FRONTIERS OF PLANT MORPHOGENESIS

Organizers: Richard J. Cyr and Barry A. Palevitz

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Plenary Sessions *Page*

Poster Sessions

Current Perspectives in Plant Morphogenesis

J5-001 CURRENT PERSPECTIVES IN PLANT PHOTOMORPHOGENESIS, Winslow R. Briggs, Department of Plant Biology, Carnegie Institution of Washington, Stanford, CA 94305.

In the past five years the most significant developments in plant photomorphogenesis have revolved around studies using mutants. Although photomorphogenic mutants have been available for many years, only recently have many been characterized at the molecular level. Among available mutants involving the phytochrome photoreceptor family are mutants in the chromophore synthesis pathway, and mutants in the structural genes for both PhyA and PhyB. These mutants plus transgenic plants over-expressing one of these two phytochromes have been extremely useful in sorting out the roles of these two phytochromes. Mutants in the signal transduction pathway have also been described and these show promise as tools for understanding some of the important early events following phytochrome phototransformation. Phytochrome mutants have also provided the first conclusive evidence that the Pr form of phytochrome might possess biological activity. Mutants in processes regulated by blue light have resolved different types of responses as having genetically independent pathways and independent photoreceptors, have implicated more than one photoreceptor chromophore in the blue and UV-A region of the visible spectrum, have provided evidence that a plasma membrane protein is an initial component in one response, and have led to the identification of the gene for a putative blue light photoreceptor in another. The current status of these studies will be discussed.

J5-002 CYTOSKELETAL DYNAMICS IN LIVING PLANT CELLS, Peter K. Hepler, Department of Biology, University of Massachusetts, Amherst, MA 01003-5810.

Microtubules and microfilaments have been imaged in living plant cells and their dynamic changes recorded during division, growth and development. Carboxyfluorescein labeled brain tubulin has been injected into living cells that are maintained in an active state in a culture chamber on the microscope stage. Subsequent imaging with the confocal microscope reveals microtubules in the preprophase band, the mitotic apparatus, the phragmoplast and the cortical array. The dynamic features of these microtubules are demonstrated by their depolymerization in elevated calcium, low temperature, and in the drug oryzalin, and by repolymerization when returned to normal conditions. Further information on their turnover has been determined using the procedure of fluorescence recovery after photobleaching. With half times of recovery of 60-70 seconds for the cortical array, the preprophase band, and the phragmoplast, and 31 sec for the mitotic apparatus, these studies demonstrate that plant microtubules turn over extremely rapidly. Finally, current studies, in which maturation promoting factor is coinjected with fluorescent tubulin, provide additional evidence for differential microtubule lability.

Microfilaments have been labeled with fluorescent phalloidin that has been injected into stamen hair cells at very low concentrations such that it does not prevent the dynamic changes of these structures. Imaging with the confocal microscope reveals hitherto undisclosed aspects of the preparation of the division site and dynamics of the phragmoplast cytoskeleton. During prophase microfilaments occur throughout the cell cortex with those in the region of the preprophase band becoming transversely aligned. At nuclear envelope breakdown, these specifically disassemble, leaving a circumferential zone from which microfilaments remain absent throughout division. During cytokinesis microfilaments arise within the phragmoplast, oriented parallel to the microtubules, but excluded from the zone where the microtubules overlap and where cell plate vesicles aggregate. The phragmoplast microfilaments, in a manner similar to the microtubules, shorten in length, expand in girth, and eventually disassemble when the cell plate is complete. These observations thus indicate that microfilaments are a major component of the phragmoplast, and that, together with microtubules, they might control the process of cell plate formation.

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Cytoskeleton and Morphogenesis

J5-003 *ACETABULARIA*: A PRIMITIVE; UNICELLULAR ORGANISM FOR THE STUDY OF PLANT CELL MORPHOGENESIS, **Diedrik Menzel,** Max-Planck-Institut für Zellbiologie, Rosenhof, D-68526 Ladenburg, Germany.

The architecture of the giant unicellular green alga *Acetabularia* is remarkably complex. Morphological features such as rhizoid, central stem, whorls of assimilatory branches and multi-chambered fruiting structures could well be mistaken for organs of more highly advanced multi-cellular plants. Yet, in *Acetabularia*, all these structures are formed through morphogenetic activities of a single cell. The cytoskeleton plays a pivotal role in this process. Each morphogenetic stage is characterized by specific cytoskeletal assemblies involving microtubules and actin filaments and transitions from one stage to the next require major reorganizations in these assemblies. To date, the factors which control cytoskeletal dynamics in *Acetabularia*, and in other plant systems as well, are essentially unknown. In order to characterize the molecular components determining the structure of the cytoskeleton and its dynamic behavior, we have determined the sequences of actins, myosin heavy chains and ser/thr-protein phosphatases (PPases) on the cDNA and genomic levels. For each of these proteins we discovered at least two isoforms and two classes of ser/thr-PPases are present in *Acetabularia*. On the basis of pharmacological evidence, we suggest that protein phosphatase 2A is involved in reorganization of actin filament assemblies and that myosin is its primary target.

J5-004 THE CYTOSKELETON AND ARCHESPORIAL CELLS, GAMETOPHYTES AND EMBRYOS. William F. Sheridan, Bing-Quan Huang, Inna Golubovskaya and Janice K. Clark. Biology Department, University of North Dakota, Grand Forks, North Dakota 58202.

Sexual reproduction in maize and other flowering plants depends on the differentiation of meiocytes, their meiotic division, formation of the haploid gametophytes and restoration of the sporophyte generation at fertilization followed by embryogenesis. We are using Robertson's *Mutator* stocks to simultaneously mutate and transposon tag genes regulating these developmental processes. We are using fluorescent microscopy and antitubulin antibody to examine the behavior of the tubulin cytoskeleton in normal and mutant cells. The *multiple archesporial cells1 (mac1)* gene appears to regulate the shift from the vegetative to the sporogenous pathway in the ovule by controlling the differentiation of hypodermal cells into archesporial cells. In contrast the *plural abnormalities of meiosis1(pam1)* mutation results in the usual single archesporial cell containing multiple nuclei which proceed through meiosis asynchronously.

The *ameiotic1(am1)* mutant allele blocks cells from entering meiosis and *am1* meiocytes are known to form a preprophase band. This allele identifies the meiotic DNA Switchpoint. The meiocytes of the *am1*485* allele advance further into premeiotic interphase and this allele identifies the Enter Meiosis Decision Point, while the meiocytes of the *am1-pral* allele proceed into the early zygotene stage and *am1-pral* identifies the Prophase I Decision Point. The behavior of the tubulin cytoskeleton in the latter two mutants will be reported. We have reported the patterns of tubulin cytoskeleton behavior in normal maize female gametophyte development. We will present results of the study of the mutation *lethal ovule2* which blocks embryo sac formation and also of the mutation *indeterminate gametophyte1* which disturbs embyro sac formation as well as seven new putative female gametophyte lethal mutations. The normal egg cell is polarized in the distribution of its nucleus and cytoplasmic organelles, as is the zygote. We will present our initial results on studying the the tubulin cytoskeleton in normal and selected *embryo-specific* mutant embryos blocked very early in development.

J5-005 THE CYTOSKELETON IN CELL ELONGATION AND DIVISION: A MUTATIONAL APPROACH. Jan Traas, Philippe Nacry, Emmanuel Gendreau, Catherine Bellini, Jocelyne Kronenberger, David Bouchez, Herman Höfte and Michel Caboche, Laboratoire de Biologie Cellulaire, Institut National de la Recherche Agronomique, Route de Saint Cyr, 78026 Versailles cedex, France.

In higher plants the cytoskeleton has been associated with two important morphogenetic processes. First, the microtubular system seems to be involved in the control of cell expansion by orienting the cellulose microfibrils in the cell wall. Secondly, the cytoskeleton appears to be directly responsable for tissue patterning by controling the orientation of division planes. In spite of extensive research very little is known on how exactly the plant cytoskeleton functions in these processes. Therefore we are now using a mutational approach by studying cytoskeletal organization in a collection of developmental mutants in *Arabidopsis* and *Nicotiana plumbaginifolia* obtained after EMS mutagenesis and T-DNA tagging. These mutants have been isolated previously in our laboratory using abnormal post-embryonic development at the seedling level as a general screen. These mutants were subsequently analysed more in detail. We have now a collection of mutants perturbed in (a) timing and dynamics of cell elongation in the hypocotyl and/or the root, (b) polarity of cell expansion (c) division plane alignment. Here we will mainly present the last category.

The division plane mutants isolated so far fall into different classes. First, we have found mutants were only very few cells or cells at specific positions make mistakes. One such an example is the *hyp2* mutant in *N. plumbaginifolia* which has one extra cell layer in the primary root due to one extra longitudinal, periclinal cell division in the cells giving rise to the endodermis. A second group of mutants shows frequent mistakes in their division plane alignment, but are still able to form a growing plant. A member of this class is the *ACL4* mutant in *Arabidopsis* which was isolated from our T-DNA tagged collection of mutants. *ACL4* is of particular interest, since the phenotype is correlated with an abnormal organisation of the cytoskeleton.

A third subgroup of mutants shows frequent mistakes in their division plane alignment, but are not able to form organized tissues, but rather grow as callus.

An extensive analysis of these mutants should provide important information on the factors controling division plane alignment.

Mitosis and Division Planes

J5-006 TOWARDS A MOLECULAR UNDERSTANDING OF PRECISE PATTERNS OF GROWTH AND CELL DIVISION IN YEAST, John Chant, Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, 02138

During vegetative division by budding, yeast cells exhibit two precise patterns of cell surface growth followed by cell division. In the axial pattern of haploid **a** or α cells, buds form at the junction of the previous division site producing an orthogonal-type division pattern. In the bipolar pattern of diploid **a**/α cells, cells form buds at the poles of their ellipsoidal shapes leading to a longitudinal-type division pattern. Several genes are important for producing these two patterns of cell division: *BUD1-BUD5*. Immunolocalization experiments have demonstrated that BUD3 protein marks sites for axial budding and division. Experiments are being performed to understand how the assembly of BUD3 protein is spatially and temporally regulated. For the position of BUD3 protein to produce a bud and cell division in axial positions, its position must be communicated to the cytoskeleton. Two GTPase cycles and associated regulators, (the BUD1 GTPase cycle and the CDC42 GTPase cycle) likely provide this link. Progress in our understanding of these cycles will be presented. Finally additional ongoing work in my lab is being performed to investigate how the poles of ellipsoidal diploid cells are recognized for bipolar budding and how cell shape is generated.

J5-007 THE ROLE OF CHROMOSOMES IN MAIZE MEIOTIC SPINDLE MORPHOGENESIS , R. Kelly Dawe[1] and W. Zacheus Cande[2], [1]Dept. Botany, Univ. Georgia, Athens, GA 30602, and [2]Dept. Mol. and Cell Biol., Univ. California, Berkeley 94270.

In most organisms, mitotic spindles are thought to be organized by centrosomes at the spindle poles which nucleate microtubules and establish the bipolarity of the spindle. However, there are no centrosomes in higher plants and few clues about what structures in plants may fulfill these important roles. Interestingly, like plants, many animals do not have centrosomes during the meiotic cell divisions. Evidence such as the fact that "minispindles" form around isolated meiotic chromosomes suggests that in animals the chromosomes have a direct role in organizing spindle polarity and/or morphology.

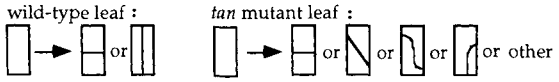
Existing literature indicates that similar mechanisms may be operating during plant meiosis. In triploid and haploid maize plants (where one entire set of ten chromosomes lacks pairing partners) massive spindle abnormalities are observed including "mini-spindles" and multipolar spindles. Using indirect immunofluorescence, we have demonstrated that severe spindle abnormalities also occur in mutant plants with incomplete pairing (partly published in Staiger and Cande, 1993, In "Molecular and Cell Biology of the Plant Cell Cycle", Ormrod and Francis, Eds., p. 157-171, Kluwer Academic Publishers, Netherlands). One explanation for these findings is that unpaired chromosomes disrupt the spindle because they have only a single functional kinetochore. If this is true, then under at least two conditions unpaired chromosomes should *not* affect spindle formation: #1) *when unpaired* chromosomes have two functional kinetochores; and #2) when unpaired chromosomes (fragments) have no kinetochores. Chromosomes meeting these criteria were generated using the meiotic mutation *absence of first division1* and a standard paracentric inversion. Under both conditions (#1 and #2) there were no affects on spindle morphogenesis.

The data support the hypothesis that bipolar meiotic spindle formation requires the presence of bipolar kinetochores – either paired univalents with one functional kinetochore each, or single univalents with two functional kinetochores. Inasmuch as neither plant meiosis nor plant mitosis involves the action of centrosomes, it is likely that the basic mechanisms underlying meiotic spindle formation are also used in mitosis. A significant role for chromosomes in mitotic spindle formation has also been proposed by Palevitz (1993, *Plant Cell* 5, 1001-1009). We emphasize that while chromosomes may be involved in establishing and/or reinforcing spindle bipolarity, chromosomes are unlikely to direct spindle orientation or direct the planes of division. A new mutation called *mms22* will also be introduced which interferes with the orientation of the spindle within the meiocyte.

J5-008 THE ROLE OF THE MAIZE GENE, *TANGLED*, IN THE SPATIAL CONTROL OF CELL DIVISION

Laurie G. Smith[1,3], Sarah Hake[1] and Anne W. Sylvester[2], [1]U.S.D.A./U.C. Berkeley Plant Gene Expression Center, 800 Buchanan St., Albany, CA 94710, [2]Department of Biological Sciences, University of Idaho, Moscow, ID 83843, [3]present address: Department of Biology, University of North Carolina, Chapel Hill, NC 27599.

Recessive *tan* mutations of maize alter the shape and arrangement of cells in mature tissues throughout the plant. Further investigation of this mutant phenotype indicated that these alterations in the cell pattern of mature tissues reflect abnormalities in spatial aspects of cell division during development. In wild-type maize leaf primordia, elongated cells in all tissue layers 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; see diagram below). In all tissue layers of *tan* mutant leaves at all stages of development examined, transverse divisions occur normally, but longitudinal divisions are largely substituted by a variety of abnormal divisions (see diagram below): divisions that join the two long sides of the cell in a crooked or curved path, divisions that join the end of the cell to one of the two long sides, and other divisions that don't fall into these categories. These observations indicate that the wild-type *Tan* gene is selectively required in order for elongated leaf cells to divide longitudinally. The cytoskeletal basis of the *tan* phenotype is currently being analyzed to ask whether abnormal divisions result from a failure to establish longitudinal division planes before mitosis (as indicated by longitudinal preprophase band formation), or a failure of phragmoplasts to interact appropriately with the cell cortex to achieve longitudinal wall formation at cytokinesis.



Cell Cycle (Joint)

J5-009 NOVEL CYCLINS ARE INVOLVED IN EARLY EVENTS IN THE CELLCYCLE, Laszlo Bögre, Irute Meskiene, Marlis Dahl, Dang Cam Ha, Manfred Pirck, Karin Zwerger, Erwin Heberle-Bors and Heribert Hirt, Institute of Microbiology and Genetics, Biocenter Vienna, Dr. Bohrgasse 9, 1030 Vienna, Austria.

In all eukaryotes, transition through the cell cycle is regulated by the activation of protein kinase complexes composed of catalytic cdk (cyclin-dependent kinase) and regulatory cyclin subunits. Protein kinase activity of these complexes is controlled at different checkpoints through the action of kinases and phosphatases and association with other regulatory proteins. In alfalfa, two cyclins, cyc1 and cyc2, were isolated that show homology to animal A and B type cyclins but transiently accumulate only at G2/M. Two novel types of cyclins were identified by either complementation of yeast G1 cyclins or suppression of pheromone-induced cell cycle arrest. Whereas cyc3 shows highest similarity to animal A and B type cyclins, cyc4 has highest homology to animal D type cyclins. Both cyclins show fluctuating transcript patterns in synchronously dividing cells. Whereas cyc3 is peaking at G1/S, cyc4 reaches maximal levels in S phase. Upon stimulation of quiescent cells, cyc3 mRNA levels rise severalfold within minutes. In agreement with other data, reentry into the cell cycle also stimulates cyc3 transcript levels but maximal levels are observed only after entry into S phase. Cdk kinase complexes have been identified that are active at the G1/S, S and G2/M transitions of alfalfa cells. Presently, antibodies against the different cdks and cyclins are used to study the composition and regulation of the complexes during the cell cycle. Dominant negative cyclin constructs have been transferred into alfalfa cells and into transgenic plants. Expression under an inducible promoter should reveal the effect of these genes in their natural context at different developmental stages and environmental conditions.

J5-010 HORMONAL INTERACTIONS WITH PLANT CELL PROLIFERATION CONTROLS, Peter C.L. John, Kerong Zhang, Ludger Diederich, Chongmei Dong, Plant Cell Biology, R.S.B.S., Australian National University, PO 475, Canberra City, ACT 2601, Australia.

Changes in $p34^{cdc2}$ level and activity are consistent with participation in control of the cessation[1] and resumption[2] of division under phytohormonal control, which allows cells to abstain from division and differentiate, or conversely to resume division in formation of lateral primordia, secondary thickening, or wound response. An indication that low $p34^{cdc2}$ relative to other proteins can enforce exit from the cell cycle was first seen in seedling wheat leaf where a fifteen fold decline in $p34^{cdc2}$ correlated with cell enlargement without division prior to differentiation into photosynthetic cells[1]. Auxin-induced division could only be elicited in wheat tissue that contained active $p34^{cdc2}$ histone H1 kinase[3,4]. In dicotyledonous tissues prior $p34^{cdc2}$ accumulation was necessary for resumption of cell division in phytohormone-stimulated cells of leaf[2], root[3] and stem pith[4]. In excised carrot cotyledon tissue fifteen fold elevation of $p34^{cdc2}$ levels, restoring the level to that in meristematic cells, preceded the resumption of proliferation in large photosynthetic cells. These changes are consistent with low $p34^{cdc2}$ being used by plants as an economical restraint of division in cells that are sufficiently large and active to divide but must switch to differentiation to allow organogenesis. In excised tobacco stem pith[5] auxin was capable of inducing $p34^{cdc2}$ -like protein but not division. In pith the additional presence of cytokinin was necessary for induction of catalytic activation of the $p34^{cdc2}$ kinase and cell proliferation. Cytokinin was specifically required for initiation of mitosis in suspension cultured *N. plumbaginifolia* cells, which in medium with 2,4-D but without cytokinin arrested in G2 phase and on supplementation with kinetin rapidly activated $p34^{cdc2}$ H1 histone kinase and entered nuclear division. We are using enzymes that can change $p34^{cdc2}$ phosphorylation *in vitro* and *in vivo* to investigate the mechanism of cytokinin activation. When division is hormonally terminated an additional control that involves removal of $p34^{cdc2}$ can operate. In pea root tissue stimulated by IAA to form lateral primordia[6] the addition of zeatin riboside caused rapid removal[3] of $p34^{cdc2}$ and sensitivity of the process to phenylmethylsulphonylfluoride indicates that the mechanism of $p34^{cdc2}$ decline involves proteolysis. Phytohormones may therefore exert effects on cell division during plant development by influencing $p34^{cdc2}$ accumulation, activation or breakdown.

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Extracellular Matrix and Morphogenesis

J5-011 **CELL ENLARGEMENT AND THE ACTION OF EXPANSINS**; Daniel J. Cosgrove, Simon McQueen-Mason, Daniel Durachko, Tanya Scherban, Jun Shi, Mark Guiltinan, Z.-C. Li, D. Blecker; Department of Biology, Penn State University, University Park, PA 16802

Prior to maturation, plant cells typically expand 10- to 1000-times in volume by concomitant enlargement of the vacuole and extension of the cell wall. Such cell enlargement is normally limited by wall relaxation, which reduces cell water potential and thereby enables cells to take up water. The biochemical mechanisms underlying wall relaxation are still poorly understood. Using in-vitro extension assays in combination with reconstitution methods, we have identified a family of novel wall proteins that mediate the "acid-growth" responses of tissues and walls. These proteins, named "expansins", catalyze the extension of walls when pH is less than 5.5. Cucumber hypocotyls contain at least two forms of expansins, which have slightly different biochemical characteristics. When expansins are added to Arabidopsis hypocotyls, they enhance cell elongation.

Biochemical mode of action: Expansins appear to lack hydrolase activity against the wall matrix polymers; rather, they can act by disrupt hydrogen bonding between the cellulose microfibril and one or more matrix hemicelluloses. Pure crystalline cellulose acts a poor substrate for expansin binding, but expansin binding is greatly enhanced by coating the cellulose with matrix hemicelluloses. Contrary to the conventional models of the wall, xyloglucans are not effective in raising expansin binding, however. This leads us to propose that a hemicellulose other than xyloglucan serves as the tether that holds cellulose microfibrils together.

Cytolocalization: We have raised rabbit polyclonal antibodies against the two cucumber expansins, called Ex29 and Ex30 after their apparent molecular size, and used them to identify the location of expansins at the tissue and cell levels. Cucumber hypocotyl cross sections were probed with anti-Ex29 and anti-Ex30 antibodies and stained with alkaline phosphatase conjugated to a secondary antibody. The label appears in virtually all of the cell walls seen in the cross section, with little or no evidence for tissue-specific localization. With immuno-gold labeling and electron microscopy, the protein is found specifically and uniformly in the walls, with little or no stratification to particular wall regions. Golgi-derived vesicles are also sometimes found to be heavily labeled, indicating that the protein is packaged and processed in the Golgi before secretion to the wall.

Cloning: Peptide sequences of the purified proteins were used to design degenerate oligonucleotide primers for PCR amplification of cDNA from growing cucumber hypocotyls. The specific PCR product was used to isolate expansin clones in a cDNA library from growing cucumber hypocotyls. From searches of the sequence databanks, we have found no sequence similarity with other proteins of known function (e.g. expansins do not share sequence similarity with cellulases, xyloglucan endotransglycosylases, etc.).

J5-012 DEVELOPMENTAL PLASTICITY OF THE GOLGI APPARATUS, L. Andrew Staehelin, Thomas H. Giddings, A. Lacey Samuels, Zev M. Winicur, and Guo Feng Zhang, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO 80309-0347.

The Golgi apparatus of plants serves as the site of assembly of the oligosaccharide side chains of glycoproteins and of the complex polysaccharides of the cell wall matrix. During cell growth and development many cell walls not only increase in size but also differentiate structurally and compositionally. These changes are the end products of alterations in the synthetic activities of enzymes associated with the endoplasmic reticulum and the Golgi apparatus. We are interested in studying by means of structural, biochemical, and molecular techniques how plant cells re-tailor the Golgi apparatus during growth and development and how this re-tailoring is regulated.

A typical example of Golgi re-tailoring can be found during the developmental differentiation of root cap meristematic cells first into columella and then into young and old peripheral cells. At the structural level, these changes manifest themselves as changes in the morphology, the staining patterns, and the ratios of *cis*, medial, and *trans* Golgi cisternae, as well as in the presence/absence of a trans Golgi network and the appearance of intercisternal filaments. Immunocytochemical studies have demonstrated concomitant changes in the distribution of unesterified pectins between different cisternae and the appearance of tissue specific arabinogalactans. To study the biochemical basis of these changes, we have developed a system that enables us to experimentally induce the synchronized differentiation of a uniform population of tobacco BY-2 suspension culture cells. In particular, we have found that auxin-depletion of tobacco BY-2 cultures can induce a differentiation of the Golgi stacks that appears to mimic the changes seen during the development of mucilage-secreting epidermal and peripheral root tip cells. The nature and kinetics of this inducible Golgi re-tailoring system will be discussed.

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J5-013 RADIAL SWELLING MUTANTS DEFICIENT IN CELLULOSE BIOSYNTHESIS, Tony Arioli[1], Andreas Betzner[1,2,4], Liangcai Peng[1,2,3,5], Tobias Baskin[2,6], Werner Herth[7], Ann Cork[1,2], Rosemary Birch[1], Barry Rolfe[3,5], John Redmond[3,5], Richard Williamson[1,2,3], [1]Cooperative Research Centre for Plant Science, [2]Plant Cell Biology Group, [3]Cell Surface Glycan Unit, [4]Biocem Pacific, [5]Plant-Microbe Interaction Group, Research School of Biological Sciences, Australian National University, Canberra, ACT 2601, Australia , [6]Biological Sciences, University of Missouri, Columbia and [7]Zellenlehre, Heidelberg, Germany

Cellulose biosynthesis is a major biosynthetic pathway in plants that, through its influence on wall mechanical properties, controls many of the most distinctive features of plant morphogenesis. We are using mutants of *Arabidopsis* that show a temperature-sensitive deficiency in cellulose biosynthesis to analyse the mechanism. Using ems-mutagenised seed, we selected *rsw* mutants whose roots underwent a temperature-sensitive radial swelling when transferred from 21 to 31°C. Seedlings of these mutants were incubated with ^{14}C-glucose at the permissive and restrictive temperature and incorporation of ^{14}C into pectins, hemicelluloses and into acetic-nitric acid soluble and insoluble (cellulose) fractions was measured. Mutations have been identified at 3 loci that reduce incorporation into cellulose only and a mutation at a 4th locus inhibits biosynthesis of cellulose and other polysaccharides. Many other mutants, most of which have not yet been assigned to loci, cause swelling without major biosynthetic defects and are being studied for cytoskeletal changes. Freeze etching of seedlings transferred to the restrictive temperature shows that particle rosettes (putative cellulose synthase complexes) rapidly disappear from the plasma membrane of one cellulose-deficient mutant but not from the wild type. A positional cloning strategy is being used to clone the genes from the cellulose-specific mutants. One locus has been mapped between two flanking markers that are an estimated 400 kB apart. YACs hybridising to those markers are known and polymorphisms are being identified among YAC end probes. With these and two molecular markers that others have recently placed between our flanking markers we expect that we will soon fine map the region to the point where we can attempt to clone the gene by complementing the mutant.

