MOLECULAR BIOLOGY OF PLANT GROWTH CONTROL

J. Eugene Fox and Mark Jacobs, Organizers

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Keynote Address

F0 THE HYDROXYPROLINE-RICH GLYCOPROTEINS OF PLANTS, J.E. Varner, Department of Biology, Washington University, St. Louis, MO 63130

Higher plants have at least three kinds of hydroxyproline-rich glycoproteins.[1] Two of these, the extensins and the arabinogalactanproteins, are localized in the extracellular matrix. Both the extensins and the arabinogalactanproteins are coded by multigene families and for both gene expression appears to be tissue specific. Wounding [2,3], infection[4], oligosaccharide elicitors[5], and ethylene[5] increase gene expression and/or accumulation of the extensins. One extensin molecule from aerated carrot slices[3] is well characterized as is one extensin gene from carrots[6]. The most abundant tryptic peptides of two different extensins from tomato contain the sequence $-SerHyp_4-$[7]. Base sequences encoding the $-SerPro_4-$sequence occur repetitively in the carrot genomic sequence, in a tomato partial genomic sequence and in tomato and petunia cDNA sequences. The hydroxylation and the glycosylations that convert the proline-rich peptide product of the extensin gene into the hydroxylated and glycosylated extensin monomer are only partially characterized. The details of how the secreted extensin monomers are associated with each other and with other cell wall components to comprise the wall architecture remain to be discovered. The amino acids (lysine, tyrosine, histidine and threonine) which occur frequently in the extensin sequences between the $-SerPro_4-(SerHyp_4-)$ repeats have sidechains rich in functional groups that could form electrovalent, reversible covalent, and irreversible covalent links with other wall components.

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Hormone Modulated Gene Expression — I

F1 HORMONAL, GENETIC AND ENVIRONMENTAL REGULATION OF ENZYME SYNTHESIS IN THE ALEURONE LAYERS OF CEREAL GRAINS,
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The synthesis of two groups of α-amylases and a group of thioproteinases in barley and wheat aleurone layers is regulated by gibberellic acid (GA), abscisic acid (ABA), trans-acting genetic elements and environmental stress conditions. The GA-induced α-amylase consists of two groups of isozymes which are encoded by two different sets of structural genes. The high pI isozyme group is induced by the hormone within 3-4 hr and reaches a maximum at 16 hr before declining. In contrast, the low pI isozyme group is preexisting at a low level yet its synthesis is enhanced by GA. The enhanced synthesis of low pI isozymes plateaus at 16 hr and remains high for another 20 hr. The timecourse of GA induction of thioproteinases appears to be very similar to that of the low pI α-amylase. The synthesis of amylase isozymes corresponds with the levels of their specific mRNA indicating a potential transcriptional regulation. In addition, the α-amylase mRNA are very stable in GA treated tissue probably due to their association with the GA-induced ER. When the cells are under heat stress there is a concomitant destruction of ER and decline of the stability of mRNA encoding secretory proteins such as α-amylase and proteinase. The effect of ABA on the inhibition of α-amylase synthesis is dependent on the transcription of other genes. Transcription inhibitors such as cordycepin prevent the action of ABA. It has been shown that ABA induces several new proteins. The timing of the ABA induction of these proteins correlates with the self-induced conversion of ABA to phaseic acid. Since phaseic acid is as active as ABA on the inhibition of α-amylase synthesis we suggest that phaseic acid is the active component of ABA action in aleurone layers.

F2 STUDIES ON GIBBERELLIN AND ABSCISIC ACID ACTION IN BARLEY ALEURONE CELLS, John V. Jacobsen, Division of Plant Industry, Commonwealth Scientific and Industrial Research Organization, Canberra, A.C.T. 2601 Australia

Gibberellic acid (GA_3) promotes and abscisic acid (ABA) inhibits the synthesis of α-amylase in barley aleurone cells and this system has been used extensivey to study the mechanisms of action of both hormones.

The α-amylase consists of a number of isozymes which can be divided into two groups, those with isoelectric points (pI) around pH 4.6 (low pI group) and those with pI values around pH 6.0 (high pI group). The two groups of isozymes differ in a number of ways and their genes occur in different chromosomes. Expression of both groups of genes is controlled by GA_3 and ABA but control is differential.

Cell-free mRNA translation studies have shown that the GA_3-promoted increase in α-amylase synthesis and its inhibition by ABA are associated with corresponding changes in the levels of translatable α-amylase mRNA. Subsequent studies using hybridization of cloned α-amylase cDNA probes to aleurone RNA have shown that the abundance of α-amylase mRNA increases in response to GA_3, and that this increase is inhibited by ABA. The increased abundance of mRNA could be due to increased rate of transcription, increased stability of newly formed transcripts or both. The transcription possibility has been examined further. Nuclei have been isolated from GA_3-responsive aleurone cell protoplasts and used for run-off transcription experiments. These experiments have shown that GA_3 promotes and ABA-inhibits the accumulation of α-amylase gene transcripts. Conversely GA_3 inhibits the accumulation of ribosomal RNA and ABA blocks the effect. These studies indicate that the two hormones act, at least in part, by regulating the transcription of specific genes.

F3 AUXIN-REGULATED GENE EXPRESSION: AN HISTORICAL PERSPECTIVE AND CURRENT STATE OF KNOWLEDGE, Joe L. Key, Department of Botany and Biochemistry, University of Georgia, Athens, GA 30602

During the 1960's a wide range of studies provided an information base which led to the suggestion that auxin-regulated cell processes - especially cell elongation - may be mediated by auxin-regulated gene expression (see Key, Ann. Rev. Plant Phys. 20,449, 1969.) (This possibility was earlier recognized by Professor Folke Skoog in the 1950's.) Indirect evidence from our work, based on the influence of inhibitors of RNA (e.g., actinomycin D) and protein (e.g., cycloheximide) synthesis on auxin-induced cell elongation coupled with correlations of the influence of auxin on RNA synthesis and cell elongation provided the basis for this suggestion. With the availability of techniques for DNA/DNA and DNA/RNA hybridization, mRNA isolation/translation in vitro/2D gel analysis of the translation products, and ultimately the cloning by recombinant DNA technologies of genomic DNA and cDNAs made to poly(A) mRNAs, we and others have provided direct evidence for the influence of auxin or the expression of a few genes out of some 40,000 different gene products (i.e. poly(A) RNAs.) Our laboratory has provided evidence for auxin both down-regulating (e.g., Baulcombe and Key, J. Biol. Chem. 255, 8907, 1980) and up-regulating (e.g., Walker and Key, Proc. Natl. Acad. Sci. 79, 7185, 1982) the level of a few poly(A) mRNAs out of a population of about 40,000 sequences that are not significantly affected by auxin (see Baulcombe et. al., in "Genome Organization and Expression in Plants", Plenum Press, p. 175, 1980.) In our studies on auxin-regulated cell elongation, two cDNA clones (pJWC1 and pJCW2) were isolated which corresponded to poly(A) mRNAs which responded during growth transitions in a way consistent with a potential role of their protein products in cell elongation. These mRNAs are most abundant in the elongating zone of the soybean hypocotyl. Upon excision and incubation in the absence of auxin, these mRNAs deplete in concert with a decreasing rate of cell elongation. Addition of auxin to the medium results in both increased levels of these mRNAs and enhanced rates of cell elongation. These mRNAs do not deplete if auxin is added to the medium at the onset of excised incubation and cell elongation rates remain high. We have isolated and sequenced genomic clones that are homologous to these cDNAs. Of the two genes sequenced, both genes are members of small multigene families. There are regions of high amino acid homology even though the nucleotide sequences are sufficiently different in these regions that cross hybridization of the clones is not observed. More recently, others, especially Guilfoyle's laboratory (e.g., Zurfluh and Guilfoyle, Proc. Natl. Acad. Sci., 77,357, 1980; Hagen, et. al., Planta, 162,147 1984), have shown that auxin selectively and rapidly influences the level of certain mRNAs and proteins. The question now is not whether auxin selectively alters gene expression and thus the biology of responsive tissues, but the mechanism by which auxin alters expression of specific genes. Additionally, the function of these gene products remains a "mystery". Fortunately, experimental approaches are available to answer these questions and are actively being pursued.

Hormone Modulated Gene Expression — II

F4 OLIGOSACCHARINS--MOLECULES THAT CAN REGULATE GROWTH, DEVELOPMENT, AND REPRODUCTION, AND DEFENSE AGAINST DISEASE IN PLANTS, Peter Albersheim, Alan G. Darvill, Keith R. Davis, Steven H. Doares, David J. Gollin, Roger O'Neill, Patrick R. Toubart, and William S. York, Complex Carbohydrate Research Center, Dept. of Biochemistry, University of Georgia and U.S. Department of Agriculture (Mailing address: Complex Carbohydrate Center, Russell Laboratory, P.O. Box 5677, Athens, GA 30613)

The shapes of the cells and, ultimately, the shapes of the plants are set by the relatively rigid wall that surrounds each cell. The information that controls what these shapes will be and what organ(s) will form must be delivered via chemical messages moving through the plant. The five well-characterized regulatory molecules of plants, abscisic acid, auxin, cytokinin, ethylene, and gibberellin, are pleiotropic. Each of these has so many simultaneous effects--some beneficial, some harmful--that none is of great commercial value. To enhance crop yields, regulatory molecules must be capable of controlling specific plant functions. We hypothesize that the regulatory molecules like auxin and gibberellin catalyze the release of other, more specific chemical messages.

Our laboratory team has long pursued two seemingly unrelated lines of research: one, elucidating the structure and function of the matrix polysaccharides of the walls of growing plant cells: and the other, studying the manner by which plants defend themselves against disease. Results from each project led us, serendipitously, to discover that structurally defined fragments of cell-wall polysaccharides are chemical messages with highly specific regulatory properties. These regulatory molecules--oligosaccharins--are released from the cell wall by means of enzymes. This lecture will describe the evidence that the cell walls are the repository of numerous, different oligosaccharins that can regulate not only triggering of plant defenses against pathogens and other types of stress, but also can regulate rate of growth, and differentiation into roots, flowers, and vegetative buds.

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F5 REGULATION OF STORAGE PROTEIN GENES IN EMBRYOS OF BRASSICA NAPUS, Martha L. Crouch, Alice J. DeLisle, Anne E. Simon, and Ruth R. Rinkelstein, Department of Biology, Indiana University, Bloomington, IN 47405.

Levels of abscisic acid (ABA) are quite high in embryos throughout seed development in Brassica napus. ranging from 5 to 20 μM (1). When embryos are removed from the seed and cultured on basal medium, endogenous ABA levels drop to about 1 μM, and the embryos germinate precociously. Exogenous ABA will inhibit precocious germination of embryos excised from the seed before 40 days post anthesis (dpa), but the higher ABA levels required to inhibit germination of older, desiccating embryos are toxic. Also, before 40 dpa exogenous ABA will maintain or stimulate high levels of storage protein mRNA. We postulate that ABA plays a role in maintaining embryogeny during the beginning of maturation, but not after desiccation begins.

