytokinin and light response systems have mutual and additive effects on the inhibition of hypocotyl elongation. It was found in testing long hypocotyl (*hy*) mutants that functional light response pathways are not required for normal cytokinin responses. This indicates that cytokinin and light response pathways are not coupled, but act in parallel.

A3-407 CHLOROPLAST PROCESSING PEPTIDASE SHARES A ZINC-BINDING MOTIF WITH A NEW FAMILY OF METALLOPROTEASES, Gayle Lamppa, Pamela VanderVere, Thomas Bennett and John Oblong, Department of Molecular Genetics and Cell Biology, University of Chicago, 920 E. 58th St., Chicago, Il 60637

Two proteins of 145 and 143 kD were identified in pea that co-purify with a chloroplast processing activity which cleaves the precursor for the major light-harvesting chlorophyll binding protein (preLHCP), removing the transit peptide. Antiserum generated against the 145/143 kD doublet recognizes only these two polypeptides in a chloroplast soluble extract. In immunodepletion experiments the antiserum removed the doublet, and there was a concomitant loss of cleavage of preLHCP as well as of precursors for the small subunit of Rubisco and the acyl carrier protein. The 145 and 143 kD proteins co-eluted in parallel with the peak of processing activity during all fractionation procedures, but they were not detectable as a homo- or heterodimeric complex, suggesting they are structurally similar and function independently during precursor cleavage. The 145 and 143 kD proteins were separately used to affinity purify immunoglobulins; each preparation recognized both polypeptides, showing that they are antigenically related. They may be products of two genes, or arise through posttranslational modification of one protein. Wheat and Arabidopsis chloroplasts also contain a soluble species similar in size to the 145/143 kD doublet. cDNAs from pea have been characterized that code for the processing peptidase, and reveal that it contains a zinc-binding motif that places it in the newly recognized family of metalloproteases, the pitrilysins, which includes E. coli protease III, insulin-degrading enzyme from animals, and the ß-subunit of the mitochondrial processing peptidase from plants, fungi and animals. Southern blot analysis indicates that the chloroplast processing peptidase is encoded by a small gene family in pea and Arabidopsis. The pattern of expression of the processing peptidase is being explored. It is present and active in dark and light-grown leaves, as well as in plastids from roots, supporting the conclusion that it is involved in the maturation of a large diversity of precursors targeted to the organelle.

A3-408 **RELATIONSHIP OF A 33 KD PUTATIVE CYSTEINE PROTEINASE WITH FALL ARMYWORM (*Spodoptera frugiperda*) RESISTANCE IN MAIZE,** D. S. Luthe [1], B. Jiang[1], U. Siregar[1], K. O. Willeford[1], & W. P.Williams[2]. [1]Dept. of Biochem. & Mol. Biol. & [2]USDA Crop Sci. Res. Lab. ARS, Miss. State Univ., Miss. State, MS 39762

The protein patterns of calli from maize genotypes resistant (Mp704, Mp708) and susceptible (Tx601, Ab24E) to fall armyworm and several other lepidoptera were analyzed by 2-D gel electrophoresis. All of the resistant, and none of the susceptible genotypes contained a 33 kD protein. When insect larvae were reared on non-friable callus derived from resistant plants, they were smaller than those reared on non-friable callus from susceptible plants. When callus from resistant plants changed from nonfriable to friable morphology, the callus no longer exhibited the resistant phenotype. Western blot analysis indicated that the 33 kD protein was absent in the friable callus from resistant plants. Microsequencing indicated that amino terminal had greater than 50% homology with other cysteine proteinases. Analysis of F_2 calli from Mp704 x Tx601 indicated that the concentration of the 33 kD protein and larval weight were negatively correlated ($r=-0.59$, $p<0.01$).