The Genetic Analysis of Morphogenesis

J5-014 MUTATIONAL ANALYSIS OF STOMATAL DEVELOPMENT IN *ARABIDOPSIS*, Fred Sack, Ming Yang, and Matt Geisler, Dept Plant Biology, Ohio State University, Columbus Ohio 43210

Stomata consist of two guard cells surrounding a pore that regulate gas exchange through the epidermis of aerial plant organs. Little is known about the genes that regulate the processes of stomatal patterning, determination and differentiation. Seedlings from EMS-mutagenized *Arabidopsis* seeds were screened by light microscopy to identify morphological mutants. Two mutants were isolated, *too many mouths* (*tmm*) and *four lips* (*flp*), that produce extra adjacent stomata. Both are nuclear recessive. The phenotypes are best described if each cluster of stomata or single isolated stoma is termed a "unit"; thus a wild-type (WT) unit consists of two guard cells (one stoma). In a *tmm* cotyledon, about 60% of units contain clusters of 2-28 adjoining stomata with all clusters containing an even number of guard cells. The clusters appear to have the spacing pattern characteristic of the WT with each cluster positionally equivalent to a single stoma in the WT. Stomatal clusters are present on all leaves but their frequency decreases in later-produced leaves. Clusters are also present, to varying degrees, on petioles, anthers, sepals, styles, and the apical ends of pedicels. In the cotyledon *tmm* produces extra stomatal precursor cells (meristemoids) whereas in the inflorescence stem and at the base of the pedicel it completely suppresses the formation of meristemoids and thus of stomata. In the *flp* cotyledon, about 20% of units have paired stomata, but occasionally single isolated guard cells or three adjacent guard cells can be found. This phenotype appears to be present in all leaves as well as in the cotyledons of *flp*. The double mutant has features of both mutants e.g. clusters with both even and odd numbers of guard cells in the cotyledon and no stomata in the inflorescence stem. All mutant plants look more or less normal, and seed yields are high despite the absence of stomata on stems (*tmm* and double mutant). Neither mutation seems to affect stomatal patterning nor differentiation. But both affect stomatal determination and cause proliferation of stomata from meristemoids. In addition, *tmm* regulates entry into the pathway i.e. *tmm* increases or eliminates meristemoid formation depending upon the domain. Thus *tmm* acts at several developmental levels to influence guard cell fate.

J5-015 CELL FATE AND CELL MORPHOGENESIS IN THE ROOT EPIDERMIS OF *ARABIDOPSIS*, Moira E. Galway, James D. Masucci, Daphne R. Foreman, Mark D. Kinkema, Haiyang Wang, and John W. Schiefelbein, Department of Biology, University of Michigan, Ann Arbor, MI 48109.

Plant cell differentiation encompasses several fundamental processes, including cell fate specification, cell polarity, and cell expansion. We are studying the differentiation of root epidermal cells in *Arabidopsis thaliana* to understand the molecular genetic mechanisms that regulate these processes. *Arabidopsis* mutants have been isolated that display defects in one or more of the following stages: epidermal cell fate specification, cell expansion, root-hair initiation, and root-hair tip growth. The cell fate mutants alter the normal positional control of root epidermis differentiation. In wild-type roots, cells located over radial walls between cortical cells differentiate into root-hair cells, whereas cells located directly over the cortical cells differentiate into mature hairless cells. Plants bearing mutations in the *TTG* (*transparent testa glabrous*) locus possess root-hair cells in all epidermal cell positions, and plants expressing the maize *R* cDNA generate hairless cells in all positions. The results indicate that alterations in *TTG* activity cause developing epidermal cells to misinterpret their position and differentiate into inappropriate cell types. Furthermore, the *TTG* product appears to affect the differentiation of hair-bearing cells in the root and shoot epidermis in opposite ways. Other root epidermis mutants under investigation alter root-hair cell morphogenesis. One of these, designated *rhd6*, affects cell polarity during root-hair initiation. The plant hormones auxin and ethylene were found to be associated with the *RHD6*-dependent process, since these hormones rescue the *rhd6* mutant phenotype and they influence epidermal cell polarity in wild-type roots. Several mutants have been isolated that affect root-hair elongation, including one mutant that also alters pollen tube growth. The analyses of these mutants has led to the development of a genetic pathway for the differentiation of root epidermal cells in *Arabidopsis*.

Cytoplasmic/Cell Wall Communications in Morphogenesis

J5-016 **OLIGOSACCHARIDE SIGNALS IN PLANTS: STRUCTURES AND SIGNAL PERCEPTION**, Michael G. Hahn, François Côté, Rob Alba, and Jian Yang, The University of Georgia, Complex Carbohydrate Research Center and Department of Botany, 220 Riverbend Road, Athens, Georgia, 30602-4712, USA.

Plants utilize a variety of signals and signaling mechanisms to regulate the expression of genes that are essential to their growth, development, and survival in the environment. Some of these signals are oligosaccharides; such oligosaccharides are called *oligosaccharins*. Oligosaccharins whose structures have been fully characterized include oligosaccharides that are derived from plant (homogalacturonan and xyloglucan) and fungal (glucan, chitin, and chitosan) wall polysaccharides, glycopeptides derived from fungal glycoproteins, and lipo-oligosaccharides (nod factors) synthesized by bacterial symbionts of plants. Knowledge of the structures and activities of oligosaccharins has made possible detailed investigations of the cellular signaling pathways triggered by these signal molecules. An overview of the structures and activities of the seven well characterized oligosaccharins will be given. In addition, results from recent studies directed toward the identification in plants of specific binding proteins (putative receptors) for several of these oligosaccharins will be summarized. The latter will focus on our studies of the signaling pathway leading to the biosynthesis and accumulation of anti-microbial phytoalexins in soybean. This pathway can be induced by a branched hepta-β-glucoside originally isolated from hydrolyzates of the mycelial walls of the phytopathogenic oomycete *Phytophthora sojae*. Using a radio-iodinated tyraminyl derivative of the hepta-β-glucoside elicitor, the presence of proteinaceous binding sites for this elicitor has been demonstrated in membranes prepared from all major parts of soybean seedlings. These elicitor binding proteins (EBPs) co-migrate with a plasma membrane marker (vanadate-sensitive H^+-ATPase) in linear sucrose density gradients. Binding of the radiolabeled hepta-β-glucoside elicitor is specific, reversible, saturable, and of high affinity (apparent K_d = 0.75 nM). Competitive displacement of the radio-labeled hepta-β-glucoside elicitor with a number of chemically synthesized and structurally related elicitor-active and -inactive oligoglucosides demonstrated a direct correlation between the ability of an oligoglucoside to displace the labeled elicitor and its elicitor activity. Thus, the EBPs recognize the same structural elements of the hepta-β-glucoside elicitor that are essential for its phytoalexin-inducing activity, suggesting that the EBPs are physiological receptors for the elicitor. The EBPs have been solubilized using the non-ionic detergent, *n*-dodecylsucrose, and the solubilized EBPs purified on an affinity column containing immobilized hepta-β-glucoside elicitor. The *n*-dodecylsucrose-solubilized, affinity-purified EBPs retain the binding affinity (apparent K_d = 1.3 nM) for the radiolabeled elicitor and show the same specificity for elicitor-active oligoglucosides determined previously for the membrane-localized EBPs. Affinity purified EBP preparations contain multiple polypeptides and experimental evidence suggests that a high molecular weight protein complex is required for elicitor-binding activity. Current research is directed toward the identification of the polypeptides essential for elicitor-binding activity and purification of sufficient quantities of those polypeptides for detailed characterization. [*Supported by NSF grant MCB-9206882*]

J5-017 ROLE OF THE CYTOSKELETON AND CELL WALL IN THE ESTABLISHMENT OF POLARITY, Ralph Quatrano, Sidney Shaw, Francois-Yves Bouget, Susanne Gerttula, Crispin Taylor, John Fowler, Leigh Brian, and Janice Davis, Department of Biology, University of North Carolina, Chapel Hill, NC 27599-3280.

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. The 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 in the cytoplasm to the extracellular matrix[3]. The ASC has been proposed to immobilize plasma membrane asymmetries established during axis formation and to provide the structural basis for directed tip growth of the rhizoid. Results of specific assays to establish the temporal and spatial basis for the initial asymmetries (e.g. plasma membrane and mRNA probes, and localized vesicle markers) will be used to generate a model for establishment of polarity. 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 this model will also be discussed. These results with the cytoplasmic components of the ASC will be presented along with those that characterize the localized macromolecules in cell walls that may play a role in adhesion of the embryo [4] and rhizoid differentiation [5,6]. 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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Cell-Cell Communication (Joint)

J5-018 HOST CELL RESPONSES TO INVASION BY *RHIZOBIUM* DURING PEA NODULE DEVELOPMENT

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Rhizobium is a soil microbe that metamorphoses into a nitrogen-fixing endosymbiont. This transformation is achieved as a result of a network of interactive signals and responses between bacteria and host plant cells. There are three phases of interaction: pre-infection signalling; the establishment of *Rhizobium* as an intercellular endophyte; and an intracytoplasmic phase where the capacity for biological nitrogen fixation is progressively developed. At each stage, both positive and negative signalling elements are recognizable. The nature and specificity of cell-signalling is related to topological position. At the pre-infection stage, nodule induction is the consequence of the secretion of specific flavonoid molecules which can act as inducers (or anti-inducers) of the lipochito-oligosaccharide (LCO) biosynthetic pathway encoded by *Rhizobium nod* genes. In the appropriate host legume, LCO's specifically stimulate root cortical cell division and organogenesis, apparently by modifying cell-cycle control and the relative concentrations of plant growth regulators. Further development of nodule primordia is regulated by ethylene and other systemically acting feedback control systems. Tissue invasion by *Rhizobium* depends on reorientation of plant cell wall growth, forming an infection thread under the control of the cytoskeleton. Bacteria are embedded in a plant intercellular matrix that may serve as a potential antimicrobial system, being capable of conversion from a fluid to a solid phase following oxidative crosslinking of glycoproteins. This cross-linking could occur if non-host rhizobia elicit host defence responses that probably induce an oxidative burst - the sudden and local release of hydrogen peroxide. In order to avoid eliciting this host defence response, the bacteria apparently need to have appropriate cell wall components, e.g., acidic extracellular polysaccharide which complexes Ca^{++} ions, and lipopolysaccharide which may have both a surface-masking effect based on the O-antigen component and a host-elicitor function based on the core polysaccharide component. Following endocytosis into the host cytoplasm, the bacteroids take on organelle-like status, being individually enclosed by a plant-derived peribacteroid membrane. Differentiation of bacteroids is then controlled by host plant cell physiology, e.g. the supply of oxygen in a microaerobic niche, and by regulation of metabolic exchanges across the peribacteroid membrane. During differentiation of the "symbiosome" compartment, the composition of the peribacteroid membrane gradually changes from that of plasma membrane to a hybrid of this with tonoplast membrane. Genetic, immunological and biochemical analysis of peribacteroid membrane differentiation and vesicle targeting is currently being investigated. Components of the peribacteroid fluid, for example a nodule-specific lectin and a nodule-specific thiolprotease, may be involved in the control of bacteroid senescence.

J5-019 PLASMODESMATA, MACROMOLECULAR TRAFFICKING AND THE SUPRACELLULAR NATURE OF PLANTS, William J. Lucas, Section of Plant Biology, Div. of Biol. Sci., University of California, CA 95616.

Plasmodesmata form an important symplasmic pathway for cell-to-cell communication in higher plants (1, 2). The formation and complex structure of primary and secondary plasmodesmata (3, 4) will be discussed in terms of the creation of specialized cytoplasmic microchannels that permit the coordination of physiological and developmental processes at the tissue (2), organ and whole-plant level (4). These microchannels permit the cell-to-cell exchange of small molecules, such as metabolic intermediates and hormones, as well as various ions; this process is driven by diffusion (1). In addition, we now know that plasmodesmata have the capacity to engage in macromolecular trafficking of proteins as well as nucleic acids (see 5); this transport function appears to be highly effective, in terms of the macromolecules that can move from cell-to-cell, and the overall process appears to involve an active, energy-dependent, step. This new aspect of plasmodesmal biology will be discussed in terms of our molecular and cellular studies on viral and endogenous plasmodesmal movement (transport) proteins (6, 7, 8). These studies provide the experimental foundation for the hypothesis that, in higher plants, plasmodesmal trafficking of macromolecules plays a central role in coordinating plant growth and development (4). In our model, nuclear pore transport and plasmodesmal transport function in concert, to establish a unique system that allows plants to regulate their developmental and physiological functions at a supracellular rather than a multicellular level (2, 5).

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Signal Transduction and Morphogenesis

J5-020 BLUE LIGHT REGULATION OF GENE EXPRESSION

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The rate of transcription for several nuclear-coded genes can be altered by a single, short pulse of low-intensity blue light. The process does not require protein synthesis and the response is immediate. We are examining the signal transduction mechanism responsible for this inductive response using the Cab (Lhcb) gene family in both peas and Arabidopsis. Specific members of the Cab gene family which are blue-light regulated have been identified in both systems and are being used for stable transgenic (Arabidopsis) and transient expression (pea) studies. From such studies we hope to identify DNA sequences necessary for blue-light effects on transcription, the cells responsible for mediating the response and possible mutants in the signal transduction system. Separately, we have identified a blue-light-activated G-protein associated with the plasma membrane of etiolated pea buds. The location and activation characteristics of this protein are coincident with the effects of blue light on Cab gene transcription. The treatment of etiolated pea, wild type or transgenic Arabidopsis seedlings with known G-protein activators (eg. mastoporan) induces expression of the Cab gene family in a dose dependent manner immediately upon treatment. We are attempting to further characterize this G-protein.

J5-021 PHYTOCHROME REGULATION OF TIP GROWTH IN THE APICAL CELL OF THE FERN PROTONEMA

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Tip growth is the basic growth patterns of many plant cells, including pollen tubes, root hairs, fern and moss protonemata and their rhizoids, most filamentous algae, and fungal hyphae. However, the cellular mechanisms of the tip growth is largely unknown.

The fern gametophyte is one of the best experimental systems for studying the physiological basis of photomorphogenesis not only because it exibits many light-regulated responses, but also because it grows as a simple filamentous protonema under red light. Since the cells of the fern protonema are not surrounded by other cells, light can directly reach the photoreceptive sites in these cells, and the resulting photomorphogenetic responses can easily be observed under a microscope.

We have studied the effect of light on various phenomena in gametophyte development, such as cell growth, phototropism, cell swelling, cell division, etc., and we have characterized the photoreceptive site and the changes of cytoskeletal arrangement responsible for these responses. In this symposium,we shall focus on the mechanisms of tip growth as controlled by phytochrome in the protonema of the fern *Adiantum capillus-veneris*.

Under red light, the apical cell of the ptoronema grows at its apical tip, and the nucleus migrates towards the tip keeping a constant distance behind it. Microtubule (Mt) and microfilament (Mf) strands connect the nucleus to the ring-like structure composed of Mt and Mf located in the lateral cortex of the apical cell. Double staining of MT and MF revealed the co-localization of each cytoskeletal element. Cytoskeleton depolymerizing agents were used to establish the different roles that the Mt and Mf play in nuclear positioning during tip-growth. When a red-light grown protonema is transferred to the dark, apical cell ceases tip growth and undergoes one cell division. After the transfer back into red light, the nucleus in the apical cell starts to migrate within 1 hr, the ring-like structure of Mt and Mf is reconstructed in 3-6 hr, and finally the apical cell resumes tip growth at about 9 hr.

If the red-light-grown protonema is irradiated with white light for 7 hr, the nuclei in the apical cell is easily centrifuged toward the base of this cell by 2000 x *g* for 20 min, after which cell division occurs at that site in the dark. When the protonema is irradiated with red light following the centrifugation treatment, a new apical tip develops at a side wall of the protonema. The ability of red light to mediate the resumption of apical growth and the development of a new apical tip provides a good experimental model for studying the cellular mechanisms of tip growth. The cellular event and the role of the cytokekleton in these phenomena will be described in this presentation.

Hot Topics

J5-022 CYTOSOLIC ION ACTIVITIES AND CYTOSKELETON IN PLANT CELL POLARITY, Darryl L. Kropf, University of Utah, Salt Lake City, UT 84112.

We are investigating establishment and expression of cellular polarity in zygotes of the brown alga *Pelvetia*. The fertilized egg undergoes a dramatic reorganization that establishes a developmental axis which determines the orientation of growth and division. Our research focuses on ionic, cytoskeletal and molecular controls of early development, with the aim of understanding how these regulators act coordinately in regulating cellular reorganization. Cortical F-actin is fundamental to polarity establishment and tip growth, and is a component of wall-membrane adhesion sites localized at the rhizoid pole. Microtubules extending from the nuclear envelope into the F-actin rich cortex are important for nuclear rotation and cell division. Cytosolic H^+ and Ca^{2+} gradients, highest activity at the rhizoid pole, have been measured in zygotes and we are actively investigating the relationship between these ionic gradients and cytoskeleton. The presentation will focus on recent findings concerning cytosolic pH and its effects on cytoskeletal-based developmental events.

Recent review: Kropf, D. L. (1994). Cytoskeletal control of cell polarity in a plant zygote. Dev. Biol. in press.

J5-023 CONSERVED REGULATORS OF ACTIN CYTOSKELETAL STRUCTURES--THE RHO SMALL GTP-BINDING PROTEINS IN PLANTS, Zhenbiao Yang, Hai Li, Junko Katsuta, Yalai Wang, Yakang Lin, Doreen Ware, and Keith R. Davis, Department of Plant Biology and Plant Biotechnology Center, The Ohio State University, Columbus, OH 43210.

Plant cells contain a number of unique actin arrays that may play an important role in mediating cytoplasmic streaming, cellular morphogenesis and orientation of cell division. Little is known about the mechanism determining the organization of these actin arrays. In fungal and animal systems, a conserved mechanism involving the rho small GTP-binding proteins, members of the ras superfamily, plays a central role in the regulation of actin cytoskeletal organization. As a result, the rho proteins are key regulators of cell processes such as the establishment of cell polarity, cellular morphogenesis and cell movement that require proper organization of actin cytoskeleton. The functions of these proteins in the control of actin cytoskeletal structures are conserved in diverse organisms. As a first step in understanding the molecular mechanism mediating the organization of the plant actin arrays, we initiated identifying the rho-mediated mechanism from plants. Using a RT-PCR-based strategy, we have recently cloned and characterized the first plant rho gene from the garden pea, *Rho1Ps* (Proc. Natl. Acad. Sci. 90:8732-8736). *E. coli*-expressed Rho1Ps protein has specific GTP-binding activity and undergoes isoprenylation--a posttranslational modification required for membrane anchoring and biological activity of the rho proteins. Rho1Ps transcript is present in all organs of pea seedlings but more abundant in root tips and apical buds. In plants, rho is encoded by a multiple gene family with at least six isogenes in pea and three in Arabidopsis. cDNA clones for two Arabidopsis rho isogenes, Rho1At and Rho2At, have been isolated. Sequence analysis indicates that Rho1At is 95% identical to Rho1Ps and 88% identical to Rho2At at amino acid level. Sequence comparisons suggest that plants have evolved a unique subfamily of rho proteins; this may reflect their distinct role in controlling the organization of the unique actin arrays in plants. The existence of the rho multiple gene family raises an interesting possibility that each rho may have a disctinct role in mediating specific actin array(s). Our current effort focuses on functional analysis of the rho proteins in the regulation of the plant actin cytoskeletal structures and of cellular and developmental processes that are dependent on these cytoskeletal structures.

Current Perspectives in Plant Morphogenesis;
Cytoskeleton and Morphogenesis

J5-100 THE ORIENTATION AND POLARITY OF THE REGENERATION AXIS OF PROTOPLASTS OF THE MOSS, *CERATADON PURPUREUS,* ARE DETERMINED INDEPENDENTLY.

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Protoplasts, isolated from protonemal tissue of *C. purpureus*, regenerate by polar outgrowth to form filaments. The regeneration axis is oriented in a directional light field. Detailed kinetic studies of protoplast regeneration in different light conditions lead to the following conclusions:

(i) Protoplasts must await some cellular event before regeneration can be initiated.
(ii) Orientation and polarity of the regeneration axis are fixed independently.
(iii) At 25°, axis orientation is fixed about 8 hours before protoplast are seen to become asymmetrical.
(iv) Polarity along the oriented axis is determined later and can be affected independently.
(v) Upon removal from a uni-directional light field, protoplasts retain a memory of light direction for the subsequent determination of axis orientation but not for axis polarity.
(vi) The gradient determining axis polarity, involves phytochrome.

A model consistent with these conclusions proposes that the orientation-determining gradient is established rapidly and is steeper in red light than in blue, whereas the polarity gradient forms more slowly and is steeper in blue light than in red.

The phototropic response of protonemal apical cells is also mediated by phytochrome (Hartmann *et al.* 1983, Photochem. Photobiol. 38. 599) and involves microfilaments (Meske and Hartmann, submitted). Mutants abnormal for phototropism are affected pleiotropically in the determination of the protoplast regeneration axis, indicating that the two processes are closely related. Analysis of these mutants is in hand.

J5-101 PROTEIN BODIES IN MAIZE ENDOSPERM ARE JUXTAPOSED WITH MICROTUBULAR ARRAYS,

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When maize endosperm is homogenized in low ionic strength buffers, cytoskeletal proteins co-sediment with the protein bodies. We sought to determine whether this association has biological significance. Using indirect immunofluorescence microscopy, we localized microtubules and protein bodies in intact, developing endosperm. In young cells, which are not yet accumulating storage proteins, we observed microtubules in well known arrays such as the spindle apparatus and phragmoplast (dividing cells) as well as in a network of randomly oriented tubules throughout the cytoplasm of interphase cells. In older, non-dividing cells that are synthesizing storage proteins, microtubules occur in two arrays. The first is adjacent and oriented parallel to the cell wall, and the second is a multidirectional network located within the cytoplasm between starch grains. This multidirectional cytoplasmic array appears to be a novel architecture for microtubules. Confocal microscopy visualization of cells double labeled with antibodies against tubulin and storage protein shows the protein bodies juxtaposed with microtubules. We are currently considering two hypotheses for a functional significance of this association. First, the microtubules might organize the cytoplasm in these large, non-vacuolated cells. Second, the cytoskeleton might coordinate the factors involved in the synthesis and accumulation of storage proteins. We are in the process of expanding these localizations to include storage protein mRNAs, actin, and elongation factors to investigate the second hypothesis.

J5-102 CALCIUM-MEDIATED EVENTS DURING MOSS DEVELOPMENT,

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In the moss, *Funaria hygrometrica*, cells of the proper physiological stage respond to cytokinin by producing vegetative buds. Cytokinin is known to cause a rapid, transient increase in cytoplasmic calcium which stimulates the cascade of events leading to bud formation. Several cellular and morphological changes occur during bud development including reorientation of the cell walls, cytoskeletal redistribution, movement of cellular organelles to the presumptive bud site, swelling at the presumptive bud site, and cell division leading to the formation of the bud initial. We are interested in elucidating the signal transduction pathway involved in the perception of cytokinin through the development of buds. Because protein kinases have been identified as calcium-responsive elements in other systems, we are taking several approaches to examine potential calcium-mediated events occurring in response to cytokinin. We are using stage-specific cultures to examine cytokinin-induced phosphorylation patterns and characterize the subcellular localization of these events. We will further identify second messengers which alter phosphorylation patterns to establish their role in bud formation. To examine the spatial and temporal regulation of phosphorylation, we have isolated the moss homologs of CDPK (calcium-dependent protein kinase), casein kinase IIα and calmodulin via amplification of stage-specific cDNA using degenerate primers. In addition, several putative stage-specific cDNAs have been identified using differential display. We will use RNA blot analysis to monitor expression of these cDNAs through the developmental switch to vegetative bud formation. Also, we would like to understand the mechanisms responsible for the cytoskeletal reorganization which takes place during moss vegetative development. Since the cytoskeleton may be a target for calcium-mediated phosphorylation, we will use microinjection to link the activity of the second messengers that affect phosphorylation to a role in cytoskeletal reorganization and bud formation.