One effect of ABA in young embryos is to increase the transcription rate of storage protein mRNAs relative to total RNA synthesis. Embryos just beginning storage protein synthesis (27 dpa) were cultured for 3d with or without 10^{-6}M ABA. Nuclei were isolated and then incubated in a transcription mix with ^{32}P-GTP, and incorporation into specific RNAs was determined by DNA-excess filter hybridization. The transcription rate of the storage protein mRNAs increased 2-4 fold in ABA-treated embryos compared to embryos cultured on basal medium, which was consistent with increase in RNA levels measured in parallel experiments. Transcription rates of actin and rRNA were not affected by ABA. In reinduction experiments (2), embryos which were switched from basal to ABA-containing medium at 12 and 24 h showed an increase in transcription rate of storage protein genes to the same level as those cultured on ABA from the start. In fact, storage protein mRNA levels in precociously germinating embryos younger than 35 dpa remains responsive to exogenous ABA for several weeks. The original embryo then produces secondary embryos on its surface, which are also responsive to ABA. Thus, it appears that secondary embryo cultures retain characteristics of the stage of development from which they were derived, and do not undergo maturation (3). We are currently determining the effects of ABA on mRNA stability in responsive embryos.

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F6 ABA MODULATION OF GENE EXPRESSION DURING WHEAT EMBRYOGENESIS, Ralph S. Quatrano, John D. Williamson, Roswitha G. Hopkins, Barbara B. Ballo, James C. Litts, Randolph L. Chakerian, Greggory J. Colwell, Afroz Sultana, Department of Botany/Plant Pathology, Oregon State University, Corvallis, OR 97331-2902.

ABA prevents wheat (Triticum aestivum L.) embryos from germinating but promotes normal embryogenesis when immature embryos are cultured in vitro. Over a 3-5 day culture period, isolated embryos in the presence of ABA (10^{-6} to 10^{-4} M) increase in fresh and dry weight, undergo normal morphogenesis and accumulate acid-soluble proteins. These proteins are precociously synthesized in culture when 10-15 day embryos are treated with ABA. The same proteins are normally synthesized in situ at 30-40 days and stored in the mature embryo. Several of these proteins, whose expression is enhanced by ABA, have been characterized and include the lectin wheat germ agglutinin (1,2), an abundant protein found in the mature embryo (E_m protein) and the globulin storage proteins. Their characterization and pattern of accumulation during grain development and in culture with ABA will be discussed. Data will also be presented which shows that ABA increases the level of mRNA coding for the E_m protein and other ABA specific sequences in immature embryos (3). The mRNA levels for these proteins are maintained or increased in mature embryos cultured in the presence of ABA. In the absence of ABA, these messages are lost within 48 hours. For the E_m message, the ABA effect is independent of new mRNA synthesis. For other sequences mRNA synthesis is required. This was determined using α-amanitin, which specifically inhibits only poly A^+ RNA synthesis. Hence, the ABA effect appears to be at the level of transcription and/or mRNA stability. Embryos cultured in -ABA synthesize another unique set of proteins, including the small subunit of RUBISCO. This set accumulates in immature and mature embryos that germinate into seedlings, and is not synthesized in +ABA embryos. ABA inhibition of RUBISCO synthesis appears to be at the level of transcription or RNA processing. RUBISCO mRNA is detectable at very reduced levels in +ABA embryos when compared to the mRNA levels in -ABA embryos (4). The above data are based on translation products from total or Poly A RNA, and by hybridization of cDNA's to mRNA using northern and slot-blotting techniques. Our data suggests that one of the ABA effects on embryogenesis in wheat is at the level of modulating mRNA levels, increasing those mRNA's essential for embryo maturation while decreasing those associated with germination.

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F7 REGULATION OF GENE EXPRESSION BY AUXIN AND ETHYLENE IN HIGHER PLANTS, Athanasios Theologis, Department of Biological Chemistry, Washington University School of Medicine, St. Louis, Missouri 63110

The primary mechanism of action of the plant hormones, auxins and ethylene, is unknown. Recent advances in recombinant DNA technology have permitted investigation of whether auxin (IAA) acts at the transcriptional or posttranscriptional level concomitant with the initiation of cell elongation in pea tissue. Differential plaque filter hybridization of cDNA libraries constructed in the vector λgt10 has led to the isolation of cDNA clones to specific mRNA sequences induced by IAA (20μM) in elongating pea stem tissue (1,2). Clone pIAA4/5 hybridizes to two mRNAs encoding the previously identified translation products 4 and 5 (M_r 23Kd and 25Kd, respectively), and clone pIAA6 hybridizes to an mRNA encoding polypeptide 6 (Mr 22 Kd). The induction of the mRNAs is rapid (10-15 min) and is specific to auxins. Stress conditions are unable to cause the induction, and other plant hormones do not affect the IAA induced mRNA accumulation. Protein synthesis inhibition mimics the IAA effect, suggesting that the auxin regulated genes are under the control of a rapidly turning over protein (repressor or activator). The hormonally regulated mRNA accumulation is not due to polyadenylylation of preexisting RNA. The data indicate that auxin has the capacity to act at the transcriptional or posttranscriptional level rapidly, concomitantly with or earlier than the initiation of cell elongation and proton secretion. Auxin induced H^+ secretion appears not to mediate the mRNA induction and is viewed as a consequence of the enhanced biosynthetic activity induced by the hormone. The mRNA induction is the fastest known for any plant growth regulator and may represent a primary response to auxin. A model uniting auxin induced H^+ secretion, mRNA induction and cell elongation will be discussed. The pIAA6 and pIAA4/5 cDNAs are currently being sequenced. pIAA4/5 cDNA hybridizes to a single 2.4 Kb EcoR1 fragment of pea DNA at a single copy level of hybridization, and genomic clones containing this fragment have been isolated and characterized.

In addition studies have been undertaken to elucidate the role of ethylene in initiating fruit ripening. cDNA libraries were constructed into the λgt10 and λgt11 cloning vectors using mRNA from climacteric avocado and tomato. cDNA sequences to specific mRNAs induced by ethylene during the climacteric rise of respiration were isolated, and the characterization of the hormonal response and the function of the proteins coded by these mRNAs is under scrutiny. Understanding the ethylene induced coordinate gene expression during fruit ripening will offer a better understanding of the mechanisms governing plant senescence.

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Hormone Modulated Gene Expression — III

F8 MODULATION BY ABSCISIC ACID OF GENES ENCODING β-CONGLYCININ IN DEVELOPING SOYBEAN COTYLEDONS, Elizabeth A. Bray* and Roger N. Beachy, Department of Biology, Washington University, St. Louis, MO 63130; *Current address: Department of Botany and Plant Sciences, University of California, Riverside, CA 92521

During soybean seed development, storage proteins accumulate at defined stages of development. The accumulation of storage proteins is regulated at the transcriptional level, although the mechanism of this regulation is unknown. We are investigating the role of the plant growth substance, abscisic acid (ABA), in the regulation of storage protein accumulation in soybean cotyledons. Soybean cotyledons were cultured with or without ABA, using a culture system that has been shown to promote development that is similar to in vivo development. To prevent confusion with the additional effect of ABA on precocious germination, the embryonic axes were removed from the soybean cotyledons and the cotyledons alone were cultured. The application of ABA at 10^{-5} M resulted in increased accumulation of the β-subunit of β-conglycinin, one of the major storage proteins in soybean seeds. The accumulation of the other two subunits of β-conglycinin was relatively unaffected. The amount of ABA in the cotyledons was increased by increasing the osmotic concentration of the medium or decreased by the application of fluridone. These treatments resulted in increased or decreased accumulation of the β-subunit, respectively. Regulation of the accumulation of the β-subunit could occur at the transcriptional or translational level. The amount of the β-transcript was increased two-fold in response to ABA and decreased four- to five-fold in response to fluridone in comparison with the control. Therefore, ABA modulated the amount of the transcript for the β-subunit. The increased accumulation of the β-transcript may be due to increased synthesis of the transcript in the nucleus or due to post-transcriptional events which might result in increased stability of the message. In vitro transcription in isolated nuclei is being used to determine if β-transcript synthesis is increased in response to ABA in cultured cotyledons. Currently, preliminary results showed that cotyledons cultured in ABA had a two-fold increase in the transcription of the β-subunit. The amount of the accumulated mRNA was also shown to increase two-fold in response to ABA. Preliminary results showed that ABA modulated the accumulation of the β-subunit of β-conglycinin through increased transcription of the message, although these results do not eliminate any additional regulation by post-transcriptional events.

F9 HORMONE REGULATION OF SPECIFIC mRNA LEVELS IN BARLEY ALEURONE

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Barley aleurone cells are responsive to both gibberellic acid (GA_3) and abscisic acid (ABA). Treatment of isolated aleurone layers, or of protoplasts derived from such layers with GA_3 or ABA results in the accumulation of sets of mRNAs specific for each hormone. The GA_3-mRNA set does not appear if excess ABA is present during the GA_3 treatment, and vice-versa.

The major GA_3-induced mRNAs are those encoding particular isozymes of a-amylase. Levels of mRNAs for these isozymes have been measured in a range of conditions by primer extension analysis. Synthetic oligonucleotides specific for the low and high pI families of a-amylase were synthesized and used to assess relative levels of their corresponding mRNAs in samples of total RNA.

In isolated aleurone layers there are moderate to high levels initially of the low pI mRNAs. These increase several-fold with GA_3 treatment, although no major difference is seen between GA_3 concentrations ranging from 10^{-9} M to 10^{-6} M. High levels of these mRNAs persist for at least 48 h. In contrast, the levels of the high pI mRNAs are initially low, rise rapidly to high levels at 12 h in a GA_3 concentration-dependant fashion, and decline rapidly to low levels at 24 and 48 h. There is, therefore, in isolated aleurone layers a major difference between the GA_3 regulation of the different mRNA families for the low and high pI isozymes of a-amylase.

Surprisingly this difference is not seen when relative mRNA levels are assessed in GA_3-treated aleurone protoplasts. Furthermore in aleurone derived from seedlings germinated for 2-6 d there are increasing but approximately equal levels of mRNAs for the low and high pI isozymes. Our current experiments are aimed at trying to resolve some of these apparently divergent aspects of the GA_3 induction of a-amylase mRNA.

F10 ETHYLENE INFLUENCED GENE EXPRESSION IN FRUITS AND STORAGE ORGANS AND IN SLICES THEREOF. George G. Laties, Department of Biology, University of California, Los Angeles 90024.

Fruit ripening with its associated respiratory climacteric has been considered a quintessential manifestation of ethylene action. Evidence based on nucleic acid and protein synthesis during ripening suggests ethylene to be an inducer in the genetic sense. Ripening of avocado is attended by a spate of polysome synthesis and an array of new messages. A cDNA library from ripe fruit has yielded a number of ethylene related clones one of which, pAV5, represents cellulase. Whereas at least three cellulase species are demonstrable in ripe avocado extracts by immunoblotting, hybrid release with pAV5 yields but one product on translation. With two clones of near full length transcripts (provided by R.E. Christoffersen and M.L. Tucker, respectively), however, hybrid release at low stringency with either clone yields some six polypeptides of differing pI's and common molecular weight, whereas at higher stringency each clone provides a non-overlapping sub-set of three members. Hybridization assays during the course of ripening indicate that different members of the putative cellulase gene family are expressed at different times. Immature avocados respond to ethylene with a sharp respiratory surge unaccompanied by ripening changes. Studies are under way to compare ethylene-induced effects at the molecular level in immature and mature fruits. Further studies will determine whether ripening slices may serve as a suitable model for fruit ripening—the virtue of slices being that they permit intervention and kinetic studies impossible with fruit.

In carrot roots as in avocado fruit the ethylene induced respiration climax is attended by a proliferation of polysomes and the appearance of new mRNAs and the disappearance of others. New messenger levels arise and subsequently fall in response to ethylene, while transcription of ethylene induced genes rises and falls even sooner. At least one induced polypeptide product continues to build with time well after transcription ceases. Whereas pure oxygen synergizes ethylene action with respect to respiration augmentation, polysome increase and induced messenger enhancement, subsequent step-down procedures where ethylene is removed, or O_2 replaced with air, cause an immediate drop in respiration while leaving messenger levels unchanged. Thick carrot slices behave much as intact roots, whereas thin slices show a pronounced wound-induced respiration followed some three days later by a sharp response to ethylene. Thus carrot slices offer a single object in which to compare wound and ethylene evoked events.