The susceptible genotype Ab24E contained a 36 kD protein that cross-reacted with antibody to the 33 kD protein. Both of these proteins have been purified from callus using classical biochemical techniques, both cross-reacted with the antibody to 33 kD protein, and both had cysteine proteinase activity. However, the specific activity of the 33 kD protein from the resistant genotype was an order of magnitude greater than that of the 36 kD protein isolated from the susceptible genotype. The presence of a 33 kD protein with a high cysteine proteinase activity appears to be required for resistance in callus. The presence of a similar protein, with lower enzymatic activity does not appear to confer resistance. The subcellular location of these proteins is currently unknown.

A3-409 THE GENETICS OF CIRCADIAN CLOCK REGULATION IN ARABIDOPSIS, Andrew J. Millar[1,2], Martin Straume[1], Isabelle A. Carré[1], Carl A. Strayer[1], Joanne Chory[3], Nam-Hai Chua[2] and Steve A. Kay[1], [1]NSF Center for Biological Timing, University of Virginia, Charlottesville, VA 22903; [2]Laboratory of Plant Molecular Biology, Rockefeller University, New York, NY 10021; [3]Laboratory of Plant Biology, The Salk Institute, San Diego, CA 92186.

Transgenic Arabidopsis plants expressing a luciferase gene fused to a circadian-regulated *CAB* promoter exhibit robust rhythms in bioluminescence. The cyclic luminescence has a 24.7 hour period in white light (LL) but 30-36 hour periods under constant darkness (DD). Either red or blue light shortens the period of the wild type to 25 hours. The phytochrome-deficient mutation *hy1* lengthens the period in red light but has little effect in blue light, whereas seedlings carrying mutations that activate light-dependent pathways in darkness (*det1*, *det2* and *cop1*) maintain shorter periods in DD. These results suggest that both phytochrome and blue-responsive photoreceptor pathways control the period of the circadian clock. The cycling bioluminescence of *cab::Luc* Arabidopsis plants was also used to identify mutant individuals with aberrant cycling patterns. Both long- and short-period mutants were recovered. A semi-dominant, short-period mutation, timing of *CAB* expression (*toc1*), has been mapped to chromosome 5. The *toc1* mutation shortens the period of two distinct circadian rhythms, the expression of endogenous *CAB* genes and the movements of primary leaves, although *toc1* mutants do not show extensive pleiotropy for other phenotypes.

A3-410 **ENHANCED HYPERSENSITIVE REACTION OF TOBACCO PLANTS TRANSFORMED WITH A SMALL GTP-BINDING PROTEINS**

Yuko Ohashi, Shigemi Seo*, Taka Murakami, Kouji Ishizuka*, Shohab Youssefian#, Hiroshi Sano#. ***Dept. Mol. Biol., Natl. Inst. Agrobiol. Resources, Tsukuba, Ibaraki 305, *Inst. Appl. Biochem. Univ. Tsukuba., Tsukuba, Ibaraki 305, #Biotech. Inst., Akita Pref. College Agr. , Akita 010-04, Japan***

In tobacco transformants containing a ras-related small GTP binding protein gene from rice (*rgp1*), elevated levels of endogenous cytokinins (zeatin and zeatin riboside) with abnormal phenotypes such as a distinct reduction in apical dominance with increased tillering were found. Analysis of the expression of several genes known to be affected by cytokinins identified a clear increase in the transcript levels of genes encoding acidic pathogenesis-related (PR) proteins in both transgenic plants and their progenies. This increase was directly attributable to elevated levels of the acidic PR protein inducer, salicylic acid (SA) and its conjugated form (SA-glucoside), due to an abnormal and sensitive response of the transgenic plants to wounding. In contrast, transcript levels of the gene for proteinase inhibitor II, which is normally induced by wounding, were generally suppressed in the same wounded plants. The altered levels of SA and PR proteins in the transgenic plants resulted in a distinct increase in their resistance to tobacco mosaic virus infection. In normal plants, the wound and pathogen-induced signal transduction pathways are considered to function independently. However, the wound induction of SA in the transgenic plants suggests integration of *rgp1* somehow interferes with the normal signal pathways, and results in cross-signaling between these two transduction systems.