J5-103 POST-TRANSLATIONAL MODIFICATION OF TUBULIN FROM DEVELOPING COTTON FIBERS CELLS.

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During cotton fiber development, cortical microtubules exhibit specific changes in orientation. These changes in microtubule orientation are associated with nearly identical changes in the orientation of cellulose microfibrils in the fiber cell wall and also coincide with changes in the rate of fiber elongation. These observations suggest an involvement of microtubules in the deposition of cellulose microfibrils and in the process of fiber cell elongation. The reorientation of microtubules during cotton fiber development is associated with changes in the accumulation of both the α- and β-tubulin isotypes that compose the cortical microtubules[1]. These changes in tubulin isotype accumulation may be attributed to differential gene regulation and/or post-translational modification. Several post-translational modifications of tubulin have been described: acetylation, tyrosination/detyrosination, glutamylation and phosphorylation. Both acetylation and tyrosination/detyrosination of α-tubulins have been described in plants. Two monoclonal antibodies with specificity to acetylated α-tubulin (6-11B-1) or tyrosinated α-tubulin (YL 1/2) were used as probes to determine the extent of these post-translational modifications during two distinct stages of cotton fiber development, 10 and 20 days-post-anthesis (DPA). The monoclonal antibody specific for acetylated α-tubulin failed to detect acetylated α-tubulin at either stage of fiber development in two related cotton cultivars. In contrast, all of the α-tubulin isotypes present in elongating fibers at 10 DPA are tyrosinated. This result was observed for both cultivars examined. At 20 DPA, when fibers are synthesizing secondary cell wall, the number of tyrosinated α-tubulin isotypes decreased dramatically. This developmental difference in α-tubulin tyrosination and its significance in cotton fiber development are being examined further.

[1]Dixon, Seagull and Triplett, (1994) Plant Physiol. 105:1347-1353.

J5-104 β-TUBULIN EXPRESSION AND RICE COLEOPTILE ELONGATION, Silvia Gianì, Anna Maestroni, Daniela Giussani and Diego Breviario, Istituto Biosintesi Vegetali, CNR, Via Bassini 15, 20133, Milano, Italy.
Rice coleoptile elongation can be controlled by different external stimuli and enviromental conditions. We are interested to identify those elements that may be part of these signal transduction chains including tubulins since correlation between tubulin expression, microtubules orientation and cell elongation has been shown.We have begun to isolate, from a λZAPII cDNA library, rice cDNA clones encoding for α and β tubulins. Insofar three cDNA clones (OSTB-16, OSTB-50 and OSTB-34) containing rice β-tubulin sequences have been isolated and characterized. OSTB-16 and OSTB-50 are full-length cDNAs and both contain an open reading frame of 1340 bp encoding polypeptides of 447 aa. OSTB-34 represents a 5' incomplete β-tubulin cDNA clone. Predicted OSTB-16 and OSTB-50 protein sequences share 94% of aa identities and more than 90% similarity with other higher plant β-tubulins. Northern blot analyses performed on total RNA extracted from rice coleoptiles identify a unique 1.7 kb long β-tubulin transcript. The amount of this transcript is strongly decreased in segments treated for 24 hrs with 50μM ABA, condition that results in the arrest of coleoptile elongation. Similarly, anaerobic treatment of rice seedlings resulted in reduction of β-tubulin mRNA in both roots and coleoptiles. Stronger reduction is observed when a 3' specific OSTB-16 ^{32}P-labeled fragment is used instead than a more aspecific rice β-tubulin probe. With the use of OSTB 3'-specific probes we will provide additional information about tissue rather than enviromentally-mediated expression of these different rice β-tubulin isotypes.

J5-105 DEVELOPMENT OF CHIMERIC PRIMORDIA AND SHOOTS WITH THE INDUCTION OF FLOWERING IN *ARABIDOPSIS*, Frederick D. Hempel, Lewis J. Feldman, Dept. of Plant Biology, Univ. California, Berkeley, CA 94720-3102

The induction of flowering in *Arabidopsis* can occur within one leaf plastochron. During that time, the shoot apical meristem ceases producing leaves and starts producing flowers.

Within populations of wild-type plants, some individuals will produce a single chimeric flower-paraclade shoot at the lower boundary of flowering on the main axis. The frequencies of chimeric flower-paraclade shoots and the phenotypes of flower-paraclades vary between ecotypes and with changes in induction treatments.

Our data indicates that chimeric shoots are derived from young leaf anlagan/primordia present at the start of photoperiodic induction. These primordia become partially "floralized" after the start of induction.

The chimeric flower-paraclade shoots have a bilateral symmetry; with the abaxial side flower-like. This symmetry suggests that the abaxial (distal) region of a developing leaf primordium may be affected *directly* by the influx of floral stimulus during the induction of flowering.

The production of chimeric flower-paraclade shoots in wild-type plants indicates that some *Arabidopsis* primordia are not strictly determined (fated) at their inception.

J5-106 HOMEOTIC GENES IN ROOT NODULE DEVELOPMENT, Jan-Elo Jørgensen, Erik Østergård Jensen, Kjeld A. Marcker,Laboratory of Gene Expression, Department of Molecular Biology, University of Aarhus, Gustav Wiedsvej 10, DK 8000 Århus C, Denmark.

The legume root nodule is a morphological structure of plant origin which is developed during the initiation of the nitrogen fixation symbiosis between legumes and bacteria of the genus Rhizobium. Several plant genes specifically involved in structure and function of the root nodule (nodulin genes) have been isolated during the past decade. None of these have been characterized as developmental regulators. This may in part be due to the methods that have been used to isolate the nodulin genes. In other systems the developmentally regulatory genes are often expressed at a very low rate at distinctly defined time points during various developmental programs. It is therefore likely that nodule developmental genes behave in a similar way, thereby escaping initial screenings which often have been directed towards abundantly transcribed genes in the root nodule.

We have initiated experiments in which we look for homologues of homeotic genes, especially homeobox genes in the legume *Lotus japonicus*. This legume has a relatively small genome and is easily transformed and regenerated. Because of the expected low abundance of homeotic gene transcripts we have initially screened for genomic DNA sequences. The genomic sequences will then be used for expression studies of the relevant genes.

A DNA fragment carrying the homeobox from the Soybean *Sbh1* gene was generated using PCR. The *Sbh1* gene is the soybean homologue of the homeobox gene *knotted1* from maize. Similarly an *Sbh1* homologous PCR fragment was isolated from *Lotus japonicus*. This 300 bp fragment was used as a probe to screen a *Lotus japonicus* genomic library. In this way the Lotus *Sbh1* homologue, *LjH1*, and another homeobox gene, *LjH2*, was isolated. The expression patterns of these homeobox genes are currently being investigated

A second approach was screening of a *Lotus japonicus* genomic library with probes of degenerate oligunucleotides. A degenerate 32mer oligonucleotide was constructed on the basis of the DNA sequence of the currently known plant homeobox genes. The oligonucleotide represented part of the helix 3 coding region which constitutes the mostly conserved part of known homeobox gene sequences. Several clones were isolated and are currently being characterized.

J5-107 INVESTIGATING THE FUNCTIONAL DOMAINS OF *GLABRA2*, A GENE THAT CONTROLS TRICHOME DEVELOPMENT. Mary E. Kent[1*], William G. Rerie[3], and M.David Marks[2], [1]Department of Plant Biology and [2]Department of Genetics and Cell Biology, Univ. of Minnesota, St. Paul, MN, [3]Plant Research Centre, Agriculture Canada, Ottawa, ON K1A 0C6, Canada.

The development of trichomes in *Arabidopsis* is a useful model for investigating cellular morphogenesis. Trichomes are unicelluar hairs which are non-essential to the plant under laboratory conditions. Many mutations have been identified which affect trichome development. The genes defined by these mutations can be divided into two classes; there are those that appear to be responsible for the establishment of trichome cell identity and those that control the differentiation of those cells into normal trichomes. *GLABRA2* (*GL2*) is a member of this second class of genes. *gl2* plants produce a seemingly glabrous first leaf pair; however, these leaves have aborted trichomes which expand laterally instead of projecting outward from the leaf surface. On later rosette leaves, *gl2* plants have short, unbranched trichomes. Analysis of the *GL2* sequence reveals that the gene encodes an acidic domain, a homeodomain, and a potential amphipathic helix motif. We are interested in determining the function of these domains and thereby learning more about the function of *GL2*. Using the yeast reporter system, we will determine if the acidic domain is sufficient to activate trancription of a reporter gene in yeast. and if *GL2* can function as a homodimer. We will also use *GL2* as a bait in a yeast interaction trap and screen an *Arabidopsis* cDNA library to identify cDNA's that encode proteins which interact with *GL2*. (Gyuris et al, Cell 75, 791-803) These experiments will lead to a better understanding of the function of the various domains of *GL2* and enable us to ask questions regarding the function of the gene itself.

*Mary E. Kent is a Howard Hughes Medical Institute Predoctoral Fellow

J5-108 BIOTECHNOLOGICAL ADVANCES IN SUGARBEET AND GRAPE VINE *IN VITRO* CULTURE, P.G.Kovalenko, N.V.Shuman, I.M.Efimenko, T.V.Medvedeva, O.M.Panicheva, A.P.Galkin, Institute of Bioorganic Chemistry, NAS of Ukraine, Murmanska Str.1, Kiev 94, 253660, UKRAINE

In order to improve regenerative potential in *Beta vulgaris L.* cultural conditions were established for the efficient plant regeneration from callus, petiole, intact leaf explants from the four commercial important varieties of sugarbeet. Three main pathways of morphogenesis as: shoot formation, root formation and somatic embryogenesis, have been observed in the callus derived from various transformed explants of sugarbeet. Concentration of phytohormones for the regenerative capacity has been optimized. The electroporation methods have been developed to transfer DNA into protoplasts isolated from cell suspension. Here we report the establishment of a suspension culture of *Vitis vinifera cv.Cabernet Sauvignon* and the conditions required for high level of transient gene expression. We have optimized the conditions using electrporation taking into consideration both expression of a reporter gene & cell viability. The role of sequences reporter to alter transient gene expression in other systems are being investigated. In preparation of gene transfer experiments, we investigated factors that might effect the production of transgenicplants.

J5-109 *Bsd1* GENE ACTION IN MAIZE LEAF DEVELOPMENT, Jane A. Langdale and Lisa N. Hall, Department of Plant Sciences, University of Oxford, Oxford, UK.
Plant cells differentiate through the interpretation of positional information. As a result, the development of function in any cell relies on complex signalling pathways. How cells transmit, receive and interpret such signals is largely unknown. The differentiation of the dimorphic photosynthetic cell-types found in maize leaves provides a model system in which to dissect these cellular communication processes. Mature maize leaves exhibit a series of parallel veins that are surrounded by concentric rings of bundle sheath (BS) and mesophyll (M) cells. The *bundle sheath defective1* (*bsd1-m1*) mutation unlinks the tightly co-ordinated development of BS and M cells that is essential for C4 photosynthetic function in maize leaves. Morphological and functional characterization of mutant plants suggests that the *Bsd1* gene plays a pivotal role in the differentiation of BS cells, since BS cells fail to differentiate yet M and all other leaf cell-types develop normally. We have identified a number of *Spm*-induced alleles of *bsd1* and have used one of them (*bsd1-s1*) to clone the *Bsd1* gene. Using both classical genetics and molecular mapping techniques, we have localized the *Bsd1* gene to the short arm of chromosome 3. Progress towards delimiting the coding sequence and characterizing *Bsd1* gene structure will be reported.

J5-110 A NEW LOCUS AFFECTING *ARABIDOPSIS* TRICHOME DEVELOPMENT DETECTED AS AN ALLELIC VARIANT BETWEEN THE COL AND LER ECOTYPES. John C. Larkin, Michael Prigge, Brian Mustanski, and M. David Marks. Department of Genetics and Cell Biology, University of Minnesota, St. Paul, MN 55108.
We are using *Arabidopsis* trichome development as a model system for examining the control of plant cell fate. Many mutations affecting trichome development have been isolated, and several genes controlling trichome development have been cloned. In this work we describe the identification of a new locus involved in the initiation of trichome development that we have detected as a variant between the Col-1 and Ler ecotypes. *Arabidopsis thaliana* plants of the Col-1 ecotype have approximately 30 trichomes on the first leaf, while Ler plants have approximately 8 trichomes on the first leaf (Larkin et al.,1994. The Plant Cell 6: 1065). F1 plants from a Col-1 x Ler cross have an intermediate number of trichomes (15.6±4.3). In the F2, the low trichome number phenotype is tightly linked to the *erecta* mutation derived from the Ler parent. Data from trichome counts of the Dean recombinant inbred (RI) lines confirm the presence of a major locus for reduced trichome number linked to, but separable from, *erecta*. We are currently examining the RI data for evidence of other quantitative factors influencing trichome number. Preliminary observations indicate that Ler leaves may stop initiating new trichomes earlier than Col-1 leaves. Current experiments include the characterization of trichome development in the low trichome variant by scanning electron microscopy, and examining the effect of the low trichome locus on the expression of a GUS reporter gene under the control of *GL1* regulatory sequences. This new locus appears to define an important gene involved in early events in the trichome development pathway.

J5-111 A 90 kDa MICROTUBULE-BINDING PROTEIN FROM TOBACCO MEMBRANES. Jan Marc, Deirdre E. Sharkey, and Richard J. Cyr[1]; School of Biological Sciences, University of Sydney, Sydney NSW 2006, and [1]Department of Biology, Pennsylvania State University, University Park, PA 16802.
The organization of cytoskeletal elements into distinct geometrical arrays in the plant cell cortex is important for fundamental developmental processes such as division, differentiation and shaping of cells. The spatial organization involves protein bridges connecting microtubules to the plasma membrane. Although the bridges have been documented by electron microscopy, their molecular nature is unclear. We have used detergent extracts from a microsomal fraction of tobacco cells and affinity chromatography to isolate tubulin-binding proteins. Eluted proteins were further purified by incubation and co-sedimentation with taxol-stabilized microtubules. Analysis by SDS-PAGE showed several distinct polypeptide bands. We have isolated a major polypeptide of Mr 90 kDa from the electrophoretic gels and raised monoclonal antibodies to it. Immunofluorescence microscopy with protoplast ghosts shows that the immunoreactive material is ordered into a filamentous pattern. When using dual labeling with monoclonal antibodies against α-tubulin, the filamentous pattern appears to co-localize with cortical microtubules. In contrast to the continuous fluorescent images of microtubules, however, the filamentous pattern consists of discrete beads. Following incubation of protoplasts with taxol, the filamentous pattern becomes rearranged into prominent bundles, which again co-localize with microtubules. Therefore it appears that the antibody detects an epitope of the 90 kDa polypeptide that is distributed along cortical microtubules and that remains attached to the microtubules as they are rearranged by treatment with taxol. Further immunochemical characterization is in progress. Supported by ARC grant A19230505 to JM.

J5-112 A POTENTIAL ROLE FOR THE PLANT CYTOSKELETON IN TRANSPORT OF PROTEIN/NUCLEIC ACID COMPLEXES,
Gail McLean, John Zupan, and Patricia Zambryski, Department of Plant Biology, University of California, Berkeley, CA 94720

In addition to its structural functions, the cytoskeleton plays integral roles in numerous cellular processes such as intracellular movement of organelles, cytokinesis, and signal transduction. It also may affect gene expression by sequestering regulatory molecules such as transcription factors, mRNA, and translation complex components to various cellular locations. While these functions have been shown primarily for the cytoskeleton in animal cells, the cytoskeleton in plant cells undoubtedly shares some if not all of them. Our research addresses the hypothesis that protein/nucleic acid complexes are actively transported on plant cytoskeletal components to specific cellular targets. Specifically, research focuses on a plant viral protein, the TMV movement protein, and its association with the plant cytoskeleton. The movement protein forms complexes with single-stranded nucleic acids and directs the complex to and through the plasmodesmata so that the virus can spread cell-to-cell. Both *in vivo* and *in vitro* methods suggest the movement protein associates with a component of the plant cytoskeleton. We propose that this viral protein uses the plant cytoskeleton as a "highway" in the cell to transport the protein/nucleic complex to the plasmodesmata, the cytoplasmic bridges that mediate intercellular transport and create a "superhighway" for macromolecular traffic in the plant cell. That transport of the viral protein/nucleic acid complex during infection does not disrupt cellular functions suggests that an existing cellular pathway is being exploited by the viral protein.

J5-113 CHARACTERIZATION OF *lem7*, A COLD-SENSITIVE *ARABIDOPSIS* MUTANT AFFECTING LEAF MORPHOGENESIS AND MAINTAINANCE OF LEAF SHAPE.
Lee Meisel and Eric Lam, AgBioTech Center, Waksman Institute, Rutgers State University, Piscataway, NJ 08855
A fundamental question in developmental biology is how cells are able to coordinate their development in a multicellular organism with complex and specialized structures. We have isolated a mutant, *lem* (leaf morphogenesis) 7, in *Arabidopsis thaliana* that may help us to address this question as well as provide further insights into the mechanisms involved in maintaining structural organization during organogenesis. *lem7* is a semi-dominant mutant isolated from a screen for abnormal leaf morphology that becomes apparent during the later stages of vegetative development. Further analysis of this mutant reveals several interesting and unique characteristics. At 23°C expanding leaves that are phenotypically normal, as well as some newly emerging leaves, degenerate at later stages. The mutant phenotype includes a loss in bilateral symmetry, a roughening of the leaf surface, as well as a reduction in the number of trichomes and minor veins. This mutation is apparently temperature sensitive. When grown at 30°C, *lem7* develops and maintains phenotypically normal leaves. When *lem7* is grown at 16°C, leaf morphogenesis at the shoot apex is completely, yet reversibly, arrested. This suggests that the *lem7* mutation affects not only the maintainance of structural organization, but may also play an essential role in differentiation at the shoot apex. The temperature sensitive nature of *lem7* may provide a useful system for the study of downstream loci that are involved in leaf morphogenesis.

J5-114 CLONING OF THE *STALKLESS* LOCUS OF *ARABIDOPSIS* AND ITS ROLE IN TRICHOME MORPHOGENESIS,
David Oppenheimer, and M. David Marks*, Department of Biological Sciences, Univ. of Alabama, Tuscaloosa, AL 35487 and *Department of Genetics and Cell Biology, Univ. of Minnesota, St. Paul, MN 55108

During the differentiation of most plant cell types, precisely controlled cell expansion determines the cell's final shape. What are the genetic and biochemical components which control this process? My lab is using the development of trichomes (hairs) on the plant *Arabidopsis thaliana* as a model to address this question. As a protodermal cell differentiates into a trichome, a marked change in cell shape occurs leading to a trichome with a stalk and two to four branches (depending on the genetic background). Mutations at at least 15 loci are known to disrupt trichome cell expansion and severely affect the final trichome cell shape. Plants homozygous for the *stalkless* (*stl*) mutation have trichomes with a greatly reduced stalk and only one or two branches.

The wild-type *STL* locus has been cloned from *Arabidopsis* by T-DNA tagging (using K. Feldmann's seed-transformed lines). Preliminary DNA sequence evidence strongly suggests that the *STL* locus encodes a member of the kinesin gene family of microtubule motor proteins. It is known that the direction of cell expansion is governed by the orientation of the cellulose microfibrils in the cell wall, and that this orientation is influenced by the orientation of the cortical microtubules present during cellulose synthesis. We now may have a link between the cortical microtubules and the control of the orientation of the cellulose microfibrils. The progress toward complementation of the *stl* mutation with the wild-type copy of the gene, the isolation of suppressor mutations, and genetic interactions will be presented.

J5-115 SPECIFIC GENES EXPRESSED DURING COTTON FIBRE DEVELOPMENT.
Sharon J. Orford and Jeremy N.Timmis, Department of Genetics, The University of Adelaide S.A. 5005, Australia.

The commercial cotton fibre is a product of Malvaceous plants of the species *Gossypium hirsutum* L. Cotton fibres are differentiated from single cells of the outer epidermis of ovules and originate at, or soon after, anthesis. Cotton fibre differentiation, characterised by a precise and synchronous growth and uncomplicated by cell division, is a suitable experimental system in which to study developmental events.

The fibre length largely determines the quality of the resulting spun thread. In view of this, the study of factors involved in controlling the extent of fibre growth is agriculturally important. Genes expected to be important in fibre development include those involved in the biosynthesis of cell wall constituents. A differential screening experiment was designed to identify mRNAs that are preferentially expressed in fibre cells.

A fibre cDNA library was constructed in λZAP®II and 23,000 recombinant plaques were screened with labelled cDNAs from both cotton fibre and leaf. Putative fibre-specific clones (24) were purified by secondary screening and the plasmids excised. Cross-hybridisation experiments reduced the size of the population to six different clones. These were characterised by Northern, Southern and sequencing analysis, and corresponding clones were isolated from a genomic library of an Australian cotton variety, Siokra 1-2.

J5-116 DO CELL WALLS MEDIATE RECOVERY OF ORDERED MICROTUBULE ARRAYS AFTER DEPOLYMERISATION? Robyn L. Overall, Nerida J. Holdaway and Rosemary G. White[2], School of Biological Sciences, University of Sydney, NSW 2006, Australia, [2] Present address : Department of Ecology and Evolutionary Biology, Monash University, VIC, 3186, Australia

There is much evidence that in elongating higher plant cells, cortical microtubules have a role in regulating the direction of cell wall expansion, through their control of the precise alignment of the relatively inelastic cellulose microfibrils in the cell wall. However, there is little evidence to indicate how the microtubules themselves are oriented. Williamson (1) has proposed a model by which stresses in the cell wall, detected by stress-receptive portions of the microfibrils, could themselves orient microtubules via interactions with cell wall-linked transmembrane proteins. Several aspects of Williamson's model were tested, using the young expanding cells of pea root tips which have highly ordered transverse arrays of microtubules oriented perpendicular to the direction of cell expansion. Using the cellulose microfibril synthesis inhibitor, dichlorobenzonitrile, it was shown that newly-formed microfibrils, proposed as the stress-detecting system in Williamson's model, are not required for the maintenance of existing microtubule arrays, or for the re-establishment of organised transverse arrays after depolymerisation with the herbicide, oryzalin, in pea root tip cells. Furthermore, cell wall stresses themselves appeared unnecessary for either maintenance or regeneration of transverse arrays. These results contradict Williamson's model. Further investigations demonstrated that RGD-mediated wall-membrane links were also unnecessary for maintenance or recovery of ordered microtubule arrays. The possibility that a cytoplasmic component may serve to stabilise microtubule arrays or act as part of a 'memory' system for microtubule alignment, was investigated by depolymerising actin using cytochalasin B. In the absence of filamentous actin, microtubules recovered from oryzalin treatment to form transverse arrays. It was concluded that neither microfibrils, cell wall stress, actin nor RGD-mediated cell wall links were necessary for the re-establishment of ordered microtubule arrays after depolymerisation.

1. Williamson, R.E. (1990) Alignment of cortical microtubules by anisotropic wall stresses. Aust J. Plant Phys. 17: 601-613

J5-117 CELLULAR DIFFERENTIATION IN THE MAIZE LEAF, Ronelle Roth, Lisa Hall and Jane A. Langdale, Dept. of Plant Sciences, Oxford University, South Parks Road, Oxford, OX1 3RB.

In the maize leaf, the differentiation of two morphologically distinct cell types, bundle sheath (BS) and mesophyll (M), facilitates a division of labour that is characteristic of C4 photosynthesis. In the thick walled BS cells, the C4 photosynthetic enzymes RuBPCase and ME, accumulate in large agranal chloroplasts. This cell type specific compartmentation of RuBPCase away from atmospheric O_2 reduces the energy wasting process, photorespiration, which is found in C3 plants. M cells, on the other hand, accumulate MDH, PEPCase and PPdK and contain granal chloroplasts. The aim of this study is to identify and characterize genes that regulate cellular differentiation in the maize leaf. In particular, we are focusing on characterizing mutants that specifically disrupt the differentiation of bundle sheath cells. Characterization of one mutant, *bundle sheath defective 1-mutable1* (*bsd1-m1*), has revealed that the functional and morphological differentiation of BS cells is disrupted, while M cells differentiate normally (Langdale and Kidner, 1994). We have recently identified further alleles of *bsd1* as well as several other BS defective mutants [*bsd2, pale green 14 (pg14), golden 2 (g2)* and *bsd3*]. Preliminary Western analysis has shown that the BS cell-specific photosynthetic enzymes, RuBPCase and ME, are downregulated in all the mutants, while M specific photosynthetic enzymes, MDH, PEPCase and PPdK accumulate to normal levels. Data obtained from the molecular and ultrastructural characterization of these BS defective mutants will be presented.

Langdale, J.A. and Kidner, C.A. (1994) *bundle sheath defective*, a mutation that disrupts cellular differentiation in maize leaves, *Dev.* **120**, pp.673-681.