Work is under way to identify other ethylene specific clones in avocado and carrot. In addition, sequencing and restriction mapping of the cellulase genes (M. L. Durbin, R. E. Christoffersen, M. L. Tucker) followed by appropriate sub-cloning may allow preparation of probes better able to distinguish among cellulase family members.

Receptors and High Affinity Binding Moieties for Plant Hormones — I: Auxins

F11 BINDING TO THE NPA RECEPTOR/AUXIN TRANSPORT CARRIER, Mark Jacobs, Department of Biology, Swarthmore College, Swarthmore, PA 19081

The binding of ^{3}H-naphthylphthalamic acid (NPA) and ^{14}C-indole acetic acid (IAA) to the NPA receptor from etiolated zucchini hypocotyl and pea stem tissue will be described. Binding of NPA to membrane-bound receptors is compared to binding to solubilized receptors measured using the Amicon MPS-1 micropartition system, a technique we have adapted as an equilibrium method for separating free from protein-bound ligand. In zucchini hypocotyls, 0.3% Triton X-100 is the optimal detergent and concentration for solubilization; NPA binding to the solubilized receptor has a pH optimum of 5 and a K_d approximately one decade lower (10^{-9}) than the K_d for binding to membrane-bound sites (10^{-8}). Unlabeled auxins and auxin analogs can decrease ^{3}H-NPA binding to the solubilized sites in zucchini, and their specificity of action in this regard appears to parallel their activity as auxins. Since unlabeled auxins can compete with ^{3}H-NPA for binding to the NPA receptor solubilized from pea stem tissue as well, the binding characteristics and specificities for the two species will be compared.

F12 CHARACTERIZATION AND FUNCTION ANALYSIS OF HIGH AFFINITY CYTOPLASMIC AUXIN-BINDING PROTEINS, K.R. Libbenga, A.M. Mennes, H.J. van Telgen, P.C.G. van der Linde, Department of Plant Molecular Biology, University of Leiden, Botanical Laboratory, Nonnensteeg 3, 2311 VJ Leiden, The Netherlands

At present the best characterized auxin-binding proteins are membrane-bound ones. However, over the past years a few reports on cytoplasmic auxin-binding proteins have appeared (1). About ten years ago we discovered such a class of auxin-binding proteins in cultured cells and tissues from Nicotiana tabacum (2). Further investigations revealed that this protein specifically binds auxins and that it has a relatively high affinity towards the natural auxin indol-3-acetic acid (KA,IAA, 25°C≈ $1.6 \times 10^{8} M^{-1}$) (3). The protein is not only present in the cytoplasm but also in a non-covalently bound form in the nucleus. Partially purified preparations of the binding protein stimulate RNA polymerase II activity in nuclei isolated from target tissues, but only in the presence of auxin. The percentage of stimulation is directly proportional to the receptor occupancy and shows auxin specificity (4).

The apparent concentration of the binding proteins in the preparations is in general very low (< 200 fmol mg^{-1} protein), often even below the detection level of the binding assays. This has hampered rapid progress in the purification and further characterization of this putative auxin-receptor protein. However, recent results indicate that phosphorylation and dephosphorylation modulate the affinity of the binding protein towards its ligand (5). At present we make use of this interesting phenomenon to label the binding proteins in vitro with ^{32}P and to keep them in their phosphorylated high affinity form during purification steps, which include affinity chromatography. The latest results will be discussed at the meeting.

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F13 AUXIN-BINDING PROTEINS IN MAIZE : PURIFICATION AND RECEPTOR FUNCTION, Michael A. Venis, East Malling Research Station, Maidstone, Kent, ME19 6BJ, England.

High affinity binding sites for auxins are found in membrane preparations from maize coleoptiles. They can also be detected in other tissues (mesocotyls, roots), in other monocotyledons (oat, wheat, rye) and also in soluble fractions. In maize coleoptile membranes, three classes of binding site have been noted. One of these, site III, is associated with auxin transport and appears to be located in the plasma membrane. Site I has been assigned to endoplasmic reticulum membranes and site II to either plasma membrane or tonoplast. Sites I and II have been distinguished in terms of kinetics and specificity, but this separation has been disputed.

The membrane-bound sites can be solubilised without detergent and purified by various means, including FPLC. The native binding proteins are 40-45 kilodaltons in size, with isoelectric points in the range pH 4.5-5.2. Affinity labelling studies have been carried out both in the membrane-bound and solubilised states, using radioactive and non-radioactive probes. Attempts are currently being made to raise both monoclonal and polyclonal antibodies to the membranous binding proteins, and also to purify the soluble binding proteins.

The various lines of direct and indirect evidence pointing to an auxin receptor function for the maize membrane binding sites will be described and discussed.

Auxin Binding

F14 A COMMON SHARED EFFLUX COMPONENT IN THE TRANSPORT OF 2,3,5-TRIIODOBENZOIC ACID, INDOLE-3-ACETIC ACID, AND 2,4 DICHLOROPHENOXYACETIC ACID BY CUCURBITA PEPO L. HYPOCOTYL SEGMENTS.

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The effects of different nonradioactive auxins, 1-naphthylphthalamic acid (NPA) and 2,3,5-triiodobenzoic acid (TIBA) on the uptake of radiolabelled TIBA and indole-3-acetic acid (IAA) by segments of zucchini hypocotyls have been compared (1). The well established ability of TIBA and NPA to stimulate net auxin uptake as a result of efflux inhibition was confirmed. It was also shown that the net uptake of [^{14}C] TIBA itself is stimulated by nonradioactive TIBA, auxins and NPA, suggesting a saturable efflux component for TIBA, which can be blocked by auxins and NPA. The non-auxin benzoic acid had no effect on auxin or TIBA uptake. No significant metabolism, of IAA, 2,4 dichlorophenoxyacetic acid (2,4 D) occured during the experimental procedures. A model is proposed for the auxin efflux carrier in which auxin and TIBA have separate specific binding sites. This is based on the reciprocal transport interactions of IAA and TIBA and the noncompetitive inhibitory effects of TIBA and NPA on polar auxin transport in maize coleoptile segments (2). A third specific site may be necessary to account for the inhibitory effects of NPA.

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F15 AUXIN BINDING PROTEINS OF PLANT CELLS IN SUSPENSION CULTURE, Malcolm C.Elliott, A.M.O'Sullivan, J.F[illegible], H.M.Bailey, R.D.J.Barker, School of Life Sciences, Leicester Polytechnic, Leicester, LE1 9BH, U.K. K.R.Libbenga and A.M.Mennes, Botanisch Laboratorium Rijksuniversiteit Leiden, Nonnensteeg 3, NL-2311 VJ Leiden, The Netherlands

The classical assumption[1] that there was a precise correlation between auxin content and growth of plant parts has been vigorously challenged[2,3]. It has been argued [4,5] that physicochemical assays of plant growth regulators of plant cells in suspension culture might help resolve the disaccord.

It is often necessary to add an auxin to the culture medium in which plant cells are grown[6]. The notion that such cells have a growth limiting defect in their capacity to synthesise IAA has been presented[7,8,9], modified[5] and challenged[10,11]. The proposal that variations in "sensitivity" determine biological responses and that hormone concentrations are irrelevant has been vigorously presented[12].

A specific, high affinity, IAA binding site has been demonstrated in both a cytosol fraction and in isolated nuclei from Nicotiana tabacum cv. Wisconsin No.38 cells grown in suspension culture. The amount of the binding site detected in both these fractions changed during the culture cycle according to a strict pattern. When partially purified by affinity chromatagraphy and allowed to pre-incubate with IAA, the site had a significant stimulatory effect on total RNA synthesis, as measured by a cell-free assay system. The system behaves rather like the steroid hormone-receptor system in animals[13].

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F16 GROWTH-STAGE DEPENDENT OCCURRENCE OF SOLUBLE AUXIN-BINDING PROTEINS (sABP) IN PEA, Hans-Jörg Jacobsen, Institut für Genetik der Universität, Kirschallee 1, D-5300 Bonn - 1, FRG

In etiolated pea epicotyls soluble high-affinity auxin-binding proteins have been demonstrated to exhibit a growth-stage dependent pattern(1). After separarating crude cytosols using a modified preparative chromatofocusing procedure, in 7 days old etiolated pea epicotyls one high-affinity binding site can be detected ($sABP_1$, pI:5.0-5.8), while in 9-12 days epicotyls a second binding site appears ($sABP_2$, pI:6.0-6.8). Recent results indicate that also different tissues have differences in their respective sABP-patterns: Apical hooks harvested from etiolated pea seedlings show the presence of three different sABPs, with only $sABP_1$ obviously identical with that from epicotyls and two yet unidentified sites (pI: 6.0-7.0 and 7.0-8.0, respectively). The binding assays used for the detection of high-affinity binding sites were the ammonium-sulfate precipitation, PEI-treated glass fiber filters (2) and equilibrium dialysis (3).
The results indicating simultaneous presence of different binding sites in the same tissue fit quite well into the picture of multiple auxin effects. Thus it may be stated that tentative auxin receptors have identical auxin-binding structures, but differ with respect to their acceptor sites, which are suspected to be localized in the nucleus (4,5). So the auxin-dependent long-term reactions of a cell may be controlled by the presence, absence or inductive capacity to synthesize a receptor population, making the cell competent or non-competent for auxin responses.
Experiments with a morphologically deviant pea genotype exhibit a genotype-specific expression of soluble auxin-binding: Recombinant R 4111, derived from a cross between the mother line of the mutant collection of our institute and a fasciated mutant (489C), differs from the mother line by its almost doubled internode length. In the epicotyls of this mutant up to day 11 of germination only $sABP_1$ seems to be present (or active), while the parent lines at that time exhibit the presence of $sABP_2$(3).

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F17 AUXIN BINDING IN TARGET TISSUE

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The most obvious physiological response of coleoptiles to auxin is elongation growth. The cause of this cell extension was believed to be a proton secretion caused by auxin in peeled and unpeeled coleoptiles (1). In terms of the hormone receptor concept this means that all coleoptile cells should have an auxin receptor.
In our investigation on auxin binding in maize coleoptiles a single polypeptide could be isolated with a high affinity for 1-Naphthylacetic acid (dissociation constant: 57 nM)(2). By affinity chromatography an antibody against this polypeptide was obtained from polyvalent antiserum. The antibody specificity was judged by immunoprecipitation an immuno western blot. In aldehyde fixed coleoptile tissue the auxin binding protein was visible by indirect immunofluorescence microscopy only within the outer epidermal cells (3). This was consistent with physiological observations that the coleoptile epidermis reacts towards auxin much more strongly than the underlying tissue (4). Although auxin induced proton secretion was observed in peeled coleoptiles auxin induced elongation growth was inhibited by 82 % (5).
In physiological experiments the monospecific antibodies significantly reduced the auxin induced elongation growth (3). Thus the auxin binding protein had to be involved in the auxin response, and in consequence is an auxin receptor. Secondly,this auxin receptor should be localized at the plasmalemma of the epidermal cells. Besides the growth inhibition by the antibodies the pH optimum for auxin binding at pH 5.5 and the fact that the auxin receptor is glycosilated might support this view.
The outer epidermal cells of the coleoptile are one of the target tissues for auxin in maize. During maize plant development only very low auxin binding activity and receptor protein could be detected in the green part of the plant, leaves and stem. In other, fast growing tissues, roots, tassel, and silk the auxin receptor and auxin binding activity was found in high amounts.