A3-411 EXPRESSION AND PROCESSING OF BARLEY SERINE CARBOXYPEPTIDASES IN BARLEY AND YEAST, Anne Rocher, Florence Dal Degan,Verena Cameron-Mills and Diter von Wettstein, Department of Physiology, Gl. Carlsbergvej 10, DK-2500 Copenhagen Valby, Denmark.

During maturation and germination of the barley grain, a family of at least six serine carboxypeptidases (Ser-CPs) is expressed (Dal Degan, F., Rocher, A., Cameron-Mills, V., and von Wettstein, D., 1994, Proc. Natl. Acad. Sci. US, in press) . Five of these (CP-MI, II, II.1, II.2 and II.3) appear to be synthesized as precursors with a signal peptide, propeptide and linker peptide. During enzyme maturation, the linker peptide would be excised and the two resulting polypeptide chains become linked by disulfide bonds. CP-MI and II exist in a mature form as dimers, each subunit consisting of two disulfide bonded polypeptide chains. In contrast, CP-MIII is a single chain monomer sharing close homology to CP-Y from yeast.

Northern, Western and RNA PCR analysis have shown CP-MII to be the only Ser-CP to accumulate in the endosperm during grain maturation and to be present in the mature grain in an active form. All six Ser-CPs are expressed *de novo* in the germinating grain, in the scutellum and/or in the aleurone and at least CP-MI, II and III are secreted into the endosperm. This supports the participation of Ser-CPs, in concert with cysteine endoproteases, in the mobilization of storage proteins to supply small peptides and amino acids to the germinating embryo. With the exception of CP-MI, all Ser-CPs are also expressed in the roots and shoots of the growing seedling, which suggest that they play additional roles, possibly in the processing of other enzymes.

To study the processing of the Ser-CPs precursors, a full-length cDNA clone for CP-MI isolated by Drs F. L. Olsen and R. Leah (Carlsberg Research Center) has been subcloned in yeast expression vectors with or without the original signal- and/or propeptide sequence. It is examined whether yeast endo- and exopeptidases can process the pre-, pro- and linker peptides of the barley precursors and if the barley Ser-CP can substitute for CP-Y, trimming the peptide chains to their mature form.

A3-412 MODULATION OF EXPRESSION OF GLUTAMINE SYNTHETASE (GS) GENES IN A GENE MEMBER/ORGAN SPECIFIC MANNER: AN APPROACH TO UNDERSTAND THE REGULATION AND FUNCTION OF DIFFERENT GS GENE MEMBERS IN ALFALFA, Champa Sengupta-Gopalan[1], Saman Bagga[1], Nina Klypina[1], Debbie Samac[2], and Stephen J. Temple[1], [1]Department of Agronomy and Horticulture, New Mexico State University, Las Cruces, NM 88003. [2]Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108

Glutamine synthetase (GS) catalyzes the first and key reaction in ammonia assimilation. It catalyzes the ATP dependent condensation of ammonia and glutamate to yield glutamine. Higher plant GS is an octameric enzyme encoded by a small multigene family whose members exhibit organ/tissue specific patterns of expression. Two full length GS cDNA clones representing the cytosolic form of GS from alfalfa were characterized and were found to represent two distinct and major classes of GS genes. Differences observed in the expression profile at an organ specific level between the two gene classes probably represents differences both in the regulatory mechanism and functional role of the two gene classes. To improve our understanding of the functional role(s) and regulation of the different GS isoforms, we have introduced gene constructs into transgenic alfalfa and Lotus japonicus, specifically aimed at both up or down regulating specific GS gene member or GS genes in an organ/tissue specific manner. Analysis of the different transgenic plants suggest that antisense gene constructs containing gene specific regions are effective in downregulating in a gene class specific manner. Similarly, our data also demonstrates that with the use of tissue specific promoters, GS can be modulated in a tissue specific manner. Our results indicate that modulation of GS gene expression does have physiological repercussions. Molecular and biochemical characterization of the transformants suggest intricate regulation of expression of GS genes in alfalfa controlled both at the transcriptional and post-transcriptional steps.