J5-118 GENETIC AND MOLECULAR ANALYSIS OF Arabidopsis ROOT EPIDERMAL PATTERN MUTANTS, Katharina Schneider, Liam Dolan and Keith Roberts, Dept. of Cell Biology, John Innes Centre, Colney Lane, Norwich NR4 7UH, UK

The Arabidopsis root epidermis was chosen as a model system to study the morphogenesis of a particular cell type: the root hair cell. In contrast to the only other cell type in the root epidermis, the hairless cell, the hair cells are always located in a position above the 8 underlying anticlinal cortex cell walls. A genetic and molecular approach has been started to understand this patterning process. 12 EMS and fast neutron mutants with no, few and ectopic root hairs have been isolated and are being analysed genetically and structurally. This will tell us about the number of genes involved and their action. On the molecular side the work is focused on *root hairless (rhl)*, a recessive T-DNA mutant that has no root hairs at all and shows poor shoot development. The line contains only one T-DNA insert that cosegregated with the phenotype in more than 600 mutant seedlings tested so far. Flanking plant DNA sequences have been isolated that indicate the presence of a single *RHL* copy in the genome. On a Northern blot a transcript was detected with very low abundance. Molecular analysis of both cDNA and genomic clones is expected to provide insight into the function of the *RHL* gene in the patterning process of the Arabidopsis root epidermis.

J5-119 MICROINJECTION OF ANTIBODIES TO DETERMINE THE ROLE OF CYTOPLASMIC PROTEINS IN THE ESTABLISHMENT OF POLARITY IN *FUCUS*, Sidney L. Shaw and Ralph Quatrano, Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

Zygotes of the brown algae *Fucus* are being used as a model system for the study of the establishment of cell polarity. Antibody microinjection and several assays for intracellular polarity phenomena have been developed to assess the role of specific molecules in polarity establishment and expression. Effects observed after microinjection of anti-actin or anti-tubulin antibodies were identical to those observed with cytochalasin B or nocodozole treatment, respectively. Microinjection of anti-actin antibody that does not recognize *Fucus* actin had no effect on zygotes. Based on these control experiments, antibodies made against the cytoplasmic domain of human beta-1-integrin, which recognizes a 90kd protein in *Fucus* extracts, were microinjected and blocked polarity expression. The effects of anti-integrin antibodies on plasma membrane asymmetry, Golgi vesicle targeting, and polarized actin concentration at the cell cortex will be discussed in relation to a model for polarity that includes a transmembrane molecule which links the cell wall to the cytoskeleton. Work supported by Grants from NSF #MCB9318757 and ONR #N00014-93-1-0888 to RSQ.

J5-120 CYTOSKELETON COMPONENTS OF ISOLATED PLANT PLASMA MEMBRANE VESICLES: ACTIN AND TUBULIN CAN BE SELECTIVELY WASHED OFF FROM INSIDE-OUT PM VESICLES, Anders Sonesson and Susanne Widell, Section of Plant Physiology, Lund University, P.O.Box 117, S-221 00, Lund, Sweden

We have shown, by co-purification, that actin and tubulin are specifically attached to the inside of isolated plant plasma membrane (PM) vesicles[1]. To be able to investigate the links between these two cytoskeleton proteins and the PM, the inside of the vesicles has to be accessible. Our approach to this problem is to use the detergent Brij 58 that instead of solubilizing the PM turns the vesicles inside-out[2]. Both actin and tubulin remain associated with the PM after such a treatment but can be washed off selectively using different buffers. Some other proteins are solubilized under the same conditions and we are now testing whether these might be associated with actin or tubulin.

[1] Sonesson and Widell 1993 Protoplasma 177:45-52

[2] Johansson et al 1995 Plant Journal (in press)

J5-121 *COT*, A NEW *ARABIDOPSIS* TRICHOME MUTANT, DEPENDS ON CONSTITUTIVELY EXPRESSED *GL1* FOR PHENOTYPIC EXPRESSION

Pamela L. VanderWiel[1], John C. Larkin[2] and M. David Marks[2]. [2]Department of Plant Biology and [1]Department of Genetics and Cell Biology, University of Minnesota, St. Paul, MN.

The mechanisms responsible for the commitment of plant cells to specific developmental fates are largely unknown. Our laboratory is using trichome development in *Arabidopsis thaliana* as a model to elucidate these processes. The *GL1* gene, which is required for trichome initiation, has been cloned and has been shown to encode a *myb* transcription factor. Plants that constitutively express *GL1* (35S*GL1*) have a few ectopic trichomes on the cotyledons. In an attempt to find mutants with a greater number of cotyledonary trichomes, 35S*GL1* seeds were mutagenized with EMS and M2 seedlings were screened. *Cot*, a mutant isolated in this screen, produces an increased number of cotyledon trichomes. A combination of outcrosses to wild type plants and backcrosses to 35S*GL1* plants indicates that the presence of 35S*GL1* is necessary for the expression of this phenotype. Attempts to map this mutation are currently underway.

J5-122 ACTIN- AND MICROTUBULE-DEPENDENT WALL DEPOSITION IN *NITELLA* INTERNODAL CELLS

Geoffrey O. Wasteneys[1], Ilse Foissner[2], David A. Collings[1] and Richard E. Williamson[1]

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We have microinjected living *Nitella* internodal cells with fluorescent tubulin and fluorescent phallotoxins to document activity and distribution of cortical microtubules (MTs) and actin filaments (AFs) respectively during primary cell wall deposition and also during cellulosic wall apposition in response to wounding. During elongation, transverse cortical MTs prescribe the direction in which cellulose microfibrils are deposited; wall anisotropy is lost when MTs are disassembled. The cortex also contains AFs whose orientation is similar to that of MTs. Cortical AFs and MTs, however, appear to be aligned independently: disassembly of MTs with oryzalin does not disrupt AF orientation and cytochalasin treatment, which modifies AFs into short, stable rods, does not alter MT orientation. Nevertheless, MT disassembly potentiates the action of cytochalasin, such that streaming stops at normally ineffective doses.

During wound-induced cellulosic wall apposition formation, MTs become disorganized and turn over more rapidly at wound sites than at adjacent, unperturbed sites but disassembly of MTs does not impede the wound repair mechanism or affect the ultrastructure of wall appositions. AFs are extremely abundant and, like MTs, are randomly oriented at wound sites. Cytochalasin not only modifies cortical AFs into short, stable rods but also suppresses exocytosis, as judged by the reduced volume of wall appositions, even at cytochalasin concentrations that do not inhibit streaming. We therefore conclude that AFs, and not MTs, are critical for the exocytotic events of wounding responses.

J5-123 LOCATION OF ACTIN IN DEVELOPING PHLOEM, Rosemary G. White and Scott Azzopardi, Department of Ecology and Evolutionary Biology, Monash University, Victoria 3168, Australia.

Actin microfilaments are found in all young cells of the root, including developing vascular cells. Mature xylem elements are non-living, hence contain no cytoplasmic contents. It has been reported that actin is also absent from mature sieve elements, a significant result as this means that actin-based motility cannot play a direct role in phloem translocation. However, in recent work employing various fluorescent probes for actin, including rhodamine-phalloidin and anti-actin antibodies, abundant fluorescence was observed in the form of thick actin microfilament bundles in the vascular cylinder, which appeared to be localised to phloem tissues. The aim of this work was to determine which cells contained this actin. Actin was localised in sections of young root tissue (*Pisum sativum, Zea mays, Arabidopsis thaliana*), either by staining fresh hand sections with rhodamine-phalloidin and observing with a BioRad 600 confocal scanning laser microscope, or by embedding tissue in butylmethylmethacrylate resin, and staining semi-thin sections with C_4 anti-actin primary antibody followed by FITC-labelled secondary antibody after removal of the resin with 100% acetone. In fresh sections, actin was observed in all young cells, with particularly strong fluorescence from young vascular tissue. Cortical cells contained fine, transverse microfilament bundles while elongate vascular cells contained thicker, longitudinal bundles. In resin-embedded tissue, the finer actin bundles were not preserved, and thicker bundles were observed primarily in companion cells and phloem parenchyma in older tissues. The actin in these phloem cells was very resistant to disruption by cytochalasin B. We are presently carrying out parallel TEM observations of resin-embedded material to determine the arrangement of actin in these cells, and any associations with other cellular organelles.

Mitosis and Division Planes; Cell Cycle

J5-200 REDISTRIBUTION OF TOTAL AND ACTIN MRNA DURING POLAR DEVELOPMENT OF THE FUCUS EMBRYO: REQUIREMENTS AND MECHANISMS (F.-Y. Bouget, S. Gerttula, and R. S. Quatrano) Department of Biology, University of North Carolina, Chapel Hill, NC 27599-3280.

Because of the unique temporal and spatial localization pattern of actin protein during polar axis fixation, polar growth of the rhizoid and morphogenesis of the young *Fucus* embryo, we are interested in determining the distribution of total and actin mRNA during embryogenesis. We demonstrate the distribution pattern of mRNA in the developing embryo by *in situ* hybridization using a non-radioactive, whole-mount technique. We find no asymmetry of mRNA in the egg and through 8 hours of zygote development. At the time of polar axis fixation (12 hours), a gradient of total and actin mRNA is established which persists through at least the 4-cell stage of embryogenesis, with the highest concentration in the thallus area. Although total mRNA continues to be asymmetrically distributed during the completion of the first and second cell division of the zygote, there is a gradual and specific localization of most of the actin mRNA around both of these horizontal cell plates. No localization of actin mRNA was observed at the tip of the rhizoid where a large concentration of actin protein accumulates. Actin mRNA appears to co-localize with the deposition of an "actin collar" around the first two division plates of the rhizoid. The requirements and mechanism(s) responsible for the localization and stabilization of total and actin mRNA will be discussed relative to other embryonic events such as vesicles movment, cell plate and cell wall formation. Supported by a grant from NSF (MCB 9318757) to R.S.Q.

J5-201 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

Virtually the entire plant body is generated by post-embryonic organogenesis, mediated by meristems. Meristem identity, therefore the type of organs produced, and meristem activity, thus the rate at which daughter cells are produced, are controlled by developmental pathways responsive to environmental signals such as light or nutrient availability. 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 organ formation.

We are interested how regulatory programs mechanistically 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 with the developmental potential 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 cytokinesis in the incipient lateral root meristem. Cyclin transcription is therefore a candidate rate-limiting event for root organogenesis and this hypothesis is presently being tested by ectopic expression of Atcyc1 to bypass this checkpoint. We will describe lateral root founder cell division in these transgenic *Arabidopsis* plants. Furthermore, we will relate the biochemical activation of the cell cycle engine to the cytological events during founder cell cytokinesis.

J5-202 THE ROLE OF F-ACTIN IN ELONGATION OF MICROSPORES OF *BRASSICA NAPUS*, Carmen Gervais[1,2], Daina H. Simmonds[1] and William Newcomb[2], [1]Plant Research Centre, Agriculture Canada, Ottawa, Ontario K1A 0C6, [2]Department of Biology, Queen's University, Kingston, Ontario K7L 3N6.

Parallel bundled microfilament (MF) arrays form in the cortical region of the vegetative cell of *Brassica napus* cv. Topas microspores shortly after the first microspore mitosis. These arrays disappear at about the same time that the generative cell undergoes mitosis to form two sperm cells. This is also the time when microspores begin to elongate to form ovoid structures. The bundled MFs are always oriented perpendicular to the microspore furrows, the axis of elongation. Microspores isolated close to the first mitosis and cultured at 25°C for 48 h elongate and develop into tricellular pollen. Culture of microspores following treatment with the MF disrupting drug cytochalasin D (10 µg/ml, 15 min) prevents cell elongation. Cells expand isodiametrically. Cell elongation was not inhibited when the cultures were treated with the microtubule (MT) disrupting drugs, colchicine or trifluralin. Our studies indicate that in gametic development MFs may have a very prominent role in determining cell shape. Current studies are focused on the location and orientation of cell wall polymer deposition in relation to the MF and MT cytoskeleton.

J5-203 **E2F-AND E1A-ASSOCIATED KINASES ARE INVOLVED IN REGULATING ENDOREDUPLICATION DURING MAIZE ENDOSPERM DEVELOPMENT.** Gideon Grafi and Brian A. Larkins, Department of Plant Sciences, University of Arizona, Tucson AZ 85721.

In maize endosperm, endoreduplication is a normal and probably vital process required for development. We conducted a study with the goal of unraveling the factors regulating DNA synthesis during endoreduplication. Histone H1 kinase activity eluted from p13-agarose was increased during maize endosperm development and peaked at 10-12 DAP, after which it declined. These results are consistent with the reduction in mitotic activity and initiation of endoreduplication. Immunoblot analysis showed that the level of the maize $p34^{cdc2}$ in the endosperm extract is high at 10 and 12 DAP and only slightly reduced at 14 and 16 DAP, indicating that the reduction in histone H1 kinase activity at 16 DAP could be a result of $p34^{cdc2}$ phosphorylation, or cyclin destruction. Several kinases have been implicated in the onset of S-phase in mammalian systems, most of which were suggested to exert their regulatory function through interaction with cellular and viral proteins, such as E2F and the adenovirus E1A protein. To study the factors involved in DNA synthesis during endoreduplication, we used mammalian and viral GST fusion proteins: GST-E2F and GST-E1A. The maize $p34^{cdc2}$ was found in precipitates eluted from GST-E2F and GST-E1A fusion proteins and its level was higher at 16 DAP. Both precipitates possess histone H1 kinase activity with the highest level at 16 DAP. These results suggest that the kinase eluted from p13-agarose is different from the kinase eluted by GST-E2F and GST-E1A. Moreover, by using H1 kinase assay, a protein of about 50-kD was highly phosphorylated with GST-E1A/16 DAP precipitates, while another protein of about 60-kD was phosphorylated with GST-E2F/16 DAP precipitate. As these phosphoproteins may be G1 cyclins, it appears that at least two different kinases, one that will associate with E2F and one that will associate with E1A, are involved in controlling DNA synthesis during endoreduplication in maize endosperm.

J5-204 **RICE cDNA HOMOLOGS OF CYCLINS AND SUBUNITS OF 26S PROTEASOMES.**

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The basic mechanism of cell cycle control is apparently conserved in all higher eukaryotes. Based on the sequence homology to other organisms, we have isolated several genes assumed to participated in the regulation of cell cycle in plants. We have recently isolated two cdc2 gene homologs from rice [M.G.G. 233, 10-16 (1992)]. Here, we report isolation of three rice cDNAs (CycOs1, CycOs2 and CycOs3) corresponding to cyclins. CycOs2 shows highest similarity to type B cyclins. CycOs1 and CycOs3 are slightly more similar to type A cyclins. The "destruction box" (putative recognition site of ubiquitin-conjugating degradation system) is found in the predicted amino acid sequences of CycOs1 and CycOs2 as well as the PEST region in those of CycOs1 and CycOs3, respectively. The two rice cdc2 genes have been mapped to the terminus of chromosome 2, whereas the three cyclin genes have been mapped to chromosomes 1, 4 and 5.

Cyclins are degraded by the ubiquitin system in which 26S proteasomes are involved. We have cloned two rice cDNAs (TBPOs1 and TBPOs2) encoding the putative regulatory subunits of proteasomes (Plant Science, in press). TBPOs2 is a homolog of the subunit 4 of 26S proteasomes , and TBPOs1 is a homolog of HIV-1 tat binding protein(TBP1). TBP1 is now claimed as a subunit of 26S proteasomes. These two rice cDNA molecules contain the domains for putative ATPase and RNA helicase (DEAD box) activities.

Northern blot analysis showed that all above mentioned genes were highly expressed in proliferating rice cells, such as suspension-cultured cells or younger seedlings. We also analyzed induced expression of these genes upon injuring of leaves.

J5-205 CYTOLOGICAL CHARACTERIZATION OF FEMALE GAMETOPHYTE MUTANTS IN MAIZE.

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The organization of cellular structures and microtubules of female gametophytes in both wild type and mutants of maize were examined using immunofluorescence light microscopy and transmission electronic microscopy. During normal embryo sac development the megasporocyte and young embryo sac display obvious polarity of organelles and microtubules. Synchronized nuclear divisions and migrations occur at micropylar and charazal poles during second and third mitosis. Deviated from normal embryo sac development, the *indeterminate gametophyte (ig)* mutant showed no obvious polarity of embryo sac. The nuclei of embryo sac frequently undergo asynchronized nuclear division and nuclear migration at second and third mitotic division and extra mitotic divisions afterward. The abnormal nuclear division and migration result in indeterminate number of micropylar cells and polar nuclei in the embryo sacs.

In contrast to normal stock, the nuclei of megasporocyte and young embryo sac of *lethal ovule 2 (lo2)* frequently show failure of nuclear division and migration. These abnormal nuclear behavior initiately occurs as early as the first division of meiosis and frequently are found at the first mitotic division of young embryo sac. The development of *lo2* embryo sac frequently arrests at two- nucleate stage, which causes the ovule abortion.

J5-206 **PETAL PROTOPLAST CULTURE SYSTEM - A NOVEL APPROACH TO STUDY PLASTID BIOGENESIS,**

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Flower development *in vivo* is controlled by a complex array of regulatory factors that involve in tight interaction between the bud and the rest of the plant. The factors controlling chromoplast biogenesis and the molecular structure of this plastid are still largely unknown. The small amount of information available concerning the subject has come mainly from studies of fruits. The chloroplast-chromoplast conversion in corollas has been shown to accompany flower development in cucumber (*Cucumis sativus* L.). In mature, yellow, corollas only chromoplasts could be found. To enable future characterization of the factors involved in the regulation of chromoplast-chloroplast conversion, an *in vitro* petal protoplast culture system is proposed. Flowers at anthesis of *C.sativus* plants grown in vitro were used as a source of protoplasts. The regeneration procedure for petal protoplasts was the same as for mesophyll protoplasts of cucumber. Calli formed were grown either in the dark, or in the light. The light has induced the greening of the calli, so suggesting chromoplast-chloroplast transition. The maintenance of the calli in the dark was preferable for a successful morphogenesis induction. In contrast with mesophyll protoplasts, plant regeneration has not been yet achieved. *In vitro* studies have established the presence of chloroplasts in the minicalli derived from petal protoplasts. Therefore, an *in vitro* petal protoplast culture provides a useful system to study chromoplast-chloroplast conversion and chromoplast and chloroplast biogenesis. It also should contribute to an elucidation of the processes involved in the realization of cell totipotency.

J5-207 NUCLEAR MIGRATION MECHANISM OF THE STOMATA SUBSIDIARY MOTHER CELL OF THE HYPOCOTYL EPIDERMIS OF *CUCUMIS SATIVUS* L.

Haruko Kazama, Keiko Sugimoto & Rie Suzuki, Biol. Dept, International Christian Univ. Mitaka, Tokyo 181, Japan

Little is known about nuclear migration mechanisms in higher plants because of few suitable experimental systems for their investigation. The stomata complex of the hypocotyl epidermis of *C. sativus* is a powerful experimental system, because of the following advantages: 1) subsidiary cell (SC) formation can be induced by red light and the timing of division of each subsidiary mother cell (SMC) can be predicted; 2) the proximal SC is the result of an asymmetric cell division accompanied by a long distance nuclear migration. We demonstrate the role of the cytoskeleton in red light-induced nuclear migration in the SMC. The position of the nucleus in the proximal SMC was examined by DNA staining with Hoechst 33258. DNA synthesis was detected by incorporation of bromo-deoxyuridine. Microtubules and micro-filaments were visualized by indirect immunofluorescence microscopy. The following results were obtained: 1) The nucleus of the proximal SMC started to migrate towards the adjacent guard cells after red light exposure, with its final position around 35 % of the SMC cell length from the guard cells after 96 hr red light irradiation. In the dark the nucleus maintained its position. 2) DNA synthesis occurred after nuclear migration. 3) Cremart, an inhibitor of the microtubules, inhibited the red light-induced nuclear migration at a concentration of 1.5 μM. 4) With 5 μg/ml cytochalasin D (an inhibitor of microfilaments) treatment in the red light, the position of the nucleus in the cell was found to be random, whereas in the dark it had no effect on the position of the nucleus. A higher concentration of cytochalasin D (10 μg/ml) inhibited the red light-induced nuclear migration. 5) A pronounced rearrangement of microtubules was observed during the SC formation. Before nuclear migration, the cortical microtubules in the SMC were transversely oriented. During nuclear migration the longitudinally-oriented microtubules over the nucleus through the SMC were clearly seen, and then transversely-oriented microtubules were observed as a pre-prophase band. 6) After cremart treatment, fragmentation of microtubules was conspicuous. 7) Rearrangement of micro-tubules during the nuclear migration was inhibited by cytochalasin D. From these results it is suggested that microtubules as well as microfilaments are involved in the red light-induced nuclear migration in the SMC of the hypocotyl epidermis of *C. sativus*. A possible mechanism of this nuclear migration is discussed.

J5-208 GENETIC ANALYSIS OF LATERAL ROOT FORMATION, Jocelyn E. Malamy and Philip Benfey, Biology Department, 100 Washington Place, New York University, New York, NY 10003

The formation of lateral roots involves the differentiation of a subset of mature pericycle cells, which divide and give rise to all the cells of the lateral root meristem and connecting vasculature. Although lateral root buds are initiated during normal development, their number and position are strongly influenced by external stimuli. Some but not all of the lateral root buds emerge to form lateral roots, also depending on external stimuli. We are addressing the mechanism of lateral root formation in *Arabidopsis thaliana*. Patterns of lateral root formation in roots after tip excision, which stimulates both initiation and emergence of lateral roots, were compared to patterns in uncut roots. Knowledge of these patterns was used to screen for mutants in lateral root formation. In a parallel approach, enhancer trapping was used to identify genes expressed at early stages of lateral root formation. The current status of mutant and enhancer trap analyses will be presented.

J5-209 MULTI-DIMENSIONAL CHROMATOGRAPHIC ANALYSIS OF RHIZOBIAL NODULATION FACTORS, Neil P. J. Price[#] and Russell W. Carlson[*], [#]Department of Chemistry, SUNY College of Environmental Science and Forestry, Syracuse, NY 13210, and [*]Complex Carbohydrate Research Center, University of Georgia, 220 Riverbend Road, Athens, GA 30602.

Nod factors are biologically active oligosaccharide signals that are secreted by symbiotically competent bacteria of the family Rhizobacaea. Their biosynthesis is determined by rhizobial nodulation (*nod*) genes, and is specifically induced in response to flavonoids secreted from the roots of host leguminous plants. The biological activity of Nod factors on these host legumes dramatically mimics the early developmental symptoms of the *Rhizobium*-legume symbiosis including, amongst other effects, root hair deformations and nodule initiation. Structurally, all Nod factors are short oligomers of β1,4-linked *N*-acetylglucosamine residues (usually dp 4 or 5) that are N-acylated on the distal glucosamine. This common "core" structure may be modified by a number of species-specific substituents on the distal or reducing sugars; modifications that are governed by rhizobial host-specificity *nod* genes. The biological activity of purified Nod factors mirrors this host-specificity, indicating that the symbiotic host-range of individual *Rhizobium* species is, at least partially, determined by the variety of Nod factors they are able to produce. Here we describe techniques that are universally applicable to the extraction, chromatographic separation, and identification of Nod factors. We have applied these techniques to Nod factors from broad host-range species *Rhizobium fredii* USDA 257 and *Rhizobium* spp. NGR234 and the more narrow host-range *Bradyrhizobium japonicum* USDA 110, and have identified a group of novel, relatively hydrophilic Nod factors from the NGR234 species that may have implications for Nod factor biosynthesis.

J5-210 PROTEIN FARNESYLTRANSFERASE IN PLANTS: MOLECULAR AND FUNCTIONAL ANALYSIS, [1] Daqi Qian, [1]Rong Ju, [2]Dafeng Zhou, [2]Carole L. Cramer and [1]Zhenbiao Yang, [1]Department of Plant Biology and Plant Biotechnology Center, The Ohio State University, Columbus, OH 43210, [2]Plant Molecular Biology Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

Protein farnesyltransferase (FTase) is a heterodimeric enzyme that transfers an isoprenyl group to a cysteine residue at the C-terminus of protein substrates. Farnesylation is required for membrane targeting and biological activity of key regulatory proteins such as $p21^{ras}$. In yeast and mammals, cell cylce progression depends on FTase activity. Recent studies indicate that FTase is also present in plant cells (1, 2). We have isolated a cDNA encoding a pea homolog (PsFTb) of the FTase β subunit using a RT-PCR strategy (1). A similar approach is used for cloning the FTase α subunit from pea. Two sets of degenerate oligonucleotides, corresponding to conserved regions within mammalian and fungal FTase α subunits, were used as PCR primers to amplify cDNAs derived from a pea root tip cDNA library. Sequence analysis of a 132 PCR fragment reveals that it has greater than 35% identity to the mammaian FTase α subunits at amino acid level. This fragment was used as a probe to screen the root tip cDNA library. Thirty positive clones were identified from $1.5x10^6$ indepedent clones. Sequence analysis of three selected clones indicated that all three encode the same gene (*PsFTa*). Like PsFTb, the PsFTa polypeptide shows greater sequence similirity to the mammalian counterparts than the fungal one. DNA hybridization analysis suggests that PsFTa is encoded by a single copy gene in pea genome. Preliminary results indicate that FTase mRNA transiently accumulates in early log phase of tobacco BY-2 cell cultures, suggesting that FTase may also play a key role in cell cycle control in plants. To directly assess the biological function of the plant FTase, we are developing a functional assay involving transient and transgenic expression of an artificial FTase substrate protein in plants.