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Receptors and High Affinity Binding Moieties for Plant Hormones — II: Cytokinins, Gibberellins, ABA

F18 ETHYLENE BINDING PROTEINS, M.A. Hall, C.J. Howarth, I.O. Sanders, A.R. Smith, P.G. Smith, R. Starling, Z.D. Tang & R.A.N. Williams, Department of Botany & Microbiology, University College of Wales, Aberystwyth, Dyfed SY23 3DA, Wales, U.K.

Ethylene binding sites have now been detected in a number of plant species but only two have been characterised to any extent (1,2,3). The ethylene binding site from developing cotyledons of Phaseolus vulgaris is located on the endomembrane system (3,5) and is highly hydrophobic in nature. It has a K_D for ethylene of around 10^{-10}M and has low rate constants of association and dissociation. Its affinities for structural analogues of ethylene reflect the relative physiological effectiveness of such analogues. The protein has been extensively purified by procedures including detergent partitioning and FPLC and its properties will be described as will progress towards the establishment of a specific immunoassay.

The properties of other ethylene binding proteins recently discovered in epicotyls of Pisum sativum will be described and their possible relation to ethylene action in this tissue outlined.

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F19 IN VITRO [^{3}H]GA_1 BINDING BY MACROMOLECULAR CONSTITUENTS OF GA-SENSITIVE AND GA-INSENSITIVE DWARF MAIZE MUTANTS. Lawrence Rappaport and Brian Keith. Plant Growth Laboratory, Department of Vegetable Crops, University of California, Davis, CA 95616, USA.

A 100,000 x g supernatant fraction was prepared from the first and second leaf sheaths of light grown Zea mays L. cv. Golden Jubilee. [^{3}H]GA_1 binding to a high molecular weight (HMW) fraction (>500 Kdaltons) was demonstrated at 4°C using Sephadex G-200 chromatography. The HMW component was shown to be a protein and the [^{3}H] activity bound to this protein was [^{3}H]GA_1 and not a metabolite.

The binding properties of an intermediate molecular weight (IMW) fraction will also be described. The specifcity and partial purification of both HMW and IMW binding components will be described for 'Golden Jubilee' and some dwarf, single-gene mutants of corn that are either GA-sensitive (d_1, d_2, d_3 and d_5) or GA-insensitive (D_8).

Second Messengers in Hormone Action

F20 CALCIUM AS A SECOND MESSENGER IN THE RESPONSE OF ROOTS TO AUXIN AND GRAVITY, Michael L. Evans, Department of Botany, Ohio State University, Columbus, OH 43210

There is evidence that calcium (Ca) plays an important role in both the transport and action of auxin. Hertel (1) proposed that auxin movement across membranes is linked to influx of Ca into the cytoplasm. Elevated cytoplasmic Ca might then mediate the effects of the hormone either directly or through calmodulin (CaM)-regulated events. Evidence for this model includes the observations that: 1. Ca is necessary for auxin transport (2), 2. Stimulation of coleoptile elongation by auxin is accompanied by enhanced Ca efflux from the

tissue (3), 3. Auxin enhances Ca flux across microsomal vesicles (4), and 4. CaM inhibitors interfere with auxin-induced stimulation of cell elongation (5).

We investigated the role of Ca in the response of maize roots to auxin and to gravity. The role of Ca in root gravitropism (gt) was tested by examining: 1. The Ca-dependence of root gt, 2. The influence of gravity on Ca distribution in roots, and 3. The Ca-dependence of gravity-induced auxin redistribution in roots. Application of EGTA to the root cap caused roots to lose gravitropic sensitivity. This was reversible by Ca. Ca gradients across the root tip induced curvature toward the high side of the gradient, and in gravistimulated roots there was strong polar transport of Ca across the root tip toward the lower side. Also, Ca application to the lower side of gravistimulated roots strongly enhanced the lateral movement of auxin toward that side. CaM may be involved in Ca action in root gt since: 1. Root caps were found to contain high CaM activity, 2. Photoinduction of gravitropic sensitivity in maize roots was paralleled by photoenhancement of CaM activity, and 3. Application of CaM inhibitors to the root cap prevented gt.

The role of Ca in the action of auxin was tested by comparing the kinetics of the effects of Ca and auxin on root cell elongation. The kinetics of inhibition of root elongation by 0.5 mM Ca and low concentrations of auxin were nearly identical, with strong inhibition followed by recovery. In seedlings raised to contain low endogenous levels of Ca, the growth rate of the root was only slightly reduced. However, auxin caused little or no inhibition of root elongation in low Ca seedlings even at concentrations 100 fold higher than that required to inhibit elongation in control roots. The results indicate that the inhibitory action of auxin on root cells is strongly dependent on the Ca status of the cell. If this is true it may be possible to establish a gradient in auxin activity without a gradient in auxin concentration simply by establishment of a Ca gradient.

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F21 CALCIUM: A SECOND MESSENGER IN CYTOKININ AND PHYTOCHROME MEDIATED DEVELOPMENT, Peter K. Hepler, Department of Botany, University of Massachusetts, Amherst, MA 01003

Calcium (Ca^{2+}) is a second messenger in many different developmental processes in animal systems, but only recently has it been recognized that the ion contributes to the regulation of development in plants. Below a brief summary provides evidence that Ca^{2+} participates as a second messenger in cytokinin mediated bud formation in the moss Funaria, and phytochrome stimulated spore germination in the fern, Onoclea.

Application of cytokinin to growing protonemata of Funaria greatly enhances the production of new buds on the caulonema cells. A role for Ca^{2+} is implied from the following observations: 1.) The bud site stains brightly with the Ca^{2+}-chelate probe, chlorotetracycline (CTC). The increase in CTC fluorescence greatly exceeds the increase in fluorescence from a general membrane marker N-phenylnaphthylamine indicating that the relative amount of calcium has increased. 2.) Treatment of buds with the Ca^{2+} ionophore, A23187 plus Ca^{2+} stimulates the production of buds in the absence of exogenously added cytokinin. 3.) Culture of protonemata in cytokinin with the addition of Ca^{2+}-channel antagonists, lanthanum, verapamil or D-600 inhibits bud formation. Taken together these results suggest that cytokinin stimulates the entry of Ca^{2+} from the extracellular wall space into the bud site, possibly through voltage-dependent Ca^{2+}-channels.

Phytochrome stimulation of spore germination in Onoclea also appears to involve Ca^{2+}. 1.) Irradiation of spores with red light stimulates net Ca^{2+} uptake while far-red inhibits the process. 2.) Incubation of spores in the ionophore A23187 plus Ca^{2+} causes germination in the dark. 3.) Finally culture of spores in lanthanum inhibits red light stimulated Ca^{2+} uptake and germination. Additional studies reveal that Ca^{2+} entry occurs rapidly following red light irradiation and that maximum stimulation of germination takes place within five minutes. However in the absence of Ca^{2+} even briefly irradiated spores will remain poised for hours in the dark to accept the ion and then initiate germination.

Cytokinin and phytochrome thus appear to promote development by eliciting an influx of Ca^{2+}. A major difference in their respective actions can be noted in the stimulus-response timing. Whereas red light irradiation and Ca^{2+} are needed only briefly (a few minutes) to stimulate germination in spores, cytokinin and Ca^{2+} must be continuously present for hours in order to promote budding in caulonemata.

F22 LIGHT, CALCIUM AND CALMODULIN REGULATION OF ENZYME ACTIVITIES IN ISOLATED NUCLEI, Stanley J. Roux, Neeraj Datta, Yuh-Ru Chen, and Sung-Ha Kim, Department of Botany, University of Texas, Austin, TX 78713

Recent data indicate that the photoactivation of the regulatory pigment, phytochrome, changes the level of translatable mRNA for a number of different proteins (1). The mechanism by which phytochrome induces these changes in gene expression is not known. Relevant to this question is the more specific one of whether phytochrome regulates the activity of any nuclear enzymes. An abstract published by Wagle and Jaffe indicated that phytochrome modulated the activity of a nuclear ATPase present in etiolated pea seedlings (2). In that report, the actinic red and far-red light irradiations were given in vivo. We have confirmed these findings and further characterized a nucleoside triphosphatase (NTPase) activity that is photoreversibly regulated by light treatments given in vitro to isolated pea nuclei. Because NTPase and other enzyme activities in animal nuclei are controlled by phosphorylation/dephosphorylation (3), we tested whether light affected such protein modification in pea nuclei. We found that several nuclear proteins were more highly phosphorylated following a red light irradiation of the nuclei, and that this effect was reversed by far-red light (4). Indirect assays of protein kinase and protein phosphatase activity indicate that both are stimulated by red light. The light stimulations of both NTPase and phosphorylation activities were blocked by Ca^{2+} chelation (EGTA) and by low concentrations of the calmodulin inhibitors, chlorpromazine and compound 48/80. We have localized calmodulin in pea nuclei by immunocytochemistry (5), and calmodulin gel overlays reveal several nuclear proteins that bind calmodulin in a calcium-dependent fashion. Most of the NTPase activity in pea nuclei is chromatin-associated. We have purified this chromatin-associated NTPase by HPLC chromatography and are investigating whether it is the one that is light and calcium regulated. We are also testing whether pea nuclei contain any protein kinases or phosphatases that bind to and are regulated by calmodulin.

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F23 THE CELLULAR PROTON PUMPS OF CORN SEEDLINGS, Lincoln Taiz, Biology Department, Thimann Laboratories, University of California, Santa Cruz, CA 95064

Proton-translocating ATPases play important roles in energy transduction, transport and growth in plant cells. Three types of H^+-ATPases have been characterized: F_oF_1-type H^+-ATPases of the mitochondria and chloroplasts, E_1E_2-type H^+-ATPases of the plasma membrane, and the recently discovered tonoplast-type H^+-ATPase. We have characterized the tonoplast-type H^+-ATPase of corn seedlings and have shown that a similar enzyme is present on Golgi cisternal membranes. The distinguishing features of this proton pump are its neutral pH optimum, stimulation by anions, inhibition by nitrate, and insensitivity to vanadate, oligomycin and azide.

The absence of vanadate inhibition suggests that the tonoplast-type H^+-ATPase, like the F_oF_1-H^+-ATPase, does not have a phosphorylated catalytic intermediate. Structural studies indicate other points of similarity. The functional molecular mass of the corn tonoplast H^+-ATPase was determined by radiation inactivation to be ~400,000 Da. Solubilization with octylglucoside followed by partial purification on sucrose gradients led to an enrichment of two major polypeptides of M_r=72,000 Da and M_r=62,000 Da. This fraction was also enriched in an M_r=16,000 Da DCCD-binding polypeptide.

Several lines of evidence suggest that the M_r=72,000 polypeptide represents the subunit containing the catalytic site. ^{14}C-NBD-Cl, which inactivates the tonoplast ATPase in an ATP-protectable manner, labeled the M_r=72,000 polypeptide, and the binding was partially reversed by Mg:ATP. Polyclonal antibodies prepared against the M_r=72,000 Da polypeptide and the native enzyme both inhibit enzyme activity, while antibody prepared against the M_r=62,000 polypeptide does not inhibit activity. The anti-native enzyme antibody binds to the M_r=72,000 Da, but not the M_r=62,000 Da polypeptide on western blots. Finally, cross-reactivity was observed between the M_r=72,000 Da polypeptides of corn and Neurospora tonoplast ATPase preparations, but the M_r=62,000 Da showed no cross-reactivity. Thus the M_r=72,000 Da polypeptide is more highly conserved.