A3-413 A ROLE FOR C_2H_2 ZINC FINGER PROTEIN GENES IN *ARABIDOPSIS* SHOOT DEVELOPMENT. Brian W. Tague and Howard M. Goodman. Department of Molecular Biology, Massachusetts General Hospital and Department of Genetics, Harvard Medical School, Boston, MA 02114.

A family of zinc finger protein genes from *Arabidopsis thaliana* (AtZFPs) has been characterized. Each of the predicted protein sequences contains a single copy of a highly conserved TFIIIA-like C_2H_2 zinc finger motif. This motif is a well-characterized DNA binding domain, examples of which have been identified in other plant species. Outside of the zinc finger domain the proteins share little identity but homologous domains are apparent, some of which are characteristic of other transcription factors. RNA blot analysis demonstrates that the genes are expressed in different but overlapping sets of organs in *Arabidopsis*. The expression pattern of one of these genes (AtZFP1) has been examined in more detail by histochemical analysis of *Arabidopsis* lines transformed with an AtZFP1 promoter:ß-glucuronidase construct. AtZFP1 is expressed predominantly in tissues involved in the structural support of the plant. These promoter:GUS lines have also been used to analyze AtZFP1 expression in response to light in etiolated seedlings; results indicate that AtZFP1 is expressed downstream from light perception, before the emergence of the first true leaves. Conversely, overexpressing AtZFP1 in tissue culture blocks the hormonal induction of shoots. As a whole, the evidence implicates a zinc finger protein gene, AtZFP1, in the development of *Arabidopsis* shoots.

A3-414 **A Combination of Site-Specific Recombination and Transposition for the Induction of Chromosomal Rearrangements in Transgenic Tomato Plants**

M.J.J. van Haaren, J. Stuurman, J.J.M. Smit, J. Hille and H.J.J. Nijkamp, Department of Genetics, IMBW, BioCentrum Amsterdam, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands

The well established transposon tagging strategy using *Ac/Ds* elements is utilised in combination with site-specific recombination to induce chromosomal rearrangements in the plant genome. Site-specific recombination systems from *E.coli* bacteriophage P1 (Cre/*lox*), *S.cerevisiae* (Flp/FRT) and *S.rouxii* (R/RS) have been integrated in a transposon tagging construct and introduced into the tomato genome, in such a way that the Ds-element harbours one of a set of recombination sites while the second recombination site is present in the T-DNA region. The transposable element is subsequently used as a tool to relocate recombination sites within the plant genome. After trans-activation of the transposable element (*Ds-lox, FRT, RS*) to different locations in the plant genome, recombination between the recombination sites can be induced by introduction of the recombination-protein (Cre, Flp or R). In this way deletions, inversions and chromosomal translocations can be induced.

We will present features of this transposition/recombination system and the analysis of deletion events in the plant genome. Also our work on the development of an *in vitro* recombination procedure for use on genomic DNA will be presented.

A3-415 CHARACTERIZATION OF GENES CORRELATED WITH SYSTEMIC ACQUIRED VIRUS RESISTANCE

Monique M. van Oers, Huub J.M. Linthorst and John F. Bol.

Institute of Molecular Plant Sciences, Leiden University, The Netherlands

Infection of tobacco leaves with tobacco mosaic virus (TMV) induces a systemic resistance against a variety of pathogens, like bacteria, fungi and viruses. This systemic acquired resistance (SAR) is accompanied by the synthesis of so called pathogenesis-related (PR) proteins of which several groups are presently known. Although some of these PR proteins have shown antifungal activity, proteins correlated with resistance against viral infections have not been identified thus far. The aim of our study is to identify plant genes functional in inducing resistance against viruses, either in the signalling pathway or in actual defense mechanisms.