1) Yang, Z. et. al. 1993. Plant Physiology 101: 667-674.
2) Randall, S.K. et. al. 1993. Plant Cell:433-442.

J5-211 NUCLEAR INTERMEDIATE FILAMENTS AND LAMIN PROTEINS IN DIVERSE PLANT SPECIES, Mary Jane Saunders, Anthony Rycerz, Mary E. Colter and Jian Zhang, Department of Biology and Institute of Biomolecular Science, University of South Florida, Tampa, FL 33620

Lamins are type V intermediate filaments which are involved in the structural integrity and chromatin organization of interphase nuclei. During mitosis, hyperphosphorylation leads to depolymerization, concurrent with nuclear envelope breakdown. We report the presence of lamin proteins (A-type and B-type) from dicot and monocot plants as well as the green algae *Chlamydomonas*. Western blot analyses reveal the presence of at least four intermediate filament type proteins that are immunologically related to human lamin B and human lamin A/C in each species. The protein profiles differ in molecular weight between species and between tissues of the same species indicating a complex family of proteins in plant nuclear matrixes. Purified lamin protein from pea assembles into 7-10 nm filaments that bundle *in vitro* and resemble expressed lamin from animal cells. Both the amount of detectable lamin antigens and their distribution changes during the cell cycle, with the greatest amount of chromatin-associated lamin during mitosis. Endogenous interphase phosphorylation of lamin-related nuclear proteins was measured in peas grown in the presence or absence of phosphate. Lamin B was highly phosphorylated by [γ-^{32}P] ATP in plants grown without phosphate. Ca^{2+}-dependent- and casein-type-II-kinase activities were detected in pea interphase nuclei using purified plant lamins as a substrate. Further, both of these kinases affect the phosphorylation of plant lamin B in concert. We are also cloning the plant lamin B_2 gene from an *Arabidopsis* flower library using a heterologous probe from chicken. We conclude that lamins occur ubiquitously throughout the plant kingdom and that they share biochemical and structural characteristics with animal lamins.

This project is supported by USDA and USF Institute for Biomolecular Science.

J5-212 *ROOT MERISTEMLESS*: GENES REQUIRED FOR CELL DIVISION IN THE ROOT PRIMORDIA

Kevin A. Seeley, Jin-Chin Cheng, Z. Renee Sung. Plant Biology, University of California, Berkeley, CA, 97420

To investigate the genetic mechanism regulating root meristematic activity, we isolated and characterized four, single-gene, recessive mutants of *A. thaliana* called *root meristemless (rml)*. Complementation tests identified two *RML* loci: *RML1* maps to chromosome IV and *RML2* maps to chromosome III. *rml* mutants produce normal embryonic roots that undergo no or limited cell division following germination resulting in primary roots that are less that 2.0 mm in length. Although root growth is arrested, shoot growth appears normal. The *rml1* mutants can initiate lateral and adventitious root primordia that grow to a size comparible to that of the embryonic root, a size of 17-18 cells/cell file, then cell division is arrested. This suggests that this arrested root size defines the minimal root size necessary to achieve root organ identity. Although root organ identity is achieved in the *rml1* mutants, they lack the ability to activate cell division following germination. As a consequence, no root meristem is formed in the mutant seedlings. In the absence of cell division, root apical cells in the *rml* seedlings are able to undergo differentiation into epidermal, cortical, and vascular cells. The presence of normal cell division in the mutant embryo, shoot, and callus cells argues that the *RML* gene functions are not part of the general cell division processes, rather, they are involved specifically in activating cell division in root apical cells once the basic root primordium is formed. Our results, therefore, suggest that, in *Arabidopsis thaliana*, the development of a root can be regarded as occurring in two distinct phases, first, the formation of a minimal root primordia, and second, the activation of cell division in the apical region of the root primordia to form an active root meristem.

J5-213 INDUCTION OF EMBRYOGENESIS IN *BRASSICA NAPUS* WITH ANTISENSE mRNA,

John Shedden[1], Daina H. Simmonds[2] and William Newcomb[1], [1]Department of Biology, Queen's University, Kingston, Ontario, Canada. [2] Plant Research Centre, Agriculture Canada, Ottawa, Ontario, Canada.

Isolated *Brassica napus* cv. Topas microspores, when cultured at 25°C, undergo pollen development. When microspores of the late unicellular stage are cultured, a heat treatment (18-24 h at 32.5°C) will induce embryogenesis in approximately 10-20% of the microspores. The mechanisms causing the switch from gametogenesis to sporogenesis have not yet been elucidated. Preliminary results from our laboratory indicate that cyclohexamide, a protein synthesis inhibitor, can induce embryogenesis in the absence of the heat treatment. Since heat induction also inhibits protein synthesis it is suggested that pollen development is discontinued in the absence of certain newly synthesized proteins and that embryogenesis may be a default developmental pathway. We are testing a hypothesis which states that the absence of a single pollen-specific protein, expressing at the late unicellular to early bicellular stage, may be sufficient to trigger embryogenesis. This hypothesis may be tested by blocking translation of a pollen specific protein with antisense mRNA. A particle bombardment system (Biolistic® PDS-1000/He, Bio-Rad Labs) has been optimized for microspores by means of GUS transient expression and will be used to deliver antisense mRNA of pollen specific genes into *B. napus* microspores.

J5-214 HYPOCOTYL/ROOT JUNCTION IN THE EMBRYONIC AXIS OF ARABIDOPSIS, Sung, Z. R., J. -C. Cheng, and K. Seeley. Department of Plant Biology, University of California, Berkeley, CA 94720

The mature embryo of most dicotyledonous plants consists of two visible organs, the cotyledons and the embryonic axis. In Arabidopsis, the junction between the hypocotyl and root becomes visible following germination as a region rich in root hairs. However, no cellular and morphological characteristics exist to identify this junction in the embryo. We exploited the notion that anticlinal cell division may be absent in the hypocotyl during germination. Without cell division, the number of hypocotyl cells/cell file in the embryo would be the same as that of the seedling. By counting the number of hypocotyl cells along the embryonic axis, we would be able to identify the hypocotyl/root junction. Root hairs were detectable in longitudinal sections of Arabidopsis seedlings as early as one day after germination. Using the emerging hairs as a landmark for the lower limit of the hypocotyl, we found that cell number/cell file for 1-, 2-, 4-, 7-, 10-, and 20-day-old seedlings were the same, 24.0±1.8 cells/epidermal cell file and 27.0±2.3 cells/cortical cell file. Thus, hypocotyl growth from 0.68±0.03 to 3.6±0.3 mm by 20 days after germination resulted largely from cell elongation and not cell division. By subtracting the number of hypocotyl cells from total number of cells/cell file in the embryonic axis, 40.5±0.9 epidermal and 44.1±2.0 cortical cells/cell file, we determined that the embryonic root contains, minimally, about 17 cells/epidermal and 17 cells/cortical cell file. This places the hypocotyl/root junction in the embryonic axis of mature Arabidopsis embryo to a site 5-6 epidermal cells above the top edge of the root cap.

J5-215 INDUCTION OF *BRASSICA NAPUS* MICROSPORE EMBRYOGENESIS: CHANGES IN SYNTHESIS AND LOCATICON OF HEAT SHOCK PROTEINS

Michiel M. Van Lookeren Campagne[1], Jan Cordewener[1], Gerd Hause[2], Elke Gorgen[3], Ronald Busink[1], Bettina Hause[2], Hans Dons[1], André A.M. Van Lammeren[2], and Paul Pechan[3]. [1] *Dept. of Developmental Biology, Centre for Plant Breeding and Reproduction Research (CPRO-DLO), P.O. Box 16, 6700 AA Wageningen, The Netherlands.* [2] *Wageningen Agricultural University, The Netherlands.* [3] *Max Planck Institut für Biochemie, Martinsried bei München, Germany.*

Embryogenesis can be irreversibly induced in isolated *Brassica napus* microspores by elevating the culture temperature from 18°C to 32°C for about eight hours. We studied the synthesis and localization of a number of heat shock proteins (HSPs) from the 70 kDa class during the inductive period. HSPs were identified by Western-blotting of two-dimensional (2-D) gels from microspore protein extracts derived from embryogenic cultures. Two polyclonal antibodies, raised against tomato HSPs, were used: anti-HSP70 and anti-HSP68. The immuno-reactive protein spots were matched to a database of [^{35}S]-methionine labeled proteins. It was found that several of the proteins that were preferentially synthesized in the embryogenic cultures belonged to the 70 kDa HSP class.

Immunocytochemistry on cryo-sectioned microspores employing these two antibodies was used to determine the distribution of the corresponding epitopes, just after isolation, and after 8 h in culture under non-embryogenic (18°C) and embryogenic (32°C) culture conditions. Staining patterns of microspores after 8h culture at 18°C was comparable to the pattern found just atfer isolation, for both antibodies. Anti-HSP68 staining was restricted to the mitochondria, and this pattern did not change after the 8h incubation at 32°C. Anti-HSP70 clearly showed temperature induced changes in the intensity and distribution of the staining. Anti-HSP70 staining appeared to be associated with the nucleoplasm during the synthetic phase of the cell cycle, and with the cytoplasm during the remainder of the cell cycle. Under embryogenic culture conditions, staining could be found in vegetative nuclei of early bicellular pollen, suggesting that the vegetative cells had progressed from G_1-arrest to S-phase during the 8h 32°C treatment. We are currently investigating whether the 70 kDa HSPs recognized by the anti-HSP70 and anti-HSP72/73 antibodies are instrumental to this cell cycle progression of the vegetative cell.

J5-216 ESTABLISHMENT AND ABOLITION OF POLARITY IN CULTURED TOBACCO MESOPHYLL PROTOPLASTS, Jean-Pierre Verbelen, Weihai Tao and Didier Stickens, Department of Biology, University of Antwerp UIA, B-2610 Wilrijk, Belgium.

The establishment of polarity has preferentially been studied is unicellular lower plant systems where it is most obviously expressed when spherical spores or zygotes grow into a tubular structure (rhizoid, protonema, mycelium...). Also in higher plants the establishment and maintenance of polarity is considered as one of the basic rules governing morphogenesis.

In the multicellular structure of the seed plant it is however difficult to search for the cellular fundamentals of polarity.

In our single cells model system polar development can be induced , maintained and stopped at will. Protoplasts are isolated from tobacco leaves in which cell division has stopped. They are immobilised on a layer of agarose and cultured in K3A medium. Depending on the hormonal content of the medium the cells can follow different developmental pathways.

In a classic culture environment containing auxins and cytokinins cells soon start dividing and produce apolar agregates of isodiametric cells. The growth pattern in an auxin-only environment however is very different. Cells express a clear polarity in their development: they start a unidimensional growth and develop into long (more than 500 µm) tubular cells over a period of 3 to 4 weeks. Addition of cytokinins to such a culture changes the developmental pattern in just a few days:elongation stops and the huge cells start dividing. During the first divisions cell polarity is conserved: all new cell walls are parallel to each other and perpendicular to the longitudinal axis of the original cell. Soon however this ordering is lost and at random orientation of the division planes produces typical microcalli.

Summarizing one can state that in single cells auxins in the absence of cytokinins induce a polar development and cytokinins in the presence of auxins induce an apolar proliferation. This rule holds also in other developmental programs tested where stand-by conditions, expansion and division were arranged in any sequence.

Cytological characteristics of the polarized and unpolarized state will be presented and the relevance for leaf morphogenesis will be discussed.

J5-217 INDUCTION OF EMBRYOGENESIS FROM MICROSPORES OF BRASSICA NAPUS BY MEANS OF COLCHICINE, Jiping Zhao[1], Daina H. Simmonds[2] and William Newcomb[1], [1]Department of Biology, Queen's University, Kingston, Ontario, Canada. [2] Plant Research Centre, Agriculture Canada, Ottawa, Ontario, Canada.

Isolated Brassica napus cv. Topas microspores develop as pollen when cultured at 25°C. However, sporophytic development can be triggered if microspores are initially treated at 32.5°C for 8-24 h. A haploid embryo frequency of 10-20% is obtained routinely. In order to evaluate the role of heat stress in changing the developmental pathway it was desirable to optimize an alternate way of inducing microspore embryogenesis. Colchicine, a microtubule depolymerizing agent has been found to be an effective inducer of embryogenesis at the non-inductive temperature of 25°C. Optimal embryogenesis was obtained by staging microspore isolations to contain at least 80% unicellular microspores and culturing with 25 µM colchicine for 42 h. Embryogenic frequency of over 10-15% can now be obtained with this method. This suggests that reorganizing the cytoskeleton can result in a developmental change. We are currently examining the role of heat shock proteins and cytoskeletal reorganization in altering microspore development.

Extracellular Matrix and Morphogenesis;
The Genetic Analysis of Morphogenesis

J5-300 THE GENETIC DISSECTION OF CAPITULUM DEVELOPMENT IN MICROSERIS (COMPOSITAE) VIA QTL MAPPING, Konrad Bachmann and Erik-Jan Hombergen, Hugo de Vries Laboratory, University of Amsterdam, Kruislaan 318, NL-1098 SM Amsterdam, The Netherlands.

The inflorescence of the Compositae is a compact capitulum in which the morphology of the individual florets and the seeds (achenes) is determined by the radial distance of their primordia from the rim of the head meristem. Floral organs therefore develop depending on their position on the floret and the position of the floret within the inflorescence. A cross between the diploid (2n=18) annual species *Microseris douglasii* and *M. bigelovii* incorporates relevant genetic differences between the two inbred parental strains, for instance ca. 12% vs. ca 17% peripheral, hairy achenes; 5 or more vs. 5 or fewer pappus parts; and 4 vs. 2 microsporangia in the anthers. An F2 of 106 plants has been intensively mapped with molecular markers (mainly RAPDs). Single genes involved in morphogenesis, especially in floret and capitulum determination are being mapped as "quantitative trait loci" (QTLs) relative to the molecular markers, and the interaction of the QTLs in the determination of the phenotype is being analyzed. Single major genes maintaining the number of anther loculi at a constant 4 and the number of pappus parts at a constant 5 have been demonstrated. In hybrid plants homozygous for the recessive gene permitting reduced numbers, the numbers become unstable, and, at least for pappus parts, subject to quantitative modifier genes, dependent on the position of the floret on the capitulum, responsive to the environment, and especially the age of the plant (consecutive number of the capitulum) . The genes determining the differentiation gradient across the capitulum (the percentage of florets with "peripheral" morphology) interact with genes determining capitulum size (by setting up a morphogen gradient in a growing meristem?). With a largely resolved genetic background and the availability of recombinant inbreds, the quantitative interactions determining the range of phenotypes that are expressed by the same genotype become available for detailed genetic and developmental analysis.

While the molecular marker map initially has been used to dissect phenotypic differences between the parental strains of the hybrid into single-gene effects, we are now also mapping homologs of genes involved in morphogenesis that have been cloned from other systems.

J5-301 THE MAIZE *crinkly leaf4* GENE IS INVOLVED IN THE MORPHOGENESIS OF KERNEL ALEURONE AND LEAF EPIDERMIS. Philip W. Becraft and Donald R. McCarty. Horticultural Sciences Dept., University of Florida, Gainesville, FL 32611

The maize *crinkly leaf4* (*cr4*) gene is required for aleurone differentiation and normal morphology of leaf epidermis. A mutant in *cr4* causes aleurone mosaicism. The pigmented parts of the kernel contain aleurone cells while unpigmented regions do not. The kernel appears otherwise normal. The distribution of aleurone is such that the regions containing aleurone cells are more prevalent on the germinal face of the kernel. This is the area where aleurone differentiation normally initiates suggesting that initiation is unaffected and that *cr4* may be involved in propagating a differentiation wave over the surface of the kernel. The mutant also causes wrinkling of the leaves by specifically affecting the morphology of the leaf epidermis. Epidermal cells are irregular in shape and size, while internal cells appear normal. In particular, the epidermal cells tend to be abnormally elongated perpendicular to the surface, and the surface wall tends to be irregularly shaped. All or most epidermal cell types appear to be affected. We are investigating when in development the phenotype first becomes apparent. Abnormalities in LI cells of the apical meristem could have implications for theories involving the influence of surface cells on organogenesis. The *cr4* mutation arose in a *Mutator* transposon line. We have identified two *Mutator* elements linked to the mutant phenotype and both elements are being cloned. A transposon mutagenesis screen has produced new alleles that will verify which fragment represents the *cr4* gene, and will provide additional information about *cr4* function.

J5-302 ENDOGENOUS RELEASABLE CELL WALL FACTORS CONTROL CELL FATE DURING ZYGOTIC EMBRYOGENESIS IN THE MULTICELLULAR ALGA *FUCUS*, Frédéric Berger and Colin Brownlee, Marine Biological Association, Citadel Hill, Plymouth, PL1 2PB (UK).

The two-celled embryo of the brown alga *Fucus* comprises a rhizoid and a thallus cell which are morphologically and cytologically distinguishable. Previous results from experiments using laser microsurgery (Berger *et al.*, 1994) have shown that the cell wall possesses inductive properties involved in embryogenesis. Partial and gradual enzymatic digestions of the cell wall of two-celled embryos orientated the development of the embryonic cells towards a generalized rhizoid development. Cell wall extracts derived from enzymatically treated embryos and applied to two-celled embryos also transformed thallus cells into rhizoid cells. Both enzymatic treatment and application of cell wall extract can trigger the overexpression of the rhizoid developmental program. This constitutes the first demonstration of an homologous diffusible inductive molecule implicated in plant embryogenesis. The cell wall is thus strongly implicated as a major source of position-dependent, fate-determining information in plant embryos. Current progress towards identification and purification of the active fate-determining factors is described.

Berger, F. Taylor, A. R. and Brownlee, C. 1994. Science 263: 1421-1423.

J5-303 THE *EMBRYONIC FLOWER* GENES OF ARABIDOPSIS DIRECT SHOOT DEVELOPMENT

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The *EMBRYONIC FLOWER* (*EMF*) genes of Arabidopsis are essential for vegetative rosette development. Two *EMF* genes have been identified and mapped to separate regions of chromosome 5. Mutations in both *EMF1* and *EMF2* result in a loss of rosette development. After germination, seedlings produce only cauline leaves and flowers on short inflorescences. The *EMF* genes exert effects throughout embryo, rosette, inflorescence, and flower development. The most severe *emf* mutants produce only abnormal carpelloid structures. Double mutants between *EMF1* and *EMF2* have no organized shoot development, suggesting that at least one *EMF* gene is needed for reproductive development, and both genes are needed for vegetative shoot development.

Genetic analysis with early- and late-flowering, floral meristem initiation, and homeotic flower mutants showed that 1) the rosette stage of development can not be rescued in *emf* mutants, 2) *EMF* and *TFL* may interact to affect the timing of floral bud formation, and 3) the regulation of the flower homeotic genes is altered in *emf* mutants. These results indicate that the *EMF* genes have a primary role in specifying rosette development and that they also play a role in inflorescence and flower development. In particular, *EMF* and *AGAMOUS* appear to have antagonistic roles in directing shoot development.

The occurrence of allele-specific phenotypes among *emf* mutants suggests that shoot development is controlled by a temporal gradient of *EMF* gene activities. We propose a model in which 1) high *EMF* activity in the embryo and rosette sustain the vegetative state, 2) genetic and environmental factors contribute to a reduction in *EMF* activity leading to a transition into reproductive development, and 3) *EMF* activity is lowest in flowers. To further test the interactions of *EMF* with other regulatory genes, we are analyzing expression of *LEAFY*, *AGAMOUS*, *PISTILLATA*, and the *APETALA* genes in *emf* mutants. In addition, we are using map-based cloning to isolate *EMF1*. Molecular characterization of the *EMF* genes will be instrumental in our understanding of the genetic regulation of shoot development in plants.

This work was supported by the USDA (93-37301-8704) and NSF (DCB-9105603).

J5-304 TRANSGENIC PLANTS EXPRESSING ptGRP1 ANTISENSE RNA EXHIBIT ABNORMALITIES IN VASCULAR TISSUE FORMATION, C. M. Condit and A. Meriin, Department of Biochemistry, University of Nevada, Reno, NV 89557

The petunia glycine-rich protein-1 (ptGRP1) gene was the first glycine-rich gene isolated from plants that could potentially code for a cell wall protein. Immunocytochemical studies, however, suggested that this protein was more highly associated with the cell membrane than the cell wall. Recent cell fractionation studies show that this protein is part of a > 40S 1% Triton resistant complex, whose extractability is increased in the presence of 0.5% phosphatidylcholine. *In vitro* translation studies have show that ptGRP1 possesses a true signal sequence. These latter studies, thus, exclude the cell wall and the internal face of the membrane as the location of the ptGRP1 complex.

ptGRP1 expression has previously been shown to be highest in the vascular tissue, and in particular the inner phloem. Expression of this protein is developmentally regulated, being higher in young than old tissue. Transgenic experiments have shown that the presence of ptGRP1 antisense RNA affects the development of the vascular system. These experiments show that ptGRP1 antisense RNA transgenic tissue is unable to organize vascular cells into a coherent vascular system. In this transgenic tissue, delineation of cortex or pith is not observed. Transgenic tissue that is capable of differentiating into a leaf and stem structure, either are chimeras (with some cells expressing ptGRP1) or express ptGRP1 incorrectly, wherein the protein is more highly expressed in old tissue than young. These latter type of plantlets, exhibit a hypertrophy of the vascular tissue, premature aging of the vascular system (lignin deposition), and invasion of phloem cells into the pith. Transgenic plants showing normal phenotype express ptGRP1 at high levels. These experiments suggest that ptGRP1 expression is involved with vascular tissue formation and development.

J5-305 IMPORTANCE OF SECRETED PROTEINS DURING SOMATIC EMBRYOGENESIS IN *PICEA ABIES*

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Norway spruce (*Picea abies*) is one of the coniferous species which can be propagated in vitro by somatic embryogenesis. The embryogenic cell-lines have different capacity for maturation, which is reflected in the morphology of the somatic embryos. Only cell lines composed of large, well organized embryos (type A) can form plantlets, whereas cell lines composed of small embryos with poorly organized structure cannot (type B). Aggregation of embryos is a common character for type A cultures and appears to be a prerequisite for formation of well-developed embryos. The morphological diffferences are stable over time under standard culturing conditions, but can be altered by factors secreted by the embryos to the medium. The type B embryos will develop a larger embryonic region and aggregate after addition of the total mixture of type A secreted proteins. Addition of extracellular type A arabinogalactan proteins (AGP) caused aggregation of the type B embryos. In suspension culture, the profile of secreted proteins studied by in vivo labeling experiments differ between type A and B cell lines. Crossed electrophoresis and immunological studies revealed differences in the sets of AGPs present extracellularly in type A and B. Furthermore, specific chitinases appear in correlation to embryo morphology and the distribution and importance of various types of chitinases were studied for their possible function during embryo development.

J5-306 THE MECHANISM AND REGULATION OF PHYTOENE DESATURASE DURING SEED DEVELOPMENT, Whitney E. Hable[1] and Karen K. Oishi[123], Committee on Genetics[1], Department of Molecular and Cellular Biology[2], and Department of Plant Sciences[3], University of Arizona, Tucson AZ, 85721

The growth regulator abscisic acid (ABA) is required for seed maturation and prevents the premature developmental switch from dormancy to germination. The goal of our experiments is to understand the mechanism and regulation of ABA biosynthesis during seed development. There are two peaks of ABA accumulation in developing seeds, one maternally and one zygotically derived. Using the biosynthesis of carotenoids (ABA precursors) as a biochemical marker for zygotic ABA synthesis, we are studying the spatial and temporal differences in carotenoid accumulation during maize seed development. Carotenoids and ABA accumulate in both the embryo and endosperm; however, endosperm levels are two to four-fold greater. Carotenoids are first detected in the embryo during late embryogenesis and in the endosperm during mid-maturation. In plants, phytoene desaturase (PDS) catalyzes the conversion of phytoene to phytofluene to ζ-carotene, two desaturation reactions which occur early in the pathway. In other systems the reaction catalyzed by PDS is the rate limiting step in carotenoid biosynthesis; we hypothesize that regulation of *Pds* is a critical regulatory component of carotenoid biosynthesis during seed development. Using molecular and genetic approaches, we are examining *Pds* expression at the transcriptional and post-transcriptional levels. The maize *Pds* homolog has been used to determine transcript levels in the embryo and endosperm throughout development. The PDS transcript is constituitively expressed in the embryo and developmentally regulated in the endosperm. We are using three mutants which accumulate phytoene and one which accumulates lycopene (a pathway intermediate that inhibits PDS activity) to identify the *Pds* locus and regulators of *Pds* expression.