An H^+-pyrophosphatase has also been identified on the tonoplast and Golgi of corn seedlings. The role of H^+-PPases in cellular proton transport processes has yet to be determined. Evidence has been obtained that red light modulates the activity of the H^+-ATPase and H^+-PPase in the tonoplast and Golgi of corn mesocotyls.

Plant Pathogens and Plant Hormones

F24 MANIPULATION OF T-DNA DERIVED PHYTOHORMONE BIOSYNTHETIC ENZYMES, Harry Klee, Robert Horsch, Robert Fraley and Stephen Rogers, Monsanto Company, St. Louis, MO 63198

In the past several years it has been convincingly demonstrated that the T-DNA of *Agrobacterium tumefaciens* contains genes which encode phytohormone synthetic activities. Three genes, in particular, have been shown to encode enzymes which synthesize auxins and cytokinins. The two auxin genes, encode a tryptophan monooxygenase (tms1) and an indoleacetamide hydrolase (tms2). These enzymes respectively convert tryptophan to indoleacetamide and then to indoleacetic acid. The cytokinin gene (tmr) encodes an enzyme, isopentenyl transferase, which synthesizes isopentenyl adenosine.

We are interested in understanding the roles of the various phytohormones in controlling plant differentiation. Since no endogenous phytohormone genes have been successfully cloned to date, we have chosen to utilize the T-DNA genes to endogenously manipulate the cytokinin and auxin levels Petunia plants. Each of the three genes has been genetically engineered, fused to a variety of transcriptional promoter elements, introduced into *Agrobacterium*-based plant transformation vectors and transformed into Petunia leaf discs. Regenerated plants have been obtained for many of these constructions. The phenotypic and regenerative properties of the transformed Petunia tissue containing the individual genes is currently being examined.

F25 REGULATION OF IAA METABOLISM IN PLANT TUMORIGENIC BACTERIA. Tsune Kosuge, Frank Roberto, Margaret Sanger and Louise Glass, Department of Plant Pathology, University of California, Davis, CA 95616

Pseudomonas syringae pv. savastanoi (P. savastanoi) is one of a number of phytopathogenic bacteria able to induce hyperplasias in plant hosts via the production of plant hormones. In contrast to the mechanism used by Agrobacterium tumefaciens whereby the plant is genetically transformed by bacterial genes including genes for plant hormone production, P. savastanoi appears to incite galls on olive and oleander plants simply by the overproduction of indole-3-acetic acid(IAA) and cytokinins which are secreted constitutively by the bacterium.

Indoleacetic acid is produced by the oxidative decarboxylation of tryptophan in virulent strains of P. savastanoi, and the genes encoding a tryptophan monooxygenase (iaaM) and an indoleacetamide hydrolase (iaaH) have been localized on a cryptic plasmid (pIAA) in oleander strains, while the genes are present on the chromosome or a megaplasmid in olive strains. These genes appear to be organized in an operon, as insertions into iaaM have polar effects on the expression of the iaaH gene product.

Tryptophan monooxygenase is competitively inhibited by the product, indoleacetamide. Additional control of IAA production may be effected by the conjugation of lysine to IAA, and otheras yet uncharacterized metabolites of IAA, which may serve to modulate the intracellular pool size and secreted levels of IAA. The IAA-lysine synthetase gene has been cloned and is located proximal to iaaM but is not part of the IAA operon. Additional studies are underway to determine if transcriptional controls are present for IAA biosynthesis and metabolism.

Significant homology has been found between the deduced amino acid sequences of the product of iaaM and the tms-1 locus of A. tumefaciens, and limited homology also exists between iaaH and tms-2.

Cytokinins, including trans-zeatin, zeatin riboside, isopentenyladenosine and isopentenyladenine are produced and secreted at relatively high levels by both olive and oleander strains. Their production occurs by determinants found on a plasmid distinct from pIAA. The results of recent experiments suggest that zeatin production is necessary for gall formation.

F26 PROKARYOTIC GENES CODING FOR CYTOKININ BIOSYNTHESIS, R.O. Morris, G.K. Powell, J.S. Beaty and N.G. Hommes, Department of Agricultural Chemistry, Oregon State University, Corvallis, OR 97331

Agrobacterium tumefaciens contains two genes which code for enzymes capable of catalyzing cytokinin biosynthesis. The first (tmr) is located within the T-region of all Ti plasmids so far examined. It specifies a prenyl transferase (dimethylallylpyrophosphate:5'-AMP transferase) capable of the synthesis of iPA 5'-phosphate from dimethylallylpyrophosphate and 5'-AMP. A similar gene, tzs, is present only on nopaline plasmids. It is located outside of the T-region close to vir. Cloning and expression of tzs into E. coli results in secretion of zeatin (1). Extensive sequence homology exists between the two genes and is manifested in common function.

Pseudomonas savastanoi secretes high levels of cytokinins and contains cytokinin biosynthetic genes. The 106 Kb plasmid of P. savastanoi, strain 1006, carries a gene whose sequence is 50% homologous to Agrobacterium tumefaciens tzs. Upon cloning and expression in E. coli it causes secretion of zeatin. Examination of cytokinin production by a number of wild type P. savastanoi strains indicates a considerable diversity in the nature of cytokinins produced.

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F27 PLANT GROWTH CONTROL BY TI PLASMID AND RI PLASMID GENES, Robert B. Simpson, Kitisri Sukhapinda, Linda Margossian, Anna Wyszogrodzka, and Elias A. Shahin, ARCO Plant Cell Research Institute, Dublin, CA 94568

Agrobacterium tumefaciens containing a Ti plasmid can transform normal plant cells into tumor cells. The basis for this transformation is the transfer of a specific portion of the Ti plasmid, the T-DNA, and the expression of the T-DNA in the plant cells. Enzymes encoded by the T-DNA include two that result in the synthesis of indole acetic acid (1-3) and one that results in the synthesis of isopentyl adenosine (4-6). These enzymes are directly responsible for the hormone autonomy of the transformed cells in vitro. The plant cells transformed by Agrobacterium rhizogenes are also hormone autonomous based on the presence of a distinct T-DNA from the bacterial Ri plasmid. Tissue from A. rhizogenes transformation frequently forms hairy roots in contrast to the calli usually formed by A. tumefaciens transformation. The hairy roots from several plant species can be regenerated into plants. It is likely that plant growth regulators are produced either directly or indirectly by enzymes encoded by the Ri T-DNA. We will report the results from our attempts to understand and control the Ti and Ri plasmid-encoded genes that regulate plant growth.

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Molecular Biology of Light Regulation

F28 MOLECULAR EVENTS IN PHOTOREGULATED GREENING IN BARLEY LEAVES,
Winslow R. Briggs, Carnegie Institution of Washington, Stanford, CA 94305, Egon Mösinger, and Eberhard Schäfer; Biologisches Institut der Universität II, D-7800 Freiburg i. Br., Federal Republic of Germany.
A brief pulse of red light reduces the lag phase for chlorophyll accumulation in subsequent white light in dark-grown barley leaves. It also induces an increase in the mRNA abundance for the light-harvesting chlorophyll a/b-binding protein (LHCP) and a decrease in the mRNA for the NADPH-protochlorophyllide oxidoreductase (reductase). Finally, it induces an increase in transcription as measured in nuclei isolated from irradiated plants for the LHCP mRNA and a decrease in transcription for the reductase mRNA. Fluence-response studies at the levels of chlorophyll accumulation, mRNA abundance, and transcriptional activity of nuclei from irradiated plants indicate that all three parameters show both very low fluence and low fluence components, spanning eight orders of magnitude of light dose. The low fluence response persists for all three parameters for many hours, as does the very low fluence component for mRNA abundance and chlorophyll accumulation. The very low fluence response for the transcriptional activity is transient. The very low fluence responses, where measured, do not show far-red reversibility, while the low fluence responses do. Kinetic and quantitative considerations indicate that while phytochrome regulation of mRNA abundance - and and hence eventually greening in the light - may be partly at the transcriptional level - phytochrome regulation must occur at other levels as well. Pertinent literature is discussed.

F29 PHYTOCHROME AND ITS GENES, Peter H. Quail, Departments of Botany and Genetics, University of Wisconsin, Madison, WI 53706.

We are attempting to understand the molecular mechanism by which phytochrome regulates gene expression. Our approach is to study genes whose expression is rapidly altered in response to Pfr formation and to define structural properties of the phytochrome molecule potentially related to its regulatory function. We have isolated from Zea and Avena a set of cDNA clones homologous to mRNAs whose steady-state levels change positively or negatively within 1 h or less of a red-light pulse. Run-off transcription in isolated nuclei is being used to determine whether control is exerted at the transcriptional level. One of the Avena genes for phytochrome itself, known to be rapidly down-regulated at the transcriptional level, has been fully sequenced and the transcription start site determined by S1 mapping. The gene is 5.9 kb long with five introns, two of which are in the untranslated regions. The full length amino acid sequence of Cucurbita phytochrome has been determined from cDNA sequencing and compared to that for Avena. The two apoproteins exhibit regions of high homology in the major NH_2-terminal domain but diverge substantially in the COOH-terminal region. Proteolytic peptide mapping is being used in conjunction with spectral measurements and monoclonal antibodies to spatially discrete epitopes to localize regions of the polypeptide involved in protein-chromophore interaction and in dimerization.

F30 PHYTOCHROME-REGULATED NUCLEAR GENES FOR CHLOROPLAST PROTEINS, E. M. Tobin, S. Flores, G.A. Karlin-Neumann, B.D. Kohorn, L. Leutwiler, J. Silverthorne, and C.F. Wimpee, Biology Department, University of California, Los Angeles, CA 90024

Phytochrome action regulates expression of some nuclear genes, and in a number of cases has been shown to affect the transcription of such genes (1). Many of these nuclear genes encode proteins that are transported to and function within chloroplasts. We have studied the genes for two such proteins--the small subunit of ribulose 1,5-bisphosphate carboxylase (SSU) and the light-harvesting chlorophyll a/b-binding protein (LHCP) associated with Photosystem II. The transcription of genes encoding each of these proteins is increased by the action of phytochrome in the aquatic monocot, Lemna gibba. Cytokinin, which can interact synergistically with red light, also affects the expression of these genes. However, in contrast to the action of phytochrome, we have found that its effect is apparently post-transcriptional, possibly on mRNA stability in the cytoplasm.

Both SSU and LHCP are encoded by multigene families in Lemna and in many other species examined. We have isolated and characterized a number of different genomic clones containing these genes (cab genes) in Lemna and all three genes for LHCP of Arabidopsis thaliana. We have sequenced these three cab genes from Arabidopsis and two from Lemna. The Arabidopsis cab genes are clustered and encode identical mature proteins; there is one variant amino acid in the transit sequence. The cab genes from Arabidopsis and the two Lemna genes analyzed have a predicted amino acid sequence similar to those of other species with the exception of the N-terminal region of one of the Lemna genes (2). The Lemna SSU genes exhibit some clustering. The coding region of these SSU genes is also highly conserved, but there is substantial divergence in the 3' untranslated regions. The transit peptides of both SSU and LHC proteins share three regions of homology; presumably, these regions have functional importance in the uptake, processing, and/or assembly of the mature polypeptides into their functional units in the chloroplast.

The fact that there are multiple genes encoding phytochrome regulated proteins raises the question of whether each of the individual genes is regulated in the same way. We have made use of the divergence in the 3'untranslated regions of the Lemna SSU genes to address this question. Subclones of these regions from 6 of the ca. 12 genes act as gene specific probes which do not cross-hybridize with each other. We have used these probes to demonstrate that a) the different genes are expressed to greatly different extents in light-grown plants, b) each of the six sequences responds to the action of phytochrome, although to differing extents, and c) there are organ specific differences in the expression of some of the genes.