The strategy was adopted to compare the mRNA set of tobacco plants showing SAR with the mRNA content of non-resistant plants. Therefore, mRNA was isolated from the upper leaves of both TMV-inoculated plants showing SAR, and mock-inoculated plants, and cDNA libraries were constructed. To identify and isolate from these libraries those genes that are differently expressed in resistant and non-resistant tobacco leaves, DNA probes are currently obtained. To this aim, subpopulations of the termed mRNA sets are screened for differences in mRNA content by PCR technology. Differently expressed genes identified with this method will be presented.

A3-416 A DROUGHT- AND ABSCISIC ACID (ABA)-INDUCIBLE H1 HISTONE GENE FROM A DROUGHT-TOLERANT TOMATO, *Lycopersicon pennellii*: EFFECTS OF OVER-EXPRESSION IN TRANSGENIC TOBACCO, Tao Wei & Mary O'Connell, Graduate Program in Molecular Biology, New Mexico State University, Las Cruces, NM 88003

In response to abiotic stress gene expression is altered in plants. Recently, H1 histone has been modeled as a molecular switch for chromatin opening, an essential step prior to eukaryotic gene expression. We have isolated and characterized a drought- and ABA-inducible gene, *pen20*, from a drought-tolerant tomato species, *L. pennellii*, which has a significant sequence homology to plant H1 histones. The structure of the gene was determined experimentally; primer extension was used to map the transcript initiation site, and S1 nuclease protection and RT-PCR assays were used to map the 3' and 5' intron splicing sites. The nucleotide sequence predicts a protein of 202 amino acids interrupted by a 130 bp intron. The size of the predicted protein is smaller than other plant H1 histones described to date, however, the sequence in the conserved globular domain is 50-60% identical. The 5' flanking region contains sequence elements similar to H1 histone-specific promoter elements as well as an ABA responsive element. Genomic blots have demonstrated that *pen20* is present as a single copy sequence, therefore, PEN20 is probably a H1 subtype. While *pen20* is drought-inducible in leaf tissue, the gene is constitutively expressed in roots. Transcripts for *pen20* increase in leaf tissue prior to the visible appearance of wilt. To determine if *pen20* is involved in the alteration of gene expression observed in drought-stressed tomato plants, we have introduced the entire coding region of *pen20* behind the 35S CaMV promoter into tobacco. Alterations in the growth, development and drought-responsiveness of the transgenic plants are under investigation. A discussion of the role of H1 histones and alteration in gene expression in response to abiotic signals will be presented.

Late Abstracts

CELL WALL PROTEINS INDUCED BY WATER DEFICIT IN BEAN (*Phaseolus vulgaris* L.) SEEDLINGS. **Alejandra A. Covarrubias, Blanca García, Wualt Ayala, Magda Hernández, and Alejandro Garciarrubio. *Department of Plant Molecular Biology, Instituto de Biotecnología. Universidad Nacional Autónoma de México, Apdo. Postal 510-3. Cuernavaca, Morelos 62271, México.***

The ability to contend with water deficit is an important factor for both the natural distribution of plants and for crop distribution and productivity. Accordingly, an understanding of the mechanisms that confer adaptation to water limited environments possess much theoretical and practical value. In thiswork, we analyze the electrophoretic patterns of basic proteins from beans (*Phaseolus vulgaris* L.) seedlings and mature plants subjected to water deficit. Three major basic proteins accumulate in bean seedlings exposed to low water potentials with apparent molecular masses of 36-kilodalton, 33-kilodalton and 22-kilodalton that we refer to as p36, p33 and p22, respectively. Leaves and roots of mature plants (21-days after germination) grown under low water availability conditions accumulate only p36 and p33 proteins. In mature plants, subjected to a fast rate of water loss both p33 and p36 accumulate approximately at the same levels, whereas mature plants subjected to a gradual loss of water, p33 accumulates at higher levels. Both p36 and p33 are glycosylated and are found in the cell wall fraction. In contrast, p22 is not glycosylated and is found in the soluble fraction. Accumulation of these proteins is also induced by application of abscisic acid (0.1-1.0 mM), as could be expected for proteins induced by water deficit. The p36 and p33 amino acid composition is simmilar to that of a vitronectin-like protein from lily (Wang *et al.*., Plant Physiol. 104:711.1994) A cDNA clone was isolated by immunoscreening a λgt11 cDNA library, prepared from bean plants subjected to water deficit, using an anti-p33 antibody. The isolated gene presents putative GTP- and actin-binding domains and shows homology with a vitronectin-like protein from tobacco (Zhu *et al*., Plant Cell 6:393.1994). The role of this proteins during the plant response to water deficit or osmotic stress will be discussed.