J5-307 **DEVELOPMENTAL AND TISSUE-SPECIFIC DISTRIBUTION OF CELL WALL POLYSACCHARIDE EPITOPES IN WILD-TYPE AND MUTANT ARABIDOPSIS ROOTS**, M.G. Hahn[1], G. Freshour[1], W.-D. Reiter[2], C.C.S. Chapple[2], C.R. Sommerville[2], M.S. Fuller[1], P. Albersheim[1] and A.G. Darvill[1], [1]Univ. of Georgia, Complex Carbohydrate Research Center, Athens, GA 30602-4712; and [2]Michigan State Univ., DOE Plant Research Laboratory, East Lansing, MI 48824, USA.

The plant cell wall is a dynamic structure that has essential functions in plant growth and development, and in the interactions of plants with their environment and other organisms. We have generated several monoclonal antibodies that recognize different carbohydrate epitopes in plant cell walls. These antibodies have been used to localize the epitopes in roots of developing *Arabidopsis thaliana* seedlings. In wild-type roots, an epitope exclusively found in the pectic polysaccharide rhamnogalacturonan I (RG-I) is present only in the walls of epidermal and cortical cells, and then only in mature parts of the root, suggesting that different forms of RG-I exist in different cells. Terminal α-(1→2)-linked fucosyl residues, which are present in RG-I and xyloglucan, are detected in almost all walls throughout the root, and, interestingly, are confined to the outer three-fourths of the thicker external epidermal wall. An arabinosylated β-(1→6)-linked galactan epitope is present in all root cells except for the lateral root cap cells, and is localized to the plasma membrane-wall junction, the expected location of arabinogalactan proteins.

Roots of the *mur1* mutant have 40% less fucose than wild-type roots, due to a defect in the biosynthesis of fucose. The distribution of the fucosyl epitope in *mur1* roots is not simply reflective of an overall reduction in fucose. Although the root tip has a wild-type distribution, the fucosyl epitope is found only in the epidermis and pericycle of the more mature parts of the root. Thus, Arabidopsis roots have at least two genes controlling fucose biosynthesis that exhibit different temporal and spatial patterns of expression. The distributions of the RG-I and arabinogalactan epitopes are the same in *mur1* and wild-type roots.

These and other results demonstrate that carbohydrate epitopes are not uniformly distributed within the walls of individual cells or across walls separating different cell types. Furthermore, wall polysaccharide epitopes are distributed in a cell type- and developmental stage-specific manner. [*Supported by DOE grant DE-FG05-93ER20115 (to AGD), and, in part, by DOE grant DE-FG05-93ER20097 (to the CCRC).*]

J5-308 A SINGLE RECESSIVE MUTATION AFFECTING MERISTEM ORGANIZATION AND ORGAN DIFFERENTIATION OF THE SHOOT IN *ARABIDOPSIS*, Susan J. Lolle, Tae Hoon Kim and Ian M. Sussex, Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138

We have identified a mutant of *Arabidopsis*, designated *disorganized growth* (*dog*), where the organization, growth and development of all lateral organs of the shoot are abnormal. Leaves, for example, are highly irregular in shape, lack bilateral symmetry and are sectored. SEMs of leaf surfaces reveal that cells usually confined to either the adaxial or abaxial epidermal surfaces are intermixed between the two. Histological analyses reveal gross changes in mesophyll tissue organization as well as in the distribution and net volume of air spaces within mutant leaves. SEMs of the shoot apical meristems indicate that the phyllotaxy of leaf and flower bud initiation is irregular and that the transition to flowering is delayed and abnormal. Flowers range from phenotypically wild-type to mosaics having variable organ and cell composition. Based on these data, we suggest that cells in *dog* plants differentiate out of correct positional context due to a disassociation of cell autonomous development from non-cell autonomous organizational events.

J5-309 CELL WALL PHENOLICS AND PLANT REGENERABILITY, Lozovaya, V., Gorshkova, T.*, Yablokova, E.*, Zabotina, O.*, Azeeva, M.*, Rumyantseva, N.*, Waranyuwat, A., Widholm, J., Univ of IL, Dept. of Agronomy, 1201 W. Gregory, PABL, Urbana, IL 61801, USA; *Kazan Inst of Biology, Kazan P.O.B. 30, 420503, Russia.

Changes in the cell wall have been documented with *Zea mays* cultures where nonregenerable callus cell walls stained more with $KMnO_4$ than did the walls of regenerable callus (Duncan and Widholm 1989). When additional maize lines and different callus of buckwheat were stained with $KMnO_4$ the non-regenerable lines reduced more MnO_4 than did the regenerable lines. Cell walls isolated from the calli demonstrated similar reactions. During the cell wall fractionation, the walls still showed the reaction after pectin removal, but the reaction decreased markedly after extraction with 1N KOH. The precipitates obtained after neutralization of the KOH extracts from all cell types also reacted with $KMnO_4$. Since the KOH fraction should contain oligosaccharides and phenolics the differences between the regenerable and non-regenerable cells could be associated with differences in wall phenolic levels, structure and/or wall polymer linkages. Our ultrastructural histochemical observations showed that lignin (or other phenolics) was oxidized by $KMnO_4$ to produce the electron-dense MnO_2 present in both regenerable and non-regenerable buckwheat callus tissues but localized differently:being mainly, in the middle lamella in non-regenerable, and in cell corners in regenerable tissues. When the phenolaldehyde products of cell wall oxidation with $CuSO_4$ were analyzed by GC, some clear and repeatable differences were noted including the very high levels of vanillin in the regenerable callus walls in comparison with the non-regenerable walls. The compounds analyzed by GC were formed by cell wall oxidation so the high level of vanillin in the regenerable tissues could have resulted from, 1. a high level of coniferyl alcohol in the lignin, 2. a high level of ferulic acid in the cell wall or, 3. a high level of coniferyl alcohol glucoside in the cell wall. Ferulate esterified to wall polymers may be of special importance in providing the morphogenesis progression in plant cell and callus cultures.

J5-310 **A GENETIC APPROACH TO ANALYZE GAMETOPHYTIC DEVELOPMENT IN THE FLOWERING PLANT** *ARABIDOPSIS THALIANA*, **Simon Miséra (a), Martin Hülskamp (b), Gerd Jürgens (c), (a)Institute of Plant Genetics and Crop Plant Research, Correnssstr. 3, D-06466 Gatersleben, F. R. Germany, (b)Department of Developmental Biology, University of Tübingen, Spemannstr. 37-39, D-72076 Tübingen, F. R. Germany**

Genetic research on *Arabidopsis* has mostly concentrated on zygotic development. However, complex patterns of gene expression and cell division as well as morphological changes also take place after meiosis. This study aims at analyzing the postmeiotic development of the haploid gametophytes using mutants that lead to aborted or nonfunctional gametes.

Genes mutating to gametophytic lethality (*GPL* genes) must be essential to pollen or embryo sack development. Since a mutant *Gpl* gene will lead to the abortion of pollen grains or embryo sacks one is confronted with two practical problems. (1) Relying on morphological criteria only, mutant *Gpl* phenotypes are difficult to identify in large-scale mutagenesis experiments. (2) *Gpl* mutations are hard to keep over several generations because of poor postmeiotic transmission.

Taking advantage of markers that affect embryonic development, a new genetic screening system has been devised. It allows fast and reliable identification of *Gpl* mutations in M1 plants after mutagenesis of seeds. Moreover, newly induced *Gpl* alleles are automatically balanced in order not to lose the mutations in subsequent generations. Using this screening system, several *Gpl* mutations have been identified. The results demonstrate that searching systematically the *Arabidopsis* genome for *Gpl* mutations is indeed practicable.

J5-311 BIOSYNTHESIS OF PECTIN: CELL-FREE SYNTHESIS OF HOMOGALACTURONAN USING MEMBRANES FROM TOBACCO SUSPENSION-CULTURED CELLS, Debra Mohnen, Ron Lou Doong, Karen Liljebjelke and Gregory B. Fralish, Complex Carbohydrate Research Center and Department of Biochemistry and Molecular Biology, University of Georgia, 220 Riverbend Rd., Athens, GA 30605

Pectins are complex polysaccharides found in the primary wall of all plant cells. Pectin is believed to play critical roles in cell wall structure, in plant growth and development, and is a source of biologically active oligogalacturonides that regulate plant defense responses and development. The three pectic polysaccharides that have been identified are homogalacturonan, rhamnogalacturonan I and rhamnogalacturonan II. Specific carbohydrate epitopes on the pectic polysaccharides have been shown to have unique spatial distributions within the walls of different cell types, arguing for precise positional regulation of pectin metabolism during development. The structural role of pectins may be enhanced by the ability of homogalacturonan to form cross-linked gels in the presence of calcium ions and by the formation of junction zones due to columnar stacking of the methyl ester groups of methylesterified homogalacturonan. In an effort to understand the role of pectin in plant development we have identified and partially characterized the enzyme responsible for the synthesis of homogalacturonan: polygalacturonate 4-α-galacturonosyltransferase (PGA GalAtransferase, EC 2.4.1.43). PGA GalAtransferase activity has been identified in microsomal membranes from tobacco (*Nicotiana tabacum* L. cv Samsun) suspension-cultured cells. The incubation of UDP-[^{14}C]-galacturonic acid with tobacco membranes results in a time-dependent incorporation of [^{14}C]-galacturonic acid into a precipitable product. Synthesis of the product has a pH optimum of 7.8, a temperature optimum of 30°C, an apparent K_m for UDP-GalA of 10 μM. Analysis of intact product, product treated with base to remove ester linkages, and endopolygalacturonase (EPGase)-treated product by scintillation counting, TLC, and high-performance anion-exchange chromatography revealed that 35%-65% of the [^{14}C]-labeled base + EPGase-treated product can be hydrolyzed into mono-, di-, and trigalacturonic acid. This demonstrates that a large portion of the product is α-1,4-linked homogalacturonan. Maximum hydrolysis of the product requires base treatment prior to EPGase digestion indicating that some (~50%) of the product contains ester linkages that render it inaccessible to cleavage by EPGase. The results of experiments testing whether the base-sensitive linkage is a methyl ester will be presented, as will progress made toward solubilization of the enyzme. [Supported by USDA CSRS grant 94-37304-1103 and in part by DOE grant DE-FG09-93ER-20097.]

J5-312 EXPANSIN ACTION ON CELLS WITH TIP GROWTH AND DIFFUSE GROWTH, Richard C. Moore, Douglas Flecker, Daniel J. Cosgrove, Intercollege Program in Plant Physiology and Department of Biology, Pennsylvania State University, University Park, PA 16802

Expansins are a novel class of cell wall loosening proteins thought to catalyze cell elongation. Previous studies of expansin activity have been performed on heat killed cucumber hypocotyl segments, but not on living tissue. Here we examined the action of exogenous expansins on growing cells. Expansins added to the solution surrounding actively growing cucumber root hairs caused localized cell wall loosening at the tips of the hairs in a concentration dependent manner. High concentrations (3μg/ml) of expansins caused the majority of the root hairs to burst, spewing their cytoplasmic contents. As the concentration of expansins was decreased, bursting was suppressed and bulging occurred at the root hair tips. This bulging was transitory and normal tip growth resumed approximately 75 min after addition of expansins, evidently because the expansins were removed from the solution by binding to the wall. Western blot analysis suggests an expansin-like protein exists in root hair cell walls. We present a model of how expansins might be involved in localized tip growth. To test the action of exogenous expansins on diffuse growth, we used excised segments from the growing region of etiolated *Arabidopsis* hypocotyls. Expansins from cucumber walls greatly increased segment elongation (50% increase in 18 h, versus 10-15% for controls). The expansin-mediated elongation was as large as the maximal growth response to auxin (1 μM), but did not act additively or synergistically with auxin. Our results give the first demonstrations that expansins are able to influence the growth of living cells and lend support to the idea that expansins mediate a key step in plant cell enlargement.

J5-313 **THE MAIZE HOMEOTIC GENE *GLOSSY15* IS A MEMBER OF THE *APETALA2* GENE FAMILY**

Stephen P. Moose and Paul H. Sisco, Department of Crop Science, North Carolina State University, Raleigh, NC 27695.

Many plants exhibit heteroblasty, where the vegetative meristem sequentially gives rise to juvenile and adult leaves with distinct developmental identities. In maize, juvenile and adult leaves are characterized by the alternate expression of a set of morphological, physiological, and cellular characteristics. Many of these traits are specific to the epidermis. These include the production of epicuticular waxes, changes in cell wall biochemistry, and the differentiation of specialized cell types such as leaf hairs and bulliform cells. Recessive mutant alleles of the maize *Glossy15* (*Gl15*) locus condition the homeotic conversion from a juvenile to an adult epidermal identity in leaves 3 through 6. Analysis of a *dSpm*-induced mutable allele (*gl15-m1*) has demonstrated that *Gl15* coordinately promotes juvenile traits (waxes, acidic cell walls) and suppresses adult cellular differentiation (bulliform cells, epidermal hairs) in a cell-autonomous fashion. The *dSpm*-tagged gene was cloned, and the predicted gene product shows extensive similarity to the *Arabidopsis APETALA2* (*AP2*) gene. *AP2* encodes a novel regulatory protein which is required for the specification of sepal/petal identities and suppression of stamen/carpel differentiation in the outer two whorls of the flower. The similarities between these two genes in both their amino acid sequence and mode of action suggest that epidermal cell identity in the maize leaf and floral organ identity in *Arabidopsis* may be regulated via common mechanisms. The relevance of proposed models for the control of flower development in *Arabidopsis* to leaf heteroblasty in maize will be discussed.

J5-314 THE EXPRESSION AND FUNCTION OF THE *Arabidopsis Athb-1* GENE DURING PLANT DEVELOPMENT. Giorgio Morelli[1], Takashi Aoyama[2], Chun-Hai Dong[3], Sabrina Lucchetti[1], Monica Carabelli[4], Giovanna Sessa[4], I. Ruberti[4] and Nam-Hai Chua[3]. [1]Ist. Nazionale della Nutrizione, Rome, Italy, [2]Inst. for Chemical Research, Kyoto University Japan, [3]The Rockefeller University, N.Y., [4]Centro di Studio per gli Acidi Nucleici, CNR Rome, Italy.

The *Arabidopsis Athb-1* gene is a member of a gene family, whose proteins contain a homeodomain followed by a leucine zipper motif. The expression analysis revealed that the *Athb-1* mRNA is present in all the plant tissues, with the higher expression in root and flower. The expression increases in the leaf with the age of the plant and in dark adapted plants.
The analysis of the DNA binding properties of Athb-1 *in vitro* showed that the HD-Zip domain binds DNA as a dimer and it recognizes a pseudopalindrome of two 5 bp half sites that overlap on a central position. Moreover, we have found that Athb-1 behaves as a strong transcriptional activator in shooting experiments with tobacco leaves. In order to investigate the function of Athb-1 in plant development, we constructed a series of modified *Athb-1* genes which were driven by the CaMV35S promoter. In a tobacco transgenic system, some of the chimeric genes specifically affected the development of the palisade parenchyma.
Based on the expression analysis in *Arabidopsis* and in transgenic tobacco plants, the function of Athb-1 will be discussed.

J5-315 ARG-GLY-ASP-SER (RGDS) DISRUPTS MICROTUBULES BUT NOT MICROFILAMENTS IN FERN PROTONEMATA OF ADIANTUM CAPILLUS-VENERIS L., K. Nozue and M. Wada, Department of Biology, Faculty of Science, Tokyo Metropolitan University, Minami-osawa 1-1, Hachioji, Tokyo 192-03, Japan
Microfilaments (MFs) in animal cell are linked to the extracellular matrix (ECM) via transmembrane protein, called integrin. On the other hand, in plant cell interaction between cortical microtubules (MTs) and the plasma membrane is thought to be important in regulation of orientation of cortical MTs, but its molecular basis is unknown. Here we show that 1.1 mM Arg-Gly-Asp-Ser (RGDS), which is a well-known inhibitor of the interaction between ECM and the plasma membrane in animal cells, disrupts MTs but not MFs in fern protonemata. When cells were treated with RGDS for 1 hr, MTs became shorter and appeared to be thicker in part, so that they exhibited wavy and dendric form. The number of filamentous MTs were reduced. This disruption occurs within 0.5 hr after application of the peptide, but is reversible, so that the organization of MTs returns to normal 2 hr after removal of RGDS. In contrast to the changes of appearances of MTs, RGDS treatment did not change the organization of MFs. Long strands of MFs were visible even after 1 hr treatment with RGDS. Similar to the effects of RGDS on animal cells, the substitution of D (Asp) into E (Glu) abolished the disruption of MTs. These data suggest that the cortical MTs, but not MFs, may be linked to the cell wall by transmembrane proteins (such as integrin in animal cells) via the RGD-sequence.

J5-316 THE ISOLATION AND CLONING OF A GENE CONTROLLING PLANT MORPHOLOGY, Valerie B.O'Leary, Thomas F.Gallagher, Graham Wilson and Martin Steer, Department of Botany, University College Dublin, Dublin, Ireland.
The basic morphology of a plant is established during early embryogenesis.The early development of somatic embryos was studied through the development of carrot somatic tissue culture cells.The embryo progresses through several morphologically distinguishable stages i.e. *globular, heart-* and *torpedo*-shaped stages.Somatic embryos were used to identify,isolate and characterize genes which are specifically expressed during the early stages of their development.Genes which are expressed during embryogenesis were identified through subtractive library construction and by the use of subtracted cDNA probes enriched for sequences expressed in embryonic developmental stages.This initially involves the biotinylation of messenger RNA from pre-embryonic cellular masses.This messenger RNA is hybridized to first strand cDNA made from early staged embryonic messenger RNA. Streptavidin and phenol extraction were used to separate the cDNA:mRNA hybrids from the non-hybridized cDNA.These non-hybridized cDNA's represent specific embryonic gene sequences. Second strand cDNA was synthesized, ligated into a vector and then transformed.Recombinant embryo specific clones were highlighted through hybridization studies. A clone containing a 2Kb. insert has been found which hybridizes strongly to mRNA isolated from developmentally advanced embryos i.e. Plantlets and not to earlier stages.Sequence data will highlight the precise nature of the gene.

J5-317 NEW MODEL FOR THE INVESTIGATION OF GENETIC CONTROL OF IN VITRO MORPHOGENESIS, Orlov P.A., Mavrischeva E.B., Palilova A.N., Institute of Genetics and Cytology BAN, Skorina str. 27, Minsk 220734, Belarus

In vitro cultivation of plant anthers results in induction of morphogenesis in new direction. Genetic mechanisms of this process have not been studied enough and genome and plasmon effects on morphogenesis in tissue culture are one of the most interesting aspects of this problem. We have created a new model system that gives a possibility for more detailed investigation of nuclear and cytoplasm effects. This model includes 20 alloplasmic lines that have been developed during direct and reverse backcrossing of 5 wheat species (T. aestivum, T. durum, T. dicoccum, T. turgidum, T. aethiopicum). The following parameters have been studied during anther culturing: number of responsible anthers, embryoid yield, formation of different callus types, development rate of morphogenetic processes and so on. It has been found that the highest levels of responsible anthers and green regenerants were observed in T. dicoccoides (4,31 and 0,86% respectfully), T. dicoccum (5,04 and 0,26%), T. aestivum, variety Myronovskaya (5,24 and 2,47%), alloplasmic lines (durum)-T. aestivum (4,16 and 7,00%), (durum)-T. dicoccum (8,47 and 3,45%). Cytoplasmic effects have been revealed with respect to callus and embryoid formation and covering of early morphogenesis stages. Nuclear cytoplasmic interaction was the most significant for embryoid yield. Formation of friable embryogenic calli that had high growth rate was found in some alloplasmic lines.

J5-318 CONDITIONAL SCREENS FOR MERISTEM MUTANTS OF *ARABIDOPSIS* F. Bryan Pickett, Michele Marino Champagne and Ry Meeks-Wagner, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.

All structures seen in mature plants, including leaves, stems and flowers, develop post-embryonically from a stem cell population residing within the apical meristem. Leaves and flowers begin development as primordia which arise on the flanks of the meristem. We are using a genetic approach to identify genes which regulate meristem function by screening for temperature sensitive mutations which cause an arrest of new organogenesis at the meristem. Temperature sensitive mutations provide the benefits of ease of propagation of what would otherwise be lethal mutations and the ability to determine temperature sensitive periods for gene function throughout development.

Screens of 115,000 plants have yielded 36 independently isolated mutations which cause a temperature sensitive arrest in post embryonic development. Of several phenotypic classes identified by this screen, I have chosen to concentrate on a class of mutations which causes a temperature dependent arrest of organogenesis and disorganization of the meristem. One of these mutations, called *indecisive(ind)*, is caused by gene disruption and co-segregates with a molecular tag that will facilitate the molecular analysis of this gene. The *IND* gene appears to be required throughout development for meristem function, as temperature shifts during germination, vegetative growth and flowering all cause temperature sensitive phenotypes. Seven of the other mutations identified in the screen resemble some aspects of the *indecisive* phenotype and are the current focus of epistasis studies with *ind*. The combination of genetic pathway analysis of the *indecisive* like mutations and the molecular cloning of the *IND* gene will provide a basis for an understanding of the regulatory mechanism underlying meristem structure and function.

J5-319 GENETICS AND MORPHOGENESIS OF *dks8*, A SHOOTLESS MUTANT OF MAIZE, Carol Rivin, John Sollinger, Dan Strom and Kris Hardeman, Department of Botany and Plant Pathology, Oregon State University, Corvallis OR 97331-2902

We have identified a mutation in maize, termed *dks8* (defective kernel shootless-*Mu8*), that is an excellent candidate for a pattern formation gene for the earliest establishment of a functional shoot meristem. At maturity, *dks8* embryos completely lack the components of the embryonic shoot system. All other aspects of the embryo and endosperm are small, but morphologically normal. Mutant seeds have a normal constellation of maturation proteins, they are desiccation tolerant, and capable of germinating the primary root.

To determine when the *dks8* gene acts, we compared the development of *dks8* and wildtype sibling embryos. The two genotypes are indistinguishable in early phases of development, but mutants clearly deviate beginning at transition stage. At this point, wildtype embryos change in conformation from the radially symmetrical proembryo to bilateral symmetry, and the shoot apical meristem becomes apparent. Mutant embryos slowly shift to bilateral symmetry and never form a histologically identifiable shoot apical meristem. We are now using antibody to the Knotted protein to determine if there are any cells in the *dks8* embryo with a shoot apical meristem identity.

The *dks8* mutation was isolated from an active transposon stock and it co-segregates with a *Mu8* transposon. We have found what appear to be two derivative alleles of *dks8* that produce embryos having a range of displaced and abnormal shoot morphologies. Using the *Mu8* element as a tag, we are working to map and clone the *dks8* gene.

J5-320 THE ROLE OF THE *TOUSLED* PROTEIN KINASE GENE DURING *ARABIDOPSIS* FLOWER DEVELOPMENT, Judith L. Roe and Patricia C. Zambryski, Dept. of Plant Biology, University of California, Berkeley, CA. 94720

Mutations at the *TOUSLED* locus in Arabidopsis cause a complex phenotype affecting the morphology of most of the organs in the plant. Flowers produced in homozygous *tsl* plants form less than normal numbers of floral organs , and the gynoecium is split. Scanning electron microscopy analysis of early *tsl* floral meristems suggested that the *TSL* gene acts early in flower development and affects the overall morphology of the floral meristem. The shape of the floral meristem itself is more highly domed than wild-type, suggesting that meristem size may be affected. Double mutants were made with two mutations which increase meristem size in *Arabidopsis*, *clavata1* and *clavata3* (seeds kindly provided by E.M. Meyerowitz). The phenotypes of the double mutants with *tsl* appear to be additive, suggesting that *TSL* does not interact with these loci. Possibly, the size of floral meristems is reduced in *tsl*, and this is being analyzed by confocal microscopy. The development of the carpel primordia in *tsl* flowers is abnormal from a very early stage, and the gynoecium at maturity lacks a fused style, and only forms small patches of stylar epidermis and stigmatic tissue. Histological analysis of the ovary domain in *tsl* plants does not reveal any abnormal arrangement of cells when compared to wild-type gynoecium. Regions of stylar, stigmatic, and transmitting tract tissue are formed in *tsl* gynoecium, but are highly reduced in size. The phenotype of double mutants of *tsl* and other mutations affecting gynoecium development in *Arabidopsis* has revealed a role for *tsl* during the differentiation of these tissues. The double mutant of *tsl* and *ettin* results in the almost complete ablation of style, stigma, and carpel wall tissues, and only a small placenta bearing several ovules is formed in the center of the flower. Outer-whorl carpels formed in the homeotic mutation *apetela2* often form a broad band of style and stigmatic tissue on the margins of these carpels, with ovules being formed on a submarginal placenta. In the double mutant of *tsl* and *ap2*, the margins of the outer-whorl carpels show only patchy development of these "apical" cell types, as is found in *tsl* gynoecia. Furthermore, double mutants of *tsl* and *flo-89* show an almost complete absence of style and stigmatic tissue, although these tissues do form in *flo-89* flowers as a protusion from the medial sides of the apex of the gynoecia. Thus, *TSL* may be required for full elaboration of apical tissues in wild-type carpel development.