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Gene Expression

F31 **INDUCTION OF ALCOHOL DEHYDROGENASE BY 1-ACETYL-1-CYCLOPROPANE CARBOXYLIC ACID,** **D. Clark Bennett and Michael Freeling, University of California, Berkeley, Ca. 94720**

Flooding of maize roots (anaerobic stress) evokes a specific pattern of protein synthesis wherein approximately 20 proteins are produced at high levels with a concomitant cessation of all other protein synthesis. Increases in mRNA levels for alcohol dehydrogenase (ADH), aldolase and several other anaerobically induced proteins have been shown to occur during anaerobic stress. Also, increased levels of ethylene have been correlated with root flooding in several species of plants. Perfusion of maize roots with 1mM ACC in the presence of 1mM cobalt (a known inhibitor of ACC to ethylene conversion) causes a three fold increase in ADH enzyme activity in the root tissue. A similar increase in ADH activity is observed when roots are anaerobically stressed for 24 hrs. Perfusion with 1mM ACC alone does not produce any increase in ADH activity above control levels while perfusion with 1mM cobalt causes a 1.5 fold increase in ADH activity. Higher levels of ACC and cobalt separately or in combination caused no further increase in ADH levels. This data suggests that ACC (and not ethylene) is the signal responsible for increased synthesis of anaerobically induced proteins such as ADH, during anaerobic stress.

F32 "Expression of Genes for Thermotolerance in Cell Suspension Cultures," Joe H. Cherry, Purdue University, West Lafayette, IN 47907

It has been shown that the synthesis of heat-shock polypeptides (HSPs) is correlated with the development and the decay of thermotolerance in cultured animal and plant cells. The biochemical nature of this relationship in plant cells is not known but our results suggest that induction of HSP synthesis is inversely correlated with heat resistance of tobacco and cowpea cells during the growth cycle. Our data is consistent with a hypothesis which might suggest that HSPs are synthesized by heat sensitive cells and therefore confer some adaptive advantages to the cells to withstand heat.

F33 **CELLULASE EXISTS AS A MULTI-GENE FAMILY IN AVOCADO, Laura DeFrancesco, Mark Tucker*, and George Laties, University of California, Los Angeles, California, and *University of California, Berkeley, California**

In an effort to study the regulation of gene expression by ethylene in ripening fruit, we isolated a cDNA clone(pAV5) for the enzyme cellulase from a cDNA library of ripe avocado. Using that clone, it was shown that the message for cellulase is undetectable in unripe tissue, and accumulates as the fruit ripen. More recently, we have used pAV5, which is not a complete copy, to isolate a number of longer cDNA's for cellulase, two of which on the basis of their restriction maps appear to constitute distinct members of a gene family. This observation is corroborated by the results of hybrid select experiments. Using the two noted cDNA clones of full length or nearly full length, we have found that messages isolated with either clone from the mRNA population of ripe tissue give rise to a set of five to six proteins of identical molecular weight but different pI's. Under more stringent conditions, however, each cDNA clone hybridizes to a non-overlapping set of messages, resulting in two to three rather than the complete set of proteins. This result suggests that the cellulase gene family consists of at least two subclasses, each of which contains several genes. Preliminary experiments suggest that early in the ripening process, or in the development of the respiratory climacteric, not all of the genes for cellulase are active, but that as the process progresses, more of the family members can be detected. In order to look at the expression of the individual members of the gene family, subclones of the 3'-ends of the cDNA's are being prepared.

F34 ORGANIZATION OF GENES RAPIDLY INDUCED BY ETHYLENE DURING TOMATO FRUIT RIPENING, Jill Deikman, Sabine Cordes and Robert L. Fischer, Division of Molecular Plant Biology, University of California, Berkeley, CA 94720

The expression of specific genes increases rapidly when ethylene is applied to mature green tomato fruit. cDNA clones of three genes which are induced by ethylene in 30 minutes to 2 hours have recently been isolated (pE4, pE8, pE17; see abstract by Lincoln et al). The rapidity of this induction may indicate that these genes are involved in the fruit's initial response to ethylene. In order to identify the sequences involved in the ethylene response we have begun to study the structure and organization of these genes. Genomic clones for these ethylene-induced genes have been isolated from a library of tomato DNA partially digested with Sau 3A and cloned into Charon 35. pE4 appears to derive from a 1- or perhaps 2-copy gene and E8 consists of a family of genes with at least 3 members. Studies are underway to determine whether all 3 E8 genes are expressed in tomato fruit and whether they are all induced by ethylene. Sequencing of the gene that cross-hybridizes most strongly with pE8 is in progress. Genomic clones for pE17 have also been obtained and are being characterized.

F35 ETHYLENE-DEPENDENT CHANGES OF TWO FORMS OF MEMBRANE BOUND CELLULASES FROM BEAN ABSCISSION ZONES, Elena del Campillo and L.N. Lewis, Univ.of Calif.,Berkeley, CA 94720

Work in this laboratory has shown that membranes isolated from bean cells contain a bound form of cellulase. It was thought that this represented cellulase which is being transported outside the cell. We have now measured this enzyme during ethylene-induced abscission. Crude membranes prepared from bean abscission zones contain high levels of cellulase activity before ethylene treatment. Following incubation with 50 ul/l of ethylene, cellulase activity showed a rapid decline, giving over 50% decrease in the first day. Afterwards the decline was smaller but continued for 48 hrs, when abscission was fully developed. Upon analyzing the properties of the solubilized membrane bound cellulase we found two enzyme forms. Membranes from untreated tissue contain an acidic cellulase with a pI= 4.5-5.0. This enzyme form is a glycoprotein, shows a strong pH-dependent activity with an optimum at pH = 5.1, is inhibited strongly by 100 mM b-glucono lactone but only slightly by 100 mM cellobiose, and it is not immunoprecipitated by 9.5 cellulase antibody. After 48 hrs of ethylene, membranes contain a basic form of cellulase with a pI = 8.0-9.0 and only trace levels of the acidic form. The basic form is not a glycoprotein, shows a broad pH dependent activity between pH 4.0 to 8.0, is inhibited by 100 mM cellobiose, but not by 100 mM b-glucono-lactone, and it is immunoprecipitated by 9.5 cellulase antibody. This basic form of membrane associated cellulase is probably 9.5 cellulase which is being transported outside the cell. Thus, it appears that during ethylene-induced synthesis of 9.5 cellulase, there is also an ethylene-induced inhibition of the membrane associated acidic form the enzyme.

F36 REGULATION OF MANNANASE SYNTHESIS IN LETTUCE ENDOSPERM - A ROLE FOR ABA? Jacqueline Dulson* and J. Derek Bewley, *The University of Calgary, Calgary, Canada and The University of Guelph, Guelph, Canada.

Endo-β-mannanase is a hydrolytic enzyme produced by the endosperm of lettuce achenes to break down the cell wall carbohydrates of this tissue after germinataion of the seed. Previous studies have indicated a role for an inhibitor localized in the endosperm in the control of mannanase production. This inhibitor can be leached out of the endosperm tissue by incubation in a sufficiently large volume, and it can be replaced by exogenous abscisic acid (Halmer & Bewley, 1979). Using these three incubation conditions (i.e. large volume, small volume, large volume + ABA) we are describing the regulation of mannanase production in isolated endsperms. We have found that the increase in mannanase activity is not due to activation of the enzyme and is dependent on protein synthesis, thus indicating the enzyme is synthesized de novo. Mannanase activity is also sensitive to transcriptional inhibitors, suggestive of control at this level. We are currently examining mRNA populations by in vitro translation to define transcriptional changes coincident with mannanase production. We will also report our efforts to determine if ABA has a role in the endogenous inhibition of mannanase production. These include comparison of fluorographs of electrophoresed radiolabelled extracts of appropriately incubated endosperms, and attempts to induce ABA-specific proteins in other systems by incubation in the presence of the endogenous inhibitor from lettuce endosperms.

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F37 ETHYLENE INDUCTION OF PLANT DEFENSE RESPONSE GENES, Joseph R. Ecker, Ronald W. Davis, Department of Biochemistry, Stanford University, Stanford, CA 94305.

We report that ethylene can directly act as a signal for plants to induce genes involved in the biochemical defense against pathogens. One of the earliest detectable events to occur during the plant-pathogen interaction is a rapid increase in the key ethylene biosynthetic enzyme, ACC synthase. The subsequent increase in ethylene production, which follows such a biological stress, is proposed to be a signal for the plant to erect defense mechanisms against invading pathogens. These observations have led us to examine the effect of exogenously applied ethylene on two plant defense response genes; phenylalanine ammonia-lyase (PAL), the first enzyme in the phenylpropanoid pathway and hydroxyproline-rich glycoprotein (HRGP), the major protein component of the cell wall. We have treated whole carrot roots with ethylene (10 nl ethylene/ml air) and examined the effect on the accumulation of PAL and HRGP mRNAs using cloned carrot PAL and HRGP genes as probes. Northern blot analysis of poly(A)-containing RNA from 50h ethylene-treated roots revealed a ten-fold increase in the level of PAL mRNA compared to control air-treated roots. A more complex pattern of regulation of HRGP mRNA was evident from similar RNA analysis. Three mRNA species homologous to the HRGP gene of sizes 1.5, 1.8 and 4.0 kb were detectable in air-treated roots. After 50h of exposure to ethylene, there was a dramatic 50 to 100-fold increase in the level of the 4.0 and 1.8 kb mRNA species, while the 1.5 kb mRNA decreased in abundance during the same time period. To further define the complex pattern of HRGP transcripts, we are constructing a λgt10 cDNA library from ethylene-treated root mRNA to obtain specific probes for each of these ethylene-regulated HRGP transcripts.

F38 CONTROL OF RAPESEED EMBRYO MATURATION, Ruth Finkelstein and Martha Crouch, Biology Dept., Indiana University, Bloomington, IN 47405

During the final phase of rapeseed embryo development, the seeds desiccate and enter a period of developmental arrest. Embryos cultured before this phase do not mature, but precociously germinate. Our previous studies have demonstrated that both exogenous abscisic acid (ABA) and high osmoticum can maintain embryonic development and suppress precocious germination of cultured rapeseed embryos. We were interested in the possible roles and interrelationships of these cues in the seed. Sensitivity to ABA or osmoticum was assayed in terms of their abilities to both suppress precocious germination and maintain storage protein synthesis in cultured embryos. We found that endogenous ABA levels were highest at the time of maximum storage protein synthesis, just before the embryos began to desiccate, and that ABA sensitivity declined as the embryos matured (Finkelstein et al, Plant Physiol. 78:630-636). In contrast, culture on osmoticum approximated the seed environment throughout embryogeny. Measurements of endogenous ABA indicated that the osmotic effects on germination and gene expression were not mediated by elevated embryonic ABA. Comparison of the kinetics of osmotic and ABA effects on gene expression show that the osmotic effect is more rapid. Finally, we examined the effects of culture on ABA and/or osmoticum on the developmental potential of immature embryos by comparing their germination behavior with that of embryos matured *in situ*. Our results suggest that , although ABA may play a role in maintaining embryogeny prior to desiccation, it probably does not regulate these processes directly.

F39 POST-TRANSCRIPTIONAL REGULATION OF LHCP GENE EXPRESSION BY BENZYLADENINE, Susan Flores and Elaine M. Tobin, UCLA, Los Angeles, CA 90024.