PEROXISOMAL PROTEIN TARGETING SEQUENCE BINDING TO GLYOXYSOMAL MEMBRANES IS SPECIFIED BY A LOW AFFINITY SITE, Robert P. Donaldson and Nathan E. Wolins, Department of Biological Sciences, The George Washington University, Washington D.C. 20052.

Serine-lysine-leucine (SKL) at the C-terminus directs polypeptides to peroxisomes in plants, animals and yeasts. Many, but not all, peroxisomal proteins have this targeting information, including acyl-CoA oxidase. We have synthesized a variety of peptides based on the sequence of the last 12 amino acids of acyl-CoA oxidase, YHKHLPKLQSKL (SKLp), and used them to detect a receptor for proteins targeted to glyoxysomes from germinating castor bean endosperm. Both unlabeled SKLp and glyoxysomal matrix proteins compete with the binding of ^{125}I-SKLp to alkali-stripped glyoxysomal membranes. Two matrix proteins, 20 kD and 56 kD, eluted from a sodium dodecyl sulfate-polyacrylamide gel, compete with the SKLp binding. Scatchard analysis of SKLp binding to glyoxysomal membranes shows two binding sites which have K_d values of 150 nM and 8300 nM . Specific binding is about 10-fold higher in the alkali-stripped glyoxysomal membrane fraction than in similarly treated mitochondrial or microsomal fractions. Protease treatment of the alkali-stripped glyoxysomal membranes greatly diminishes binding of the SKLp. Alterations in the SKLp, replacement of the C-terminal L with a G or the deletion of the KL, in otherwise identical synthetic peptides, abolishes the low affinity binding but high affinity binding is retained. Another synthetic peptide, YHKETEPLQSKL, in which two negatively charged residues are used in place of positively charged residues in SKLp, lacks both high and low affinity binding. Two other synthetic peptides, YKARM based on the C-terminus of isocitrate lyase and YPDVNQRIARISAHLH based on the N-terminal glyoxysomal targeting sequence of malate dehydrogenase, do not compete with SKLp binding and thus these protein may be imported through a different receptor. We hypothesize that the high affinity SKLp binding site recruits proteins from the cytosol to the membrane surface and that the low affinity site specifies import for proteins having the C-terminal SKL. Support from NSF grant MCB 9219413

THE TOMATO *Cf-9* GENE FOR RESISTANCE TO *CLADOSPORIUM FULVUM*: AN INTERESTING PROBLEM IN CELL SIGNALLING, David A. Jones, Colwyn M. Thomas, Kim E. Hammond-Kosack, Saijun Tang and Jonathan D. G. Jones, Sainsbury Laboratory, John Innes Centre, Colney Lane, Norwich, NR4 7UH, U.K.

The tomato *Cf-9* gene confers resistance to infection by races of the fungus *Cladosporium fulvum* that carry the avirulence gene *Avr9*. The *Cf-9* gene was isolated by transposon tagging using the maize transposable element *Dissociation*. The DNA sequence of *Cf-9* encodes a putative membrane-anchored extra-cytoplasmic glycoprotein. The predicted protein shows homology to the receptor domain of several receptor-like protein kinases in *Arabidopsis*, to anti-fungal polygalacturonase-inhibiting proteins in several plants, and to other members of the leucine-rich repeat family of proteins involved in protein-protein interaction. This structure is consistent with a receptor that could bind Avr9 peptide. However, Cf-9 lacks any obvious cytoplasmic signalling capacity. The activation of plant defence following the interaction between Cf-9 and Avr9 therefore presents an enigmatic problem in plant cell signalling. This problem is compounded by the possible localisation of Cf-9 to the endoplasmic reticulum.