J5-321 CELL WALL AUTOLYSIS DURING FRUIT RIPENING: VISUALIZATION OF CALCIUM IONS AND PECTIN SEQUENCES.

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In fleshy fruits pectin solubilization and degradation occur simultaneously during the ripening and senescence processes. These reactions, which occur in the cell wall, lead to partial separation of cells in the pericarp and result in the expansion of the intercellular spaces. The separation of cells, which results in a loss of tissue cohesion during ripening, is a form of cell wall autolysis that illustrates the concept of "responsive mosaic" as proposed by Carpita and Gibeaut (1993, Plant J. 3:1).

The present study utilized two different approaches to investigate cell wall autolysis. First, anionic sites of the galacturonic acid were labelled with cationic colloidal gold. More specifically, the unesterified homogalacturonic sequences were labelled with the monoclonal antibody JIM5 and the esterified homogalacturonic sequences with the monoclonal antibody JIM7. These results were monitored with fluorescence, light and electron microscopy. In the second approach, analytical detection of calcium ions was performed by secondary ion mass spectrometry (SIMS). With SIMS, native calcium was localized *in muro* and could be discriminated from other calcium isotopes based on mass to charge ratios. The results from these studies indicated that cell wall architecture changed during fruit ripening. The different labeling patterns obtained at different stages of ripening suggested that controlled deesterification occured in the cell wall. The persistent triangular maps, which were obtained with SIMS, outlined opposing walls of the intercellular spaces and suggested that calcium rich zones were retained even in the ripe fruit. When ^{44}Ca was exogenously applied to visualize those triangular junctions having low calcium content, isotope ratio images distinguished two types of cell wall intersects opposing the intercellular spaces: those with a high ability for calcium uptake and others with low ability. Finally, pectic microdomains were affinodetected within the cell wall during the ripening process.

These data further document that autolysis of the cell wall is affected by calcium, junction zones and pectins.

J5-322 **THE *NAM* GENE OF *PETUNIA HYBRIDA* IS INVOLVED IN SHOOT APICAL MERISTEM FORMATION AND FLOWER DEVELOPMENT AND ENCODES A NOVEL CLASS OF PROTEINS.**

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We have isolated several mutants in plant and flower development by random transposon tagging. To this end we used the highly mutable *Petunia hybrida* line W138 that contains more then 200 copies of the non-autonomous transposable element *dTph1* (Gerats et al. (1990) Plant Cell 2, 1121-1128). One of the mutants, the *nam* (*no apical meristem*) mutant, failed to develop an apical meristem during embryogenesis. Cotyledons of the seedling are fused at their base and variably positioned. Seedlings occasionally developed an adventitious meristem on the hypocotyl. Plants developing from this adventitious meristem bore flowers with various abnormalities such as the development of extra petals in the second whorl of the flower. To reveal possible interactions with the floral homeotic gene *gp* (*green petals*) we constructed the double mutant with *nam*. In the double mutant again extra organs develop in whorl 2. Furthermore, the appearance of sepals in the second whorl of this double mutant seems to cause the development of extra organ primordia in whorl 3. Formation of the carpel is aberrant as only the placenta with ovules develop. We cloned the *nam* gene by a newly developed method based on IPCR amplification and differential hybridisation of flanking sequences of *dTph1*. To prove the identity of this clone we isolated three independent *dTph1* insertions in the *nam* gene by a PCR based method, all of them giving the same phenotype. Southern analyses showed that the *nam* gene belongs to a multigene family. By database searches we could not reveal homology with any proteins with a known function indicating that the *nam* gene seems to encode a new class of proteins. To our knowledge this is the first report of the cloning of a vital gene of plant embryogenesis.

J5-323 MOLECULAR ANALYSIS OF THE PRP FAMILY OF EXTRACELLULAR MATRIX PROTEINS, Mary L. Tierney, Elizabeth Lee and Thomas Fowler, Department of Botany, University of Vermont, Burlington, VT 05405

While it is clear that cell wall structure changes in response to both developmental and environmental signals, the critical components responsible for these changes are poorly understood. My laboratory is investigating the developmental and wound-induced expression of a family of proline-rich structural cell wall proteins (PRPs) that are thought to contribute uniquely to cell wall matrix structure in specific cell types. We have previously shown that there is a strong interaction between the developmental and wound-induced expression of two soybean PRPs (SbPRP1 and SbPRP2). We are currently analyzing a series of promoter/β-glucuronidase fusions in transgenic tobacco for for these two proteins in order to characterize the relationship between the sequences necessary for their cell-type specific developmental expression and their expression in response to physical damage. In addition, we are testing whether a putative auxin-responsive element present in the SbPRP2 promoter is necessary and/or sufficient for SbPRP2 expression in wounded seedlings. We have also isolated four *Arabidopsis* PRP genes that separate into two distinct subgroups based on DNA sequence identity, repetitive motifs, conserved intron location and domain organization. The expression of one subgroup (AtPRP1 and AtPRP2) is linked to root development while the other subgroup (AtPRP2 and AtPRP4) is expressed in leaves, shoots and flowers. This co-segregation of PRP expression and domain organization suggests different functions for these extracellular matrix proteins during growth. To test this, we are re-introducing antisense constructs for each of these genes into *Arabidopsis*. The goals of these studies are to analyze the physiological consequences linked to an alteration in the level of specific PRPs within the extracellular matrix during growth.

J5-324 MOLECULAR GENETICS OF THE SOYBEAN *I* LOCUS WHICH INHIBITS PIGMENT SYNTHESIS IN A SPATIAL MANNER, Joselyn J. Todd and Lila O. Vodkin, Plant and Animal Biotechnology Laboratory, Department of Agronomy, University of Illinois, Urbana, IL 61801

Control of the patterns of gene expression is important to differentiation and morphogenesis. We are studying the *I* locus in soybean which determines the presence or absence as well as the spatial distribution of anthocyanin pigments in the epidermal layer of the developing seed coat. The dominance relationships of the four alleles are $I > i^i > i^k > i$; the i allele conditions a self colored seed coat, i^k and i^i restrict pigment to the saddle and hilum regions, respectively. Recently, we have shown that chalcone synthase (CHS) mRNA levels and activity are reduced in yellow soybean seed coats with dominant *I* alleles (Wang, Todd, and Vodkin, Plant Physiol. 105: 739-748). Thus, the biochemical basis of yellow seed coats in soybean appears to be a block in the flavonoid pathway at the CHS step. We are currently determining which of seven CHS genes in soybean are expressed in the developing seed coat using primer extension analysis with gene specific primers and cloning reverse transcriptase-PCR products. Multiple restriction fragment length polymorphisms (RFLPs) are found in near-isogenic lines that contain different *I* alleles. We show that these CHS RFLPs cosegregate with the seed color phenotype indicating that CHS and the *I* locus are closely linked. A unique genetic resource is the existence of a large number of independent spontaneous mutations from the dominant *I* (yellow) or i^i (yellow seed coat with black hilum) alleles to the recessive *i* (self color) genotypes. Southern blot analysis of these isogenic pairs revealed that large structural changes in the CHS RFLP patterns occur in mutations from $I \rightarrow i$ and $i^i \rightarrow i$. Using PCR, we have mapped some of these changes to a 10 kb region containing three tandemly linked CHS genes. These data indicate a complex but intimate association between the *I* alleles and CHS gene structure and expression. The paradox remains of how the changes in CHS gene structure lead to reduction in the amount of CHS mRNAs in a trans-dominant manner.

J5-325 GENETIC AND MOLECULAR INTERACTIONS OF CELL WALL PROTEINS AND THE FLAVONOID PATHWAY,

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Two genes, *Prp1* and *Prp2*, encode proline-rich proteins of the cell wall. PRP1 is found in young seed coats and PRP2 is found later during seed desiccation. In some soybean varieties, both proteins are smaller as determined by immunoblotting. We found that *Prp1* and *Prp2* genes are linked by 13% recombination. The molecular basis for the allelic size differences in the PRP polypeptides is the existence of genetic length polymorphisms that result in omission of some of the repeat units (pro pro val tyr lys) from the proteins (Schmidt, Lindstrom, and Vodkin, *Plant Journal* 6: 177-186, 1994). In *Prp1*, there were two separate deletions in different parts of the gene, each being two tandem repeats in length. In *Prp2*, there was only one deletion of two tandem repeats. The nature of these genetic length polymorphisms indicates that the basic repeat unit structure is conserved and that the proline-rich proteins of the cell wall tolerate loss or gain of integral numbers of repeat units.

In addition to the qualitative variation of the cell wall proteins, the dominant *I* gene of the anthocyanin pathway affects the abundance of *Prp1* polypeptides during seed coat development. The dominant *I* (inhibitor) gene blocks expression of the anthocyanin pathway by a 90% reduction in the amount of chalcone synthase mRNAs and activity leading to yellow seed coats (Wang, Todd, and Vodkin, Plant Physiol. 105: 739-748, 1994). In contrast, the levels of soluble polypeptides and mRNAs for *Prp1*, a proline-rich cell wall protein, are 50% less in pigmented isolines with the homozygous recessive *i* genotype. Seed-coat structure is also defective in the pigmented seed coats of isolines that carry the double recessive *it* genotype, whereas *iT* pigmented lines have intact seed coats. The *T* locus putativelyencodes a flavonoid 3' hydroxylase that affects the types of anthocyanins and proanthocyanidins. The epistatic interaction of the *it* gene combination implies an interaction between the flavonoid pathway and wall structure in the seed coats.

J5-326 **EFFECT OF CALCIUM-ALGINATE IMMOBILIZATION ON POTATO PROTOPLAST DIVISIONS AND COLONY FORMATION**

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High frequency of protoplast divisions and plant regeneration from protoplast-derived colonies are necessary for the study of plant morphogenesis *in vitro*. Mesophyll protoplasts were isolated from four potato (*Solanum tuberosum* L.) cultivars. Different culture conditions for potato protoplasts were tested: 1) protoplast immobilization in thin Ca-alginate layers and cultivation in liquid nutrient medium, 2) plating in agarose blocks and cultivation in the liquid medium, 3) cultivation in the liquid medium. Calcium-alginate immobilization resulted in a 3-fold increase of the fraction of divided cells compared to culture in the liquid medium without cell immobilization, and had higher frequency of protoplast divisions than in agarose blocks. Potato microcalli immobilized in alginate gel had a compact structure, were more viable and contained less necrotic tissue than those grown in liquid medium. Alginate entrapment of potato protoplasts was preferable for successful plant regeneration. The advantage of protoplast immobilization in Ca-alginate is also confirmed in the plating efficiency test on the solid medium, where potato microcalli grown immobilized had a high and stable plating efficiency of 94%($\pm$4%). Microcalli grown in liquid medium had a lower and considerably more variable plating efficiency of 50%($\pm$20%). Improved cell survival was due to protection of immobilized cells against strong gradients in environmental conditions. Cell immobilization in alginate gel reduced diffusion of cellular metabolites away from the cells and led to maximum in conditioning effects, that significantly increased cell divisions and colony formation. Therefore, the effects of Ca-alginate immobilization on potato cell divisions provide a convenient system for the study of plant development *in vitro* from individual cells to whole plants.

J5-327 ARE HOMEODOMAINS INVOLVED IN THE TERMINAL STEP OF TUBERIZATION PROCESS?,

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Patatin, a tuber specific storage protein, has been used extensively as a biochemical marker to study the process of tuberization, as it accumulates rapidly during the early stages of tuber development. Thus investigating the mechanisms that regulate patatin gene transcription may lead to elucidation of the developmental signals that govern tuberization.

The fact that patatin can be induced in tissues other than tubers under sink limitation or by application of certain metabolites leads to the dissection of the complex tuberization event into two distinct but interconnected processes, a morphogenetic one and a biochemical one.

Analysis of the patatin promoter revealed a minimal promoter that is still able to confer tuber specificity and sucrose inducibility and encompasses three different classes of *cis* regulatory elements. The first is an AT-rich HMG like binding site which appears to have an inhibitory effect in tissues where the gene is not normally expressed. The second element is reiterated at several places in the promoter and is able to confer sucrose inducibility when is fused in a chimeric promoter.The mechanism of induction by sucrose and the metabolites that can act as intermediates in this regulatory pathway is under investigation.

The third element which is the main subject of our study, has been identified by *in vivo* footprinting and is identical to a homeodomain binding site in the promoter of *pr2* gene in parsley. DNA binding activities present in tuber nuclear protein extracts were found to interact specifically with this particular element. The existence of the homeodomain binding site in the patatin promoter raises the possibility that the tuber specific factor(s) could be a homeodomain transcription factor. In addition data from transgenic potatoes demonstrated the important role of this *cis* element in the tuber specific expression . Further experiments are in progress in order to clone the putative tuber specific homeodomain protein(s) and to elucidate its function.

Signal Transduction and Morphogenesis; Hot Topics

J5-400 **AN ARABIDOPSIS MUTANT WITH A DEFECT IN CELL ELONGATION AND AN APPARENT LIGHT-REGULATORY PHENOTYPE.**
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A number of dwarf mutants are currently under investigation in our laboratory. *dwf4*, the focus of this study, attains a height of <3 cm. at five weeks, whereas wild-type plants are >25 cm. All organs in the mutant plants are shorter than normal. In addition, development is slower in this mutant: both flowering and senescence are significantly delayed. Microscopic analysis shows that the small size of *dwf4* plants is due to a defect in cell elongation rather than a reduction in cell number. The mutation severely reduces the response to applied gibberellic acid and blocks hypocotyl elongation in phytochrome deficient *dwf4*, *hy* double mutants. *DWF4* is therefore proposed to be necessary for normal cell elongation as a response to various stimuli. As an additional effect of the mutation, dark-grown seedlings on agar exhibit opening of the cotyledons and initiation of leaf expansion. There is no additional light-regulatory phenotype, however, as shown by EM analysis of stomates and plastids, as well as by northern blots probed with light-inducible sequences. The apparent light-regulatory defect is most likely a result of the proximity of the meristem to the agar surface due to the short stature of the seedlings. We show that even wild-type seedlings grown in the dark in contact with the agar can produce leaves and flowers, as has been shown for liquid-grown plants.

J5-401 **Transposon tagging and characterisation of the tomato dwarf gene.** Gerard Bishop, Kate Harrison, Colwyn Thomas, James Keddie and Jonathan Jones. The Sainsbury Laboratory, Norwich Research Park, Colney Lane, Norwich, NR4 7UH. U.K.

A linked targeted tagging experiment of the tomato dwarf gene generated 20 dwarfs amongst 5000 progeny. Seventeen appeared to be a clonal event (d_1) whereas the other three were from two independent events (d_2, d_3). In F_2 populations of both d_1 and d_2 lines a recessive extreme dwarf phenotype segregated. This phenotype is similar to the reference stock d^X which is a strong allele of *dwarf*. Germinal and somatic reversion has been observed in F_2 progeny from the d_2 line; whereas the d_1 lines show minor somatic reversion. The two d_3 dwarf plants have reduced fertility, suggesting that they may have arisen from a large deletion in the dwarf region or some other chromosomal rearrangement. IPCR and DNA sequencing reveals that in both d_1 and d_2 the transposon has inserted into the same region of DNA. Sequence analysis of the wild type *dwarf* gene and other dwarf alleles will be discussed. Complementation was achieved using a genomic clone and phenotypes of plants containing both anti-sense and sense constructs will be reported.

J5-402 A PLASMODESMATA ASSOCIATED PROTEIN ISOLATED FROM *CHARA CORALLINA* IS ALSO FOUND IN HIGHER PLANTS, Leila M. Blackman[1], Robyn L. Overall[1] and Brian E. S. Gunning[2], [1]School of Biological Sciences, University of Sydney, 2006, Australia, [2]Plant Cell Biology Group, Research School of Biological Sciences, Australian National University, Canberra, 2601, Australia
Plasmodesmata provide symplastic connections between plant cells. In this study we have used the giant celled freshwater alga, *Chara corallina*, to identify putative plasmodesmatal proteins. In *Chara* it is possible to separate walls which do not contain plasmodesmata, that is the side walls of the giant internodal cells, from those that do in the nodal complexes separating the internodal cells. Four nodal specific wall proteins have been identified by comparing protein extracts from each wall type using SDS-PAGE. The relative molecular masses of these proteins are 58, 27, 26 and 20 kDa. Monoclonal antibodies to the 27 kDa *Chara* protein recognise higher plant cell wall proteins. Immunofluorescence studies using these antibodies show punctate labelling on the walls of adjoining cells in *Chara* nodes and in cauliflower florets and pea roots. This punctate labelling co-localises with aniline blue induced fluorescence of callose consistent with the distribution of plasmodesmata. These antibodies do not co-localise with callose at the sieve plates in pea roots, suggesting it is not specific to callose synthesis, but rather some other plasmodesmata protein.

J5-403 **OSCPK2 AND OSCPK11, TWO NEW MEMBERS OF THE RICE CDPK FAMILY, SHOW SPECIFIC FEATURES,** Diego Breviario, Daniela Giussani, Anna Maestroni and Silvia Gianì, Istituto Biosintesi Vegetali CNR, Via Bassini 15, 20133 Milano, Italy.
We have recently cloned two rice cDNAs (OSCPK2 and OSCPK11) encoding for putative Calcium-dependent Protein Kinase (CDPK) proteins. OSCPK2 and OSCPK11 predicted protein sequences are 533 and 542 amino acids long with corresponding molecular mass of 59,436 and 61,079 respectively. Structure of these two proteins is typical of recently described plant CDPKs with a kinase catalytic domain linked to a calmodulin-like regulatory domain through a sequence of 31 aa that has been recently shown to act as pseudosubstrate inhibitor. Junction domain sequences of predicted OSCPK2 and OSCPK11 proteins are highly homologous to the corresponding sequences in Arabidopsis AK1 and soybean CDPKα proteins. 4 EF-hand calcium binding loops are present in the calmodulin-like regulatory region of OSCPK2 whereas just one fully conserved EF-hand amino acid motif is observed in OSCPK11 deduced gene product. Comparison of the NH2-terminal sequence of predicted OSCPK2 and OSCPK11 proteins with that of another rice CDPK (SPK) indicates the presence of a conserved MGXXC(S/Q)XXT motif that, particularly for OSCPK2 where Ser is present at position 6, may define a consensus signal for N-myristoylation. OSCPK2 and OSCPK11 encode for different size transcripts (2.4 and 3.5 kb long respectively) that are also differentially regulated. In fact, a long white light treatment or a shift to anoxia causes, in rice coleoptiles, a significant decrease in the amount of OSCPK2 mRNA with almost no effect on the level of OSCPK11 transcript. Additional data on possible tissue and developmental specific transcriptional regulation will be presented. These studies should help to define the role that these kinases may play during rice development and in response to different external signals.

J5-404 CYTOKININ-TREATED TOMATO PLANTS SHOW MORPHOLOGICAL TRAITS OF THE AUXIN-INSENSITIVE *diageotropica* MUTANT

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The *diageotropica* (*dgt*) mutant of tomato is a single-gene, recessive mutant with a complex pleiotropic phenotype. Hypocotyl segments of *dgt* are insensitive to exogenous auxin in elongation and ethylene-synthesis assays, which has led to the proposal that the primary effect of the *dgt* mutation is to reduce the sensitivity of the tissue to auxin via an altered perception mechanism (Kelly and Bradford, 1986, Plant Physiol. 82: 713-717). It is unclear, however, whether a defect in auxin perception can explain all aspects of the pleiotropic *dgt* phenotype. Cytokinins counteract auxin effects in many physiological systems. We have investigated to what extent *dgt* morphology and physiology can be phenocopied through the application of cytokinins. Wild type tomato plants watered with benzyladenine (BA), a synthetic cytokinin, exhibited the reduced leaf complexity, dark green color, enhanced anthocyanin production, retarded senescence of the cotyledons, reduced root system and stunted growth which is typical of *dgt* plants. Pretreatment of wild type hypocotyl sections with BA reduced their response to auxin in elongation assays. Adventitious root formation in young *dgt* seedlings was delayed and reduced. Low concentrations of cytokinins mimicked this behavior in wild type seedlings. Current investigations of systems in which auxin and cytokinin are presumed to act synergistically (e.g. ethylene biosynthesis) should help decide whether cytokinin hypersensitivity or overproduction can explain the auxin-insensitivity in *dgt* or whether the defect in auxin perception leads to changes in cytokinin metabolism or sensitivity.

J5-405 FUNCTIONAL ANALYSIS AND BINDING PROPERTIES OF A *CIS*-ELEMENT IN THE NAPIN PROMOTER, Inés Ezcurra, Mats Ellerström, Kjell Stålberg and Lars Rask, Department of Cell Research, Swedish University of Agricultural Sciences, Box 7055, S-750 07 UPPSALA, Sweden.

Expression of seed storage protein genes in the developing *Brassica napus* embryo follows a defined temporal and spatial pattern, involving subsequent onsets of gene transcription in different sets of cells. The timing of these transcriptional onsets is believed to reflect early cellular events related to the establishment of apical meristems in the developing embryo (Fernandez et al.,1991, Development 111, 299-313).

As a step towards studying the mechanisms by which storage protein genes are regulated during embryogenesis, we are analyzing the promoter of the 2S storage protein gene *napA*. Previous work by our group has shown that a region between -152 and -126 is crucial for this promoters ability to drive reporter gene (GUS) expression in transformed tobacco plants. Here we show the existence of two functional elements within this portion of the *napA* promoter. The most distal (5') element is essential for quantitative expression while the second element is involved in repression in non embryonic tissues. Mobility shifts and competition studies show that at least two different factors bind to this region *in vitro*. Furthermore, we have detected binding activity to these elements in cauliflower apical meristems and are able to enrich it by affinity binding using magnetic beads. Ongoing research involves further characterisation and affinity purification of this factor.

J5-406 INFLUENCE OF AUXIN ON EMBRYONIC POLARITY DURING EARLY MONOCOT EMBRYOGENESIS. Fischer Ch., Neuhaus G., Institute of Plant Sciences (Dept. of Prof. I. Potrykus), ETH - Zürich, Universitätstrasse 2, CH - 8092 Zürich, Switzerland.

Our aim is to gain information on the signals influencing early plant embryogenesis. In particular, we focus our attention on the mechanisms underlying the shift from radial to bilateral symmetry during early monocot embryogenesis. The model plant chosen for this purpose is wheat (*Triticum aestivum* L.). In a first approach, *in vitro* culture conditions for very young (globular to early transition) zygotic embryos have been established. By this method, up to 70 % of the cultured embryos will develop through normal direct embryogenesis. The pattern of development of these embryos mimics the *in planta* development. In a second approach, we have performed experiments, in which we studied the influence of auxins on the attainment of the embryonic symmetry from the radial symmetry during embryogenesis. For this purpose, auxin as well as antiauxins have been added to the culture medium. Two auxins have been used, such as 2,4,5 T (2,4,5-trichlorophenoxyacetic acid) and 2,4 D (2,4-dichlorophenoxyacetic acid). Two classes of antiauxins have been tested: the auxin polar transport inhibitor TIBA (2,3,5-triiodobenzoic acid) and PCIB (2-(p-chlorophenoxy)-2-methylpropionic acid), a compound that is assumed to have an antagonistic effect. The auxins as well as the auxin transport inhibitor affect the embryonic symmetry, the scutellum and the shoot apical meristem differentiation. The phenotypes obtained are dependent on the concentration used and on the developmental stage of isolated embryos. By contrast, the auxin antagonist has no visible effect on embryonic symmetry. These observations indicate that auxins influence the change from the radial symmetry to the embryonic polarity during early monocot embryogenesis.

J5-407 CLONING OF AUXIN-INDUCED GENES FROM LOBLOLLY PINE, *Barry Goldfarb, *Ross Whetten and *Wesley P. Hackett, *Department of Forestry, North Carolina State University, Raleigh, NC 27695, *Department of Horticultural Science, University of Minnesota, St. Paul, MN 55108.

We are studying adventitious root formation in stem cuttings of loblolly pine. Endogenous or exogenous auxin is necessary, but not sufficient, to stimulate root meristem initiation. Cuttings taken from very young plants root readily, whereas cuttings from mature plants are recalcitrant. We are interested in: (1) elucidating the molecular events that are triggered by the presence of auxin and result in root meristem organization, and (2) determining how this pathway differs in cuttings from juvenile and mature plants. It is likely that a critical step in this pathway will be the synthesis and/or activation of transcriptional regulators in response to auxin treatment. A class of auxin-responsive genes has been cloned from annual plants that are rapidly transcribed after auxin treatment, have structural similarities to transcriptional regulators and encode short-lived nuclear proteins (e.g. PS-IAA4/5 in Abel et al, 1994, Proc. Natl. Acad. Sci. USA 91:326-330). We designed degenerate PCR primers with homologies to regions conserved among this class of genes from pea, soybean, mung bean and *Arabidopsis*. The primers were used to amplify an internal fragment from single-stranded cDNA synthesized from mRNA purified from hypocotyl cuttings of loblolly pine seedlings 10 minutes after treatment with 1.6 mM 1-naphthaleneacetic acid for 5 minutes. The fragments were cloned and 11 recombinants chosen for further study. To date, four inserts have been sequenced and each appears to be unique. All have some homology with the auxin-responsive genes mentioned above, although this is a tentative conclusion, because the internal fragment does not span large portions of the conserved domains. We are currently using the cloned fragments as probes to identify full-length cDNAs of these genes and will use them in studies of gene expression during root formation in pine cuttings.