When white light grown *Lemna* plants are placed in the dark for 6 days, levels of mRNA encoding the major chlorophyll *a/b*-binding protein (LHCP) and the small subunit (SSU) of ribulose-1,5-bisphosphate carboxylase decline to less than 10% of the initial amounts. We found that treatment of plants with a cytokinin, benzyladenine (BA), in darkness caused an increase in the abundance of both these mRNAs relative to total RNA. BA pretreatment also amplified the increase in these mRNAs due to a 1' red light pulse. However, BA treatment did not affect levels of all messages; no changes were seen in levels of RNA encoding the large subunit of RuBP carboxylase or the 32 kd herbicide binding protein, which are both made in the chloroplast, or in mRNAs encoding β-actin and an unidentified dark-specific product. When nuclei were isolated from plants harvested 2 hrs after receiving 1' red light and nuclear RNA extracted, the increase in LHCP RNA levels by BA which was consistently observed in total RNA was not seen in nuclear RNA. We therefore believe that BA is regulating the expression of this message at a post-transcriptional level, possibly by altering cytoplasmic stability. We are currently investigating possible mechanisms which might be affected by BA and could account for altered stability, such as changes in nuclease levels or differential association with polysomes. Preliminary results suggest that the increase in SSU and LHCP RNA levels after BA treatment is not a primary response to cytokinin, since pretreatments with BA for less than 8 hrs did not lead to an increase in the relative abundance of LHCP RNA relative to total RNA, either in darkness or after red light treatment.

F40 ISOLATION OF cDNA SPECIFIC TO DEVELOPING FLOWER PARTS OF TOMATO. Charles S. Gasser, Kim A. Budelier, Sheila McCormick, Dilip Shah, and Robert T. Fraley, Monsanto Company 700 Chesterfield Village Pkwy., Chesterfield, MO 63198

We are interested in studying the properties of genes which are specifically expressed during the development of flower parts. As a first step in these studies we have constructed cDNA libraries from the RNA of tomato ovaries isolated at four stages of flower development. By screening duplicate cDNA plaque filters with ovary cDNA probes and probes synthesized from seedling RNA we have identified clones specific to the reproductive organs. Northern transfers of RNA from all major plant vegetative and reproductive organs are being performed to determine which of these clones are specific to developing ovaries. The clones are to be used as probes in transcript analysis, and to isolate the corresponding genes from genomic libraries.

F41 **HORMONAL REGULATION OF α-AMYLASE GENE EXPRESSION** ,Richard Hooley,(1) and John A. Zwar, (2). (1) Weed Research Division, LARS, Yarnton, Oxford, OX5 1PF, UK (2) CSIRO, Division of Plant Industry, Box 1600, Canberra, ACT 2601, Australia

Aleurone protoplasts of Avena fatua respond to gibberellin (GA_4) by synthesizing α-amylase. This effect is antagonized by abscisic acid (ABA). Northern hybridizations using barley α-amylase cDNA reveal that α-amylase mRNA is present only in those treatments where protoplasts are secreting α-amylase, and that levels of α-amylase mRNA are correlated with levels of the enzyme.

The GA_4-induced accumulation of α-amylase mRNA, and it's prevention by ABA could result from altered gene transcription, post transcriptional processing and stability, or a combination of both of these. We have examined the effects of GA_4 and ABA on the transcription of α-amylase genes.

In an in vitro transcription system nuclei isolated from aleurone protoplasts display characteristics of a faithful DNA-dependent RNA-synthesizing system. Hybridization of ^{32}P-RNA synthesized in vitro to phage and plasmid DNA containing the barley α-amylase insert reveal that α-amylase genes are transcribed in nuclei isolated from GA_4 treated protoplasts but are transcribed at a very much lower rate, or not at all, in nuclei isolated from protoplasts incubated either without GA_4 or with both GA_4 and ABA.

F42 EXPRESSION OF GENES RAPIDLY INDUCED BY ETHYLENE DURING TOMATO FRUIT RIPENING, James E. Lincoln, Evan B. Read, Sabine P. Cordes and Robert L. Fischer, Division of Molecular Plant Biology, University of California, Berkeley, CA 94720

Ethylene biosynthesis in tomato fruits is one of the first detectable events of ripening, and is thought to initiate and coordinate subsequent ripening processes. In order to study very early fruit ripening events, we have isolated clones representing ethylene-inducible genes. To this end, we constructed a cDNA library enriched for sequences present in the ripening stage when ethylene biosynthesis has just begun. This cDNA library was differentially screened to isolate cDNA clones of mRNAs that accumulate in response to exogenously supplied ethylene. cDNA clones of three genes, which are induced by ethylene in 30 minutes to two hours, have been isolated. These three cDNA clones (pE4, pE8, pE17) are being studied for their expression in response to ethylene in fruit, as a function of fruit development, in response to ethylene in leaves, and in tomato fruit ripening mutants.

F43 Sequence Analysis of a β-Tubulin Gene from Arabidopsis thaliana Reveals an Unusual 5' Non-coding Region. M. David Marks and Donald P. Weeks, Zoecon Corporation, 975 California Avenue, Palo Alto, CA 94304.

We have begun the characterization of the structure and expression of the β-tubulin genes in Arabidopsis thaliana. In southern blot analysis, five fragments of Eco RI-cleaved Arabidopsis DNA hybridized with varing degrees of intensity to a heterologous ^{32}P labeled β-tubulin probe from Chlamydomonas reinhardtii. This suggests the possibility that the β-tubulins are encoded by a multigene family. Using the Chlamydomonas probe eight putative β-tubulin encoding clones have been isolated from an Arabidopsis genomic library (provided by Nigel Crawford, Department of Biochemistry, Stanford University). Southern analysis of Hind III cleaved DNA from four of these revealed that two appear to contain identical β-tubulin genes, whereas two others each have distinct β-tubulin genes. One of the genes has been partially sequenced. Over one third of the coding region and 150 bases of the 5' non-coding region have been analyzed. Thus far no introns have been identified and the derived amino acid sequence is 80-85% homologous to that of previously published β-tubulin sequences. The analysis revealed a very unusual 5' non-coding region. Starting at 75 bases upstream from the start codon there is a string of 12 A's, at 40 bases upstream there is a string of 10 T's and preceding the first A of the start codon there is a string of 12 A's. Complete DNA sequence analysis of this and other β-tubulin genes is underway along with S_1 nuclease analysis of transcription start sites.

F44 SEARCHING FOR MOLECULAR MECHANISMS INVOLVED IN FRUIT RIPENING, Peter H. Morgens, Jana B. Pyle, Wilbur L. Hershberger, and Ann M. Callahan, West Virginia University and USDA, Appalachian Fruit Research Station, Kearneysville, WV 25430.

Ethylene has long been associated with ripening in climacteric fruit such as tomatoes. Our goal is to understand changes in gene expression that are associated with tomato ripening well enough to attempt to modify the process by genetic engineering. During ripening there are both increases and decreases in individual RNA species as measured by in vitro protein synthesis; some of the changes are localized to either the locular area or the pericarp area of the fruit. We have isolated a number of ripening-specific cDNA clones from a large cDNA library and are characterizing their respective RNA sequences as to developmental timing and localization within the fruit. Preliminary results indicate that various developmental programs are evident. We hope to present information pertaining to the questions of whether DNA rearrangements or specific methylation of the DNA may be involved in the expression of the developmentally modulated genes. We have constructed a large genomic library from tomato fruit DNA and plan to isolate the genes and hence the promoter sequences corresponding to our ripening-specific cDNA clones. We hope to determine whether coordinately regulated genes share common regulatory sequences.

F45 ABSCISIC ACID INDUCTION IN DEHYDRATED PLANTS, John E. Mullet, Felix Guerrero, Patricia Gamble and Bob Hensen, Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas 77843.

Abscisic acid levels in excised pea plants increase 50-100 fold when dehydrated to near zero turgor. Pretreatment of plants with inhibitors of transcription (actinomycin D, cordycepin) or cycloheximide, an inhibitor of protein synthesis on 80S ribosomes, inhibited the dehydration induced increase in ABA. This result suggests that nuclear gene transcription is required for increased synthesis of ABA in dehydrated plants. The biosynthetic precursor to ABA in dehydrated plants was examined. Plants treated with fluridone (an inhibitor of carotenoid biosynthesis) and grown in the light are bleached, show chloroplast abnormalities (cf. Fedtke, 1982; Wright & Corbett, 1979) and reduced ability to synthesize ABA (cf. Hensen, 1984; Quarrie & Lister, 1984). To distinguish between fluridone effects on carotenoid biosynthesis and photobleaching damage to chloroplasts we treated barley with fluridone in darkness. Fluridone treatment inhibited carotenoid biosynthesis and the dehydration-induced increase in ABA levels without changing plastid numbers, size or polypeptide composition. This result is consistent with the hypothesis that carotenoids are precursors to ABA in dehydrated plants. Changes in poly A^+RNA populations which occur during the dehydration induced increase in ABA have been analyzed by 2D gel separation of in vitro translation products. The time course of changes in pea leaves and the elongating region of soybeans hypocotyls will be presented.

F46 THE CONSTRUCTION OF VECTORS FOR THE CLONING OF PLANT GENES BY PHENOTYPIC COMPLEMENTATION, Neil E. Olszewski, Jim Hu, Joanne Chory and Frederick Ausubel, Department of Genetics, Harvard Medical School and Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114

We have constructed cosmid-cloning vectors which contain either the right border or both the right and left border from the T-DNA region of the Ti plasmid of Agrobacterium tumefaciens. In addition to these features, these vectors contain: (i) a ColE1 replicon; (ii) a broad host range replicon; (iii) a mob region; (iv) bacterial selectable markers; (v) and a plant selectable marker. These features allow one to construct cosmid libraries in E. coli and mobilize these libraries into A. tumefaciens where the vir funtions, which are provided in trans on the Ti plasmid, direct the transfer of the cloned DNA into the plant genome.

We have demonstrated that these vectors can be used to transform plants. We have constructed genomic cosmid libraries of Arabidopsis thaliana in these vectors. We will discuss the use of these libraries to clone plant genes which code for gibberellic acid biosynthesis enzymes by phenotypic complementation of mutations in these genes.

F47 NUCLEAR AND PLASTID GENE EXPRESSION DURING TOMATO FRUIT DEVELOPMENT AND RIPENING

Birgit Piechulla and Wilhelm Gruissem, Department of Botany, University of California, Berkeley, CA 94720, USA.

Fruit development and ripening in tomato invólves a series of complex biochemical and physiological changes affecting several cell compartments. Drastic alterations occur within the plastids which change their structure and function during fruit maturation. We have studied the expression of plastid genes during tomato fruit ripening. The transcript levels for most of the photosynthetic genes encoded on the plastid genome decrease during the ripening process. However, the mRNA level for the 32 kD 'herbizide binding' protein is significantly higher in red fruit preparations compared to those of other plastid encoded photosynthetic proteins.

The concentration of various photosynthetic proteins of PSI, PSII, electron transport chain and stroma also decrease during plastid differentiation. We therefore conclude that transcription and translation of photosynthetic genes/proteins are not continued or reduced during the ripening process.

Little is known about the coordination of the plastid and nuclear gene expression during fruit maturation. We have compared transcript levels for proteins which are nuclear encoded (chl a/b binding protein - cab; small subunit of RuBP Carboxylase - rbcS) with transcript levels for their counterpart proteins in the plastid (32 kD protein - psbA, large subunit of RuBP Carboxylase - rbcL) during fruit development and ripening. The maximum of plastid and nuclear transcript levels appear to be in 14 to 30 and 7 to 14 day old tomato fruits, respectively. No nuclear gene transcripts (cab, rbcS) are detectable in mature green fruits, while significant amounts of plastid mRNAs (psbA, rbcL) are still detectable. It appears, therefore that inactivation of nuclear genes for photosynthetic polypeptides precedes the inactivation of plastid genes during fruit maturation.

F48 DEVELOPMENTAL REGULATION OF GENE EXPRESSION IN Zea mays EMBRYOS.

P. Puigdoménech, D. Sánchez and M. Pagès. Departament de Genètica Molecular, C.I.D., CSIC. Jorge Girona Salgado, 18. 08034 Barcelona, Spain.

The synthesis of polypeptides has been studied in the maturation of corn embryos, in dry embryos and in the early germination. Different sets of polypeptides can be defined according to their pattern of synthesis "in vivo" in the different periods of development studied. 2-dimensional electrophoresis of the labelled polypeptides has been used for the analysis of the products of translation. In particular a subset of proteins synthesized at the earlier times of germination (2 hours) has been studied, it appears that their mRNA is present in the dry seeds and the late stages of embryo maturation but not in the immature seed before 30 days after pollination. "In vitro" translation shows that some of the polypeptides may be a product of post-translational modification of the proteins. Their appearance id not sensitive to α-amanitin during germination but their biosynthesis can be induced by ABA treatment of the immature embryo.

F49 AUXIN MODULATION OF PROTEIN SYNTHESIS AND PROTEIN PHOSPHORYLATION DURING SEED GERMINATION, Estela Sánchez-de-Jiménez, Laura Pérez and Raúl Aguilar. Depto.de Bioquímica Vegetal. Facultad de Química. UNAM. México, D.F. 04510. México.

Exogenous auxin modifies important biochemical processes during seed germination. These alterations in the long run, cause callus formation. The effect of 2(2-methyl, 4-chloro) phenoxy propionic acid (MCPP), an analogue of 2,4-D, on protein synthesis and protein phosphorylation was analysed during maize germination. Disected maize embryonic axes were incubated in nutrient medium either with ^{14}C-amino acid mixture or ^{32}P-orthophosphate, with and without the auxin. Cytoplasmic and nuclear proteins were analysed for ^{14}C or ^{32}P incorporation into TCA precipitable material. Results indicated MCPP stimulates both protein synthesis and protein phosphorylation. The proteins synthesized in the presence of MCPP are highly modified as shown by the fluorografies of the two-dimensional gel electrophoretic patterns, the main feature being the disapearance of several proteins. The synthesis of some proteins, however, is at the same time specifically stimulated, such as histones, which were immunologically identified. The stimulatory effect on protein phosphorylation is mainly quantitative. The normal phosphorylated proteins are increased by MCPP, as judged by Shephadex G-100, and DEAE column chromatographies, as well as by analysis of these proteins with two-dimensional gel electrophoresis and fluorography.

F50 CLONING OF BEAN ABSCISSION CELLULASE mRNA USING A FULL LENGTH cDNA CLONE OF AVOCADO CELLULASE AS A PROBE, Mark L. Tucker, Lowell N. Lewis, and Mary L. Durbin, Univ. of Calif., Berkeley, CA 94720

Previous research in this laboratory had shown that antisera made against bean abscission cellulase (9.5 cellulase) also immunoprecipitates native avocado fruit cellulase. Recent studies show that the 9.5 cellulase antisera immunoprecipitates an in vitro translation product of poly(A)$^+$ RNA from bean abscission zones and ripe avocado fruit. Both immunoprecipitates have a molecular weight of approximately 53 kD. Christoffersen, Tucker, and Laties (Plant Molec Bio 3:384-392) isolated and characterized a 0.6 kb cDNA clone (pAV5) of avocado fruit cellulase mRNA. However, gel blots of bean abscission RNA probed with pAV5 gave negative results when hybridized at low stringency indicating that this avocado cDNA probe did not share homology with any bean abscission message. To overcome the possibility that pAV5 codes for a nonconserved region of cellulase mRNA we prepared and screened a cDNA library of ripe avocado mRNA to obtain a near full length clone of the avocado cellulase mRNA. A 2.2 kb avocado cDNA clone (pAV3-63) having homology with a cellulase mRNA of approximately 2.2 kb was isolated and sequenced. This longer clone was used to probe a 750 colony cDNA library of bean abscission zone mRNA. Several bean cDNA clones sharing partial homology with pAV3-63 have been identified, and we are currently characterizing these clones.

Methods

F51 THE ROLE OF THE SUSPENSOR AND GIBBERELLIN IN THE REGULATION OF PROTEIN CONTENT AND SYNTHESIS IN PHASEOLUS EMBRYOS, Tom Brady, Hamilton College, Clinton, NY 13323

Circumstantial evidence exists for the suspensor's playing a role in the nutrition of globular and heart stage embryos of the French bean, Phaseolus vulgaris. Using the Prussian blue technique, we have demonstrated that the suspensor is a major route of nutrients into the early bean embryo. In tissue culture of heart stage embryos (0.2 and 0.5mm) the attached suspensor is necessary for the maintenance of protein content in these cultured embryos, however it may be replaced by GA_3 at concentrations from 10^{-4} to 10^{-5}M (Brady and Walthall, Dev. Biol. 107:531-536,1985). The attached suspensor is also necessary for the maintenance of protein synthesis in these heart staged embryos, however the same pattern of synthesis is observed in autoradiograms of SDS-PAGE of beans cultured attached to their suspensors as those incubated in the presence of GA_3 (Walthall and Brady, Cell Dif. in press). Thus the suspensor may function in providing gibberellin, which is either synthesized in the suspensor or is transported into the suspensor from the sporophyte tissue, to the early plant embryo.

F52 MONOCLONAL ANTIBODY-BASED APPROACH TO DISTINGUISH CLASSES OF CYTOKININS David L. Brandon, Joseph W. Corse, and Arieh Maoz, USDA Western Regional Research Center, Albany, CA 94710, and ARO Volcani Center, Bet Dagan 50250, Israel.

We hypothesize that it is the amount and localization of specific cytokinins, rather than the level of total cytokinins, which determine the physiological responses mediated by these hormones. As a first step in applying immunoassay to the quantitation of individual classes of cytokinins (the purines, ribosides, ribotides, and glucosides), we have synthesized derivatives of natural cytokinins for use as haptens in eliciting antibodies which can differentiate between classes of cytokinins. 9-Carboxyethyl derivatives of isopentenyladenine, dihydrozeatin, and E-zeatin were synthesized using 6-chloropurine as starting material. The derivative of E-zeatin was coupled to proteins to produce an immunogen and ligands for solid-phase immunoassays. A series of monoclonal antibodies were prepared in this manner, and analyzed along with a panel of antibodies raised with conjugates of periodate-cleaved E-zeatin riboside. There are two major patterns of antibody specificity, one in which the purine and riboside are bound equivalently, and one in which the riboside is bound preferentially, permitting selective assay of this compound. Various assay formats have been compared, and applications to the study of plant senescence will be discussed. (Supported, in part, by BARD grant US-711-83)

F53 **Potential Role for GA_3 in the Stabilization of α-amylase mRNA of Barley Aleurone.** M. Brodl, F. Belanger, T.-H. D. Ho, Biology Department, Washington University, St. Louis, MO 63130.

In response to the phytohormone GA the aleurone layers of barley seeds synthesize and secrete α-amylases, which are encoded by a set of stable mRNAs (half life >100 h). GA also initiates the formation of ER lamellae for the synthesis and secretion of α-amylase and other hydrolases. Under heat stress the synthesis and secretion of α-amylase is suspended and ER lamellae are destroyed. Time course studies reveal that the destruction of ER lamellae and α-amylase mRNA are coincident. During recovery their reappearance is also coincident. Inhibition of heat shock protein (hsp) synthesis during heat shock reveals that hsp are not responsible for the observed destruction of ER lamellae or α-amylase mRNA. Other secreted hydrolase mRNAs are also degraded during heat stress while mRNAs for proteins translated on free ribosomes are unaffected. These observations indicate that the GA-stimulated proliferation of ER lamellae may be required for stabilization of GA-stimulated α-amylase mRNA transcripts.

F54 Auxin Resistant Mutants of Arabidopsis With a Dramatic Developmental Phenotype Mark A. Estelle and Chris R. Somerville. MSU-DOE Plant Research Lab, Michigan State University, East Lansing MI. 48824

In order to identify genes important in the metabolism and action of indole acetic acid (IAA) we have isolated 13 mutants of Arabidopsis thaliana with resistance to the artificial auxin 2,4-dichlorophenoxyacetic acid (2,4-D). All but one of these mutants has dramatic alterations in growth behavior including a reduction in secondary root initiation, loss of apical dominance, and partial to complete male sterility. Complementation analysis has shown that at least 8 of the mutants are altered at a single locus. We have named this gene aar-1 for altered auxin response.

Mutations at aar-1 alter the response of growing roots to exogenously applied 2,4-D or IAA. In addition the mutants exhibit reduced 2,4-D induced ethylene biosynthesis from mature leaves and form callus poorly under standard conditions. Evidently the 2,4-D resistance is not a trivial consequence of the altered growth habit. Rather, we believe that both resistance to auxin and the developmental aberrations are due to a change in a protein involved in some aspect of IAA metabolism or response.

To determine whether these mutations are affecting auxin metabolism or response we have examined the metabolism of radiolabelled 2,4-D and have detected no significant differences in the fate of exogenously applied compound.

The accumulated evidence suggests that mutations at the aar-1 gene affect the plant's ability to respond to auxins. We plan to proceed by analysing auxin binding components in both wildtype and aar-1, and by isolating DNA sequences which restore normal callus forming ability to mutant tissue.

F55 STUDYING PLANT CELL GROWTH AT THE CELL, TISSUE AND ORGAN LEVEL, Richard D.Firn, Department of Biology, University of York, England, YO1 5DD.

Much of our thinking about plant cell growth emerged from work conducted over the last five decades on the response of isolated organs to plant growth substances. It was widely accepted that an understanding of the way in which an isolated piece of organ responded to an added growth substance would provide useful information as to how growth was regulated in both cells and intact organs. There are now reasons to doubt whether such an approach is adequate. Firstly cell heterogeneity has usually been overlooked, indeed all too often organs have been regarded as large, single cells. Is the complex response of some organs simply the consequence of separate responses in different cells? Secondly, too much attention has been paid to ways in which cells might show a growth promotion when studies of growth rate changes causing real developmental changes often demonstrate that a reversible growth cessation or reduction is more important. Finally, studies of the growth distribution in intact plants have shown that even in organs apparently growing uniformly, cells rarely grow at a constant rate for prolonged periods. In etiolated hypocotyls, for instance, all cells follow a complex pattern of growth rate changes. What kind of controls must be postulated in such systems? These important developmental changes will represent a real challenge to those studying the molecular biology of the control of plant cell growth. However, the emerging methodologies will offer those interested in cell heterogeneity a real chance to probe developmental responses in groups of cells rather than whole organs.

F56 RAPID PLANT REGENERATION FROM NICOTIANA MESOPHYLL PROTOPLASTS, Ebrahim Firoozabady, Agrigenetics Corporation, Advanced Research Division, 5649 East Buckeye Road, Madison, WI 53716

A procedure is described for rapid plant regeneration from tobacco mesophyll protoplasts. Large numbers of diploid plants are regenerated from single colonies derived from individual protoplasts in about five weeks. This procedure avoids occurrence of somaclonal variation which is usually present in plants obtained from conventional techniques.

F57 INDUCTION OF VIVIPARY IN DEVELOPING Zea mays KERNELS BY CPTA, 2-(4-chlorophenylthio)triethylamine. Franklin