THE PLANT 90 kDa HEAT SHOCK PROTEIN EXISTS IN A HIGH MOLECULAR MASS COMPLEX AND CO-IMMUNOPRECIPITATES WITH AN HSP60-RELATED PROTEIN, Priti Krishna[1], J. Roger H. Frappier[2], Roderick F. Felsheim[3] and Melanie Sacco[1], Departments of [1]Plant Sciences and [2] Zoology, University of Western Ontario, London, Ontario, Canada N6A 5B7, [3]Department of Biochemistry, University of Minnesota, St. Paul, MN U.S.A. 55108

The 90 kDa heat shock protein (hsp90) is known to regulate the activities of steroid hormone receptors and protein kinases in animal cells. Information on the functions of hsp90 in plant cells is, however, very limited. A polyclonal antibody R_2 was raised against a fusion protein consisting of a portion of plant hsp90 fused to the trpE protein of *Escherichia coli*. This antibody was found to be specific towards plant hsp90, showing little or no cross-reactivity with mouse and human hsp90 proteins. The R_2 antibody identified an 83 kDa protein as the hsp90 homolog in cytosolic extracts of several dicot and monocot plants. Two-dimensional gel electrophoresis indicated that at least two different isoforms of hsp90 are expressed in *Brassica napus* seedlings. An examination of the native state of hsp90 by non-denaturing gel electrophoresis showed that this protein exists as a monomer and as a high molecular mass complex of approximately 680 kDa in cell extracts of spinach cotyledons, *B. napus* seedlings and wheat germ. Immunoprecipitation of ^{35}S-labelled *B. napus* cytosolic extracts with the R_2 antibody revealed that a 60 kDa protein co-immunoprecipitates with hsp90. The 60 kDa protein was identified as an hsp60-related protein on the basis of its cross-reactivity with an anti-hsp60 antibody. The results of the present study lay the ground work on which to investigate some of the functions of plant hsp90 in the future.

CHLOROPLASTIC INOSITOL SYNTHASE: REGULATION BY ENVIRONMENTAL SIGNALS. Arun Lahiri Majumder, present address (as DBT Overseas Associate) Dept. of Biochemistry & Plant Sciences, University of Arizona, Tucson, Arizona 85721, U.S.A. Fax # (602) 621 1697 / 9288 and Aniruddha Raychaudhuri, Biochemistry Laboratory, Dept. of Botany, Bose Institute, 93/1, A.P.C. Road, Calcutta - 700 009, INDIA.

Inositol, an ubiquitous cyclitol present throughout the eukaryotic system, is formed by cyclization of Glucose-6-P by inositol-1-phosphate synthase (IS) coupled with inositol -1- P- Phosphatase (I-1-Pase). In plants, IS is compartmented in cytosol and chloroplasts. The chloroplastic forms from Euglena, Vigna and Oryza appear to be a tetramer of ~65 kd protein in contrast to the trimeric association of ~65 kd protein of the cytosolic form, both having almost the same kinetic and other biochemical properties. The chloroplastic IS, in contrast to the cytosolic one is photoregulated. Moreover, in salt tolerant rice cultivars, activity of chloroplastic IS is enhanced under NaCl stress in photodifferentiated chloroplasts suggesting a convergent induction by these two environmental stimuli. This increase in IS function could presumably lead to overproduction of inositol, which could act as an osmolyte in chloroplasts or could be shuttled to form pinitol by enzymatic methylation with O-methyl-transferase (Imt). In fact, during salt stress, IS, I - 1 - Pase (homologous to osmotically induced HAL- 2) and Imt could act as a cascade leading to generation of polyols for protection of chloroplast structure and functions.