J5-408 LIPO-CHITIN-OLIGOSACCHARIDES (LCOs) AS ENDOGENOUS ORGANOGENESIS SIGNALS OF THE PLANT. Eric Kamst, Dimitris Kafetzopoulos, Ben J. J. Lugtenberg, Herman P. Spaink, Institute of Molecular Plant Sciences, Leiden University, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

The recent experimental evidence that the rhizobial nodulation signals (1), termed Nod factors, can influence the developmental processes in non-leguminous plants suggests that endogenous lipophilic chitin-oligosaccharides (LCOs) are involved in plant development (2). This hypothesis is consistent with the observation that the expression of several plant chitinases correlates with the normal development of plant organs and embryos, suggesting that these chitinases are involved in the release of endogenous chitin-like signal molecules.
Assuming that the enzyme activities involved in the biosynthesis of the plant chitin-oligosaccharide signals are similar to the biosynthetic enzymes of Nod factors in Rhizobium, we have used the observed significant similarities of the rhizobial NodC protein to the eukaryotic chitin synthases for the isolation of its plant homologue.
We have designed PCR primers which recognize nodC and its homologues from Xenopus and Myxobacterium. These primers are now being used to isolate homologous genes from plants.

1. Spaink et al., (1991), Nature, 354, 125-130.
2. De Jong et al., (1993), Plant Cell, 5, 615-620.

J5-409 SYSTEMIC ACCUMULATION OF PHOSPHATIDIC ACID AND LYSOPHOSPHOLIPIDS IN WOUNDED TOMATO AND BROAD BEAN PLANTS
Youngsook Lee, Sujeoung Suh, Sumin Lee, Richard C. Crain, Department of Life Science, Pohang University of Science and Technology, Pohang, Korea (YL, SS, SL), Department of Molecular and Cell Biology, The University of Connecticut, Storrs, CT, 06269 (RCC).

Plants establish defence systems upon exposure to pathogens or physical wounding by herbivores. Many lipid compounds are used as defence molecules or as mediators of defence response. We tested if physical wounding activates phospholipases which can facilitate defence establishment. We wounded the first leaves of tomato or broad bean seedlings at 2 leaf stages with a plier. Lipids were extracted, separated on TLC, and the phospholipids were analyzed. Wounding elevated phosphatidic acid (PA) level within 5 min in the non-wounded second leaf as well as in the wounded first leaf, indicating systemic activation of phospholipase(s). Lysophospholipids (LPL) level also increased in wounded tomato leaves and broad bean leaf disks. Our results show that a wound signal is propagated to the outside of the wounded leaf within 5 min in plants, and it activates phospholipases which can produce some important precursors of the defence molecules or second messengers in wound signal transduction of plants.

J5-410 CALMODULIN INVOLVEMENT IN POLARISATION OF *FUCUS SERRATUS* ZYGOTES. John Love and Anthony J. Trewavas, Institute of Cell and Molecular Biology, The University of Edinburgh, King's Buildings, Mayfield Road, Edinburgh, EH9 3JH, Scotland.

Zygotes of the Fucales (Phaeophyceae) have been widely used as a model system for the study of plant cell polarity. Early zygotes are apolar. During the first cell cycle, a gradual cytoplasmic re-organisation replaces the initial spherical symmetry by a highly polarised morphology.

Gradients of the Ca^{2+} binding protein, calmodulin, have been observed in the growing tips of polarised plant cells such as root hairs and pollen tubes. Moreover, calmodulin is essential for budding in *Saccharomyces cerevisiae*, and localises to the future bud site where it is thought to interact with localised microfilaments.

Both Ca^{2+} and microfilament localisations have been linked to polarity in Fucoid zygotes, but data regarding calmodulin has been lacking.

We have shown that Ca^{2+} and calmodulin are essential to polar formation during the short time when *Fucus serratus* zygotes are polarised by light (10-13 hours after fertilization). We hypothesise that localised calmodulin activity is part of the signal transduction pathway between light stimulus and cytoplasmic polarisation. Microinjections of recombinant calmodulin from the related species *Macrocystis pyrifera* are being performed in polarising *F. serratus* zygotes to address this hypothesis.

J5-411 NPA BINDING ACTIVITY IS PERIPHERAL TO THE PLASMA MEMBRANE AND IS ASSOCIATED WITH THE CYTOSKELETON, Gloria K. Muday and Daniel N. Cox, Department of Biology, Wake Forest University, Winston-Salem, NC 27109
N-1-naphthylphthalamic acid (NPA) binding activity is released into the supernatant, upon treatment of plasma membranes with high salt. This release indicates that NPA binding activity is peripherally associated with the membrane. Extraction of plasma membrane vesicles with Triton X-100, resulted in retention of the NPA binding activity in the detergent-insoluble cytoskeletal pellet. This detergent-insoluble pellet was shown to contain cytoskeletal elements by fluorescence microscopy and immunoblot. Treatment of this pellet with KI released the majority of NPA binding activity from the pellet into the supernatant. KI treatment was shown to release actin and α-tubulin monomers by immunoblot. Dialysis to remove KI led to the repolymerization of cytoskeletal elements and movement of NPA binding activity, actin, and α-tubulin into an insoluble cytoskeletal pellet. NPA binding activity partitioned into the detergent-insoluble cytoskeletal pellet obtained from both zucchini and maize membranes and was released from these pellets by KI treatment. Treatment of a cytoskeletal pellet with cytochalasin B doubled NPA binding activity in the resulting supernatant and released both actin and α-tubulin.. Together these experiments indicate that NPA binding activity is peripherally associated with the plasma membrane and interacts with the cytoskeleton in vitro.

J5-412 IMMUNOLOCALIZATION OF AGL-E, A MADS-DOMAIN PROTEIN EXPRESSED DURING EMBRYOGENESIS, Sharyn E. Perry and Donna E. Fernandez, Department of Botany, University of Wisconsin, Madison, WI 53706.

cDNA clones encoding a MADS-domain protein, AGL-E, expressed during embryogenesis were recently isolated in our lab from *Brassica napus* and *Arabidopsis thaliana*. Because the MADS-domain family includes transcription factors that play critical roles in developmental decisions, an evaluation of the function of the gene product of *AGL-E* in embryogenesis could yield important new insights into that process. Determination of the temporal and spatial pattern of protein accumulation is crucial to this understanding. To this end, antibodies that specifically recognize the nonconserved portion of AGL-E downstream of the MADS-domain were produced by immunization of rabbits with purified protein obtained by overexpression in *E. coli*. These antibodies have been used to examine the pattern of expression of *AGL-E*, both at a whole tissue level by Western analysis and at a cellular level by immunolocalization. The antibodies recognize a single protein species present in developing *B. napus* embryos, inflorescences and flower buds, but not in leaf tissue. Expression of *AGL-E* was also examined in the precocious germination system of *Brassica napus*, in which the identity of organs produced at the apex can be manipulated. The presence of AGL-E correlates with "embryonic identity" of the developing organs (*i.e.* cotyledon). Finally, evolutionary conservation of this protein has been examined by Western analysis. A cross-reacting protein has been found in a variety of dicots as well as in the monocot *Zea mays*, suggesting an important role conserved throughout evolution in the flowering plants.

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J5-413 GENETIC EVIDENCE FOR A LONG-RANGE ACTIVITY THAT DIRECTS POLLEN TUBE GUIDANCE IN *ARABIDOPSIS THALIANA*, Robert E. Pruitt, Martin Hülskamp and Kay Schneitz, Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138

The fertilization process of plants is governed by different kinds of cell-cell interactions. In higher plants these interactions are required both for recognition of the pollen grain by the female reproductive system and to direct the growth of the pollen tube inside the ovary. Despite many years of study, the signaling mechanisms that guide the pollen tube toward its target, the ovule, are largely unknown. Two distinct types of principles, mechanical or chemotropic, have been suggested to account for the directed growth of the pollen tube. The first of these two types of models implies that the guidance of the pollen tube depends on the architecture and chemical properties of the female reproductive tissues, whereas the latter suggests that the ovule provides a signal for the target directed growth of the pollen tube. To examine such a role for the ovules, we analyzed the growth path of pollen tubes in mutants defective in ovule development in *Arabidopsis thaliana*. The results presented here provide unique *in vivo* evidence for an ovule-derived, long-range activity controlling pollen tube guidance. A morphological comparison of the ovule mutants used in this study indicates that within the ovule the haploid embryo sac plays an important role in this long-range signaling process.

J5-414 SUPPRESSORS OF AN *ARABIDOPSIS phyB* (PHYTOCHROME B) MUTATION

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Under conditions of natural shade, many plants grow taller and flower earlier than in direct sunlight. In *Arabidopsis thaliana*, this "shade avoidance" response has been shown to depend on the red/far-red light photoreceptor phytochrome B (phyB). PhyB is one of a family of phytochromes in *Arabidopsis*, and normally represses elongation and flowering until inactivated by light having a low red:far-red ratio (characterisitic of natural shade). *phyB* mutants have lesions in the gene for the phytochrome B apoprotein, and consequently grow tall and flower early whatever the light conditions. In order to identify other genes involved in controlling elongation and flowering responses, we have embarked on a screen for genetic suppressors of the elongated hypocotyl phenotype caused by a *phyB* mutation. From 15,000 M2 progeny of EMS-mutagenized *phyB* seed, we have isolated 12 candidate mutants. Some of these mutants appear dwarfed, suggesting that the mutations affect some process required for cell enlargement. Other mutants appear similar to wild-type plants, and may have mutations in light signal transduction or some other environmental control of morphology. We have separated the suppressor mutations (called *shy*, for short *hy*pocotyl) from the starting *phyB* mutation, and are analyzing the growth responses to light of the resulting lines. These experiments should allow us to determine which mutants have defects in phytochrome signal transduction, and which have defects in other aspects of plant morphogenesis.

J5-415 INJECTED ANTIBODIES TO ANIMAL VINCULIN, ANKYRIN, TALIN, SPECTRIN AND INTERMEDIATE FILAMENTS FORM PUNCTATE ARRAYS CONNECTED BY A FINE "LACEWORK" IN LIVING ONION CELLS, Christophe Reuzeau, Keith W. Doolittle, James G. McNally and Barbara G. Pickard, Biology Department, Washington University, Saint Louis, MO 63130

We have postulated that "plasmalemmal control centers" similar to animal focal adhesions help integrate plant cell activities (Aust J Plant Physiol 20:439). If so, some homologies between proteins at such sites in plants and animals are predicted. Computational optical sectioning microscopy has already evidenced vitronectin-, fibronectin-, and integrin-like antigenicities in punctate array at the interface between wall and plasmalemma of onion cells (Biophys J 66:A169). We here extend that study to some antigenicities inside the plasmalemma. Cells of epidermal strips were maintained at roughly *in planta* turgidity by floating on 250 mM mannitol with dilute salts. Some samples were brought to "incipient plasmolysis" by increasing mannitol to 350 mM. Monoclonal antibodies to animal cytoskeletal proteins were conjugated with fluorochromes and injected into the cytoplasm of selected cells, which were subsequently optically sectioned. With suitable point spread functions, images were reconstructed both by linear least squares and maximum likelihood methods. These images conformed with predictions from raw data and from images deblurred by Gaussian filtering. Several techniques were used for 3-D viewing, including formation of stereo pair images. In cells treated with ~250 mM solution, antibodies to vinculin, talin and intermediate filaments were each covisualized with $DiOC_6$. All antibodies accumulated in a punctate array of bright patches under the plasmalemma. $DiOC_6$, which stains membranes maintaining a large transmembrane voltage drop (eg ER), also lit the patches. Between the bright patches, the antibodies and $DiOC_6$ co-traced fine "lines", "spots", and "rings" or "vesicles". $DiOC_6$ could also be observed in adjacent cells, making clear that injection altered its distribution and halted cytoplasmic streaming. In cells treated with ~350 mM solution, the punctate sites (tagged with the above antibodies plus antiankyrin and covisualized with antispectrin) tend to organize in circles of variable size where the plasmalemma pulls away from the wall. Speculatively, an actin net links the patches, rupturing during plasmalemmal detachment and tugging them into circles. It should be possible to image all the structures in 4-D during physiological challenge.

J5-416 THE REGULATION OF CALCIUM DURING MOSS DEVELOPMENT, Karen S. Schumaker, Michael J. Gizinski, & John S. Rohwer, Department of Plant Sciences, University of Arizona, Tucson, AZ 85721

In plants, calcium (Ca^{2+}) has emerged as the principal ionic intermediate in signal transduction, bridging both the temporal and spatial gaps that occur between stimulus perception and specific growth responses. Cellular Ca^{2+} levels are tightly controlled and small fluctuations in intracellular Ca^{2+} levels provide energy changes that can be used as molecular information. To study plant signal transduction, we are looking at the role of Ca^{2+} as an intracellular messenger in moss development. In the moss *Funaria hygrometrica*, induction of buds begins with the accumulation of the phytohormone bryokinin, an adenine-type cytokinin. A localized increase in Ca^{2+} is an intermediate in this cytokinin-induced bud formation. The immediate goal of our research is to understand how Ca^{2+} levels are regulated in the moss to allow stimulus-induced changes in cellular Ca^{2+} levels. We have characterized a dihydropyridine (DHP)-sensitive Ca^{2+} transport system in the moss plasma membrane by looking at influx of $^{45}Ca^{2+}$ into moss protoplasts. Ca^{2+} influx was stimulated by external K^+, indicating that transport is voltage-dependent. K^+-induced influx was DHP-sensitive with > 50% inhibition at 500 nM nifedipine. Ca^{2+} influx was stimulated by increasing concentrations of the DHP Ca^{2+} agonist BayK 8644 with half-maximal effects at 25 nM; this stimulation was seen only in the absence of K^+. The phytohormone 6-benzylaminopurine consistently stimulated Ca^{2+} influx with a Km of 1 nM. We are characterizing the binding of [^{3}H]azidopine (an azidolabeled DHP) to isolated plasma membranes to understand the interaction of the Ca^{2+} channel with DHPs. Azidopine binding is cation-stimulated, and DHP-specific binding (the difference in binding in the absence and presence of unlabeled nifedipine) is increased in the presence of K^+. We are also isolating molecular probes to look at the temporal and spatial distribution of the channel during bud formation. Degenerate oligonucleotide primers made to conserved regions of the DHP-sensitive Ca^{2+} channel from animal cells have been used to amplify a portion of a putative DHP-sensitive channel from moss. The fragment has been cloned and is currently being sequenced.

J5-417 ZINC-FINGER PROTEIN GENE FAMILY OF PETUNIA; POSSIBLE ROLES IN FLOWER DEVELOPMENT, Hiroshi Takatsuji[1], Norihisa Nakamura[2] and Yukihisa Katsumoto[2], [1]Department of Plant Physiology, National Institute of Agrobiological Resources, Tsukuba, Ibaraki, Japan. [2]Suntory Research Center, Osaka, Japan.

Several genes that control floral organ development have been cloned from Arabidopsis, Antirrhinum, Petunia and some other plants. An excellent model (ABC model) explains the control of organ identities by the homeotic genes. Most of these genes code for putativetranscription factors, MADS box protein. Probably, there is a hierarchical network of transcription factors functioning in the regulation of floral-organ formation.

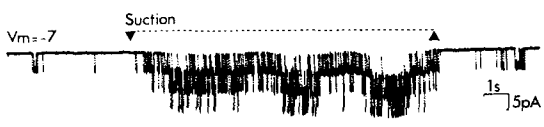
We have previously reported cloning and characterization of the genes for a family of Cys_2/His_2-type zinc-finger proteins (EPF) of Petunia. Further expression analysis revealed that there is an interesting relationship in the expression patterns of these genes and floral homeotic genes. *EPF2-5* gene is primarily expressed in petal and stamen (B region), *EPF2-7* is in sepal and petal (A region) and *EPF1* is in the petal (overlapping area of A and B regions). The onset of the *EPF* expression was later than those of the homeotic genes. Consensus binding sequence of the MADS box protein were found in the promoter region of *EPF1* and *EPF2-5*. These observations suggest that the *EPF*'s are acting downstream of the homeotic genes in the transcriptional hierarchy. Histochemical analyses using promoter-*GUS* fusion revealed that the expression of *EPF2-5* was in all cell types in the upper part of limb and gradually became restricted to upper epidermis cells towards bottom. The expression of *EPF2-7* was specific in phloem in sepal and petal. The limited localization of these expression suggest that the EPF genes plays more specialized roles than those of the homeotic genes in the flower development.

Progenies of the transformants with the *EPF2-5* promoter-GUS chimeric gene showed abnormal phenotypes in inflorescence and floral organs. In typical plants, the inflorescence gave only one distorted terminal flower at the top of the inflorescence instead of normal sympodial growth, implying possible roles of the EPF2-5 in the flower development.

J5-418 ION CHANNELS AND SIGNAL TRANSDUCTION IN THE POLARISING *FUCUS* ZYGOTE, A.R.Taylor and C.Brownlee, Marine Biological Association, The Laboratory, Citadel Hill, Plymouth, PL1 2PB, U.K.

Ion channels play a key role in stimulus-response coupling in plant cells. The polarised *Fucus* zygote is an ideal model system with which to investigate the spacial signalling mechanisms in a plant cell, in particular, regulation and modulation of ion channels during photopolarisation, growth and developmental responses. In order to address the role of plasma membrane channels in the development and maintenance of polarity in *Fucus*, localised patch clamp recordings are necessary. UV laser microsurgery has been developed and refined to enable plasma membrane patch clamp recordings from localised regions of polarising or polarised cells. At least two inward K^+ channels have been identified. Stretch activation and modulation of inward K^+ channels has been observed in membrane patches from both rhizoid and thallus regions of the zygote. K^+ channel activity in cell attached configuration was seen to increase significantly if the patch was subject to suction, reducing on cessation of the suction stimulus (fig 1). These stretch-activated channels are present in the plasma membrane of undivided and two cell zygotes in both rhizoid and thallus locations. Channels permeable to Ca^{2+} have been observed. The implication of a stretch modulated cationic channel permeable to Ca^{2+} in polarised apical growth in *Fucus* will be discussed.

Figure 1. Stretch-activated inward K^+ channels in the *Fucus* plasma membrane.



J5-419 EFFECTS OF NEOMYCIN AND FLUOROALUMINATE (AlF_4^-) ON GA_4- AND IAA-INDUCED REORIENTATION OF CORTICAL MICROTUBULES IN CUCUMBER HYPOCOTYL EPIDERMAL CELLS, Young M. Woo and Susan M. Wick, Department of Plant Biology, University of Minnesota, St. Paul, MN 55108

Using immunofluorescence microscopy, we have investigated whether well-known cellular signaling molecules such as G-proteins, phospholipase C, and protein kinase C are involved in the reorientation of cortical microtubules (cMTs) that is induced by treatment of cucumber hypocotyl segments or peels with GA_4 and subsequently with IAA. Neomycin (an inhibitor of phospholipase C), AlF_4^- (an activator of G-proteins), and 1,2-dioctanoyl glycerol (an activator of protein kinase C) were employed along with GA_4 and IAA, which normally induce transverse cMTs and concomitant cell elongation. Treatment with 1 mM neomycin alone significantly increased the number of oblique and longitudinal cMTs in the epidermal cells. Neomycin followed by AlF_4^- or by GA_4+IAA produced extensive bundling and irregularity in orientation of cMTs, whereas neomycin after AlF_4^- or after GA_4+IAA only slightly inhibited the reorientation of cMTs to the transverse orientation. The effects of neomycin on cMTs were partially reversible by subsequent treatment with 1,2-dioctanoyl glycerol. These preliminary results showing that neomycin has a striking effect on the structure and orientation of cMTs suggest (1) phospholipase C is involved in the signal transduction pathway that leads to reorientation of cMTs; (2) sequential interaction possibly exists among G-proteins, phospholipase C, and protein kinase C; (3) the induced bundling and irregular orientations of cMTs are not a result of toxicity of the treatments; and (4) AlF_4^- produces similar effects as GA_4 + IAA, even though its mode of action most likely differs from those of GA_4 and IAA.

Late Abstracts

GENETIC ANALYSIS OF CELL EXPANSION IN ROOTS OF ARABIDOPSIS; Marie-Theres Hauser[1] and Philip N. Benfey[2], [1]Center of applied Genetics, Universität für Bodenkultur, A-1180 Vienna, Austria, [2]Department of Biology, New York University, New York, N.Y. 10003, U.S.A.

Cell expansion is a key parameter in cell and organ development in plants. The *Arabidopsis thaliana* root is particularly well suited to the study of cell expansion. Major reasons are the simple architecture of the root and that cell expansion develops in a continuum along the length of the root.

We have employed a genetic approach to identifying the genes that regulate cell expansion in roots. We screened for mutants with abnormal root expansion. Some of these mutants have been described previously (Benfey *et al.*, 1993, Development **119**:57-70). Here, we present the genetic and cellular characterization of mutants representing a new locus (designated pleiades (*ple*) mutants). Three alleles were isolated. All exhibit short, thick roots with a wavy growth pattern. The alleles differ in the rate of root growth. Transverse sections through the roots revealed an irregular radial expansion of the two outer root tissues, cortex and epidermis. Since cell enlargment in flowering plants is correlated with nuclear DNA content we analysed the nuclei of the mutant roots. This analysis revealed that the expanded cells are polyenergid containing several clustered nuclei.

Since plant cells have a rigid cell wall which excludes the possibility of fusion of different cells to create polyenergid cells, these polynucleate cells probably result from a mitotic cycle with nuclear division (karyokinesis) but without cell division (cytokinesis). Therefore these mutations affect a gene involved in the regulation of cytokinesis and cell plate formation.

Further cellular and molecular characterizations are currently under way and the results will be presented.

TRANSPOSON TAGGING OF A FLORAL INDUCTION LOCUS WITH *Ds*, M. Honma[1,2], C. Waddell[1], and B. Baker[1] [1]Plant Gene Expression Center, U.S.D.A., Albany, CA 94710 and [2]Department of Botany, Duke University, Durham, NC 27708

We have established an efficient transposon tagging system in *Arabidopsis* based on the maize transposon family *Activator/Dissociation (Ac/Ds)*. Our system has two components: a stable *trans*-activator, Ac^{st}, and a *cis*-responsive *Ds* element. *Ds* transposes to new sites when *trans*-activated by Ac^{st} but is stabilized when Ac^{st} segregates away from *Ds*. *Ds* and Ac^{st} elements carry different selectable and screenable markers with which to monitor *Ds* transposition. *Ds* carries an *ALS* gene, that confers resistance to the herbicide chlorsulfuron . The Ac^{st} constructs carry a GUS marker that enables the identification of those plants in which a transposed *Ds* is stable. Ac^{st} transposase expression is driven by one of three strong promoters: the 35S promoter from CaMV, or promoters from the *Arabidopsis rbcS* or *CHS* genes.

Our results show that Ds^{ALS} transposes at a high frequency in *Arabidopsis*. *Trans*-activation of Ds^{ALS} by Ac^{st} resulted in germinal excision frequencies of up to 64% using 35S transposase fusions, up to 67% using *rbcS* transposase fusions, and up to 1% using *CHS* transposase fusions. Induction of the *CHS* transposase fusion with high intensity light increased the germinal excision frequency up to 45%. In general, the germinal excision and reintegration frequencies observed with 35S-Ac^{st} lines were somewhat higher than with *rbcS*-Ac^{st} and *CHS*-Ac^{st} lines. However, the patterns of *Ds* reintegration catalyzed by the Ac^{st} constructs was different. Sibling plants from 35S-Ac^{st} and *CHS*-Ac^{st} crosses often carried the same transposed Ds^{ALS}, while each plant from *rbcS*-Ac^{st} crosses represented a unique transposition event. These results suggest that *Ds* transposition in *rbcS*-Ac^{st} crosses occurred relatively late in development.

50 lines carrying transposed *Ds* elements were screened on agar plates and in soil for visable mutant phenotypes. From this general mutant screening, a dwarf and an early flowering mutant were found. These mutant phenotypes arose after *Ds* transposition. The early flowering *(eaf20)* mutant flowers 5 weeks earlier than wild type (10 weeks) when grown under short day conditions. Genetic and molecular studies suggest that the *eaf20* gene is tagged with Ds^{ALS} and allelism tests with other early flowering mutants are in progress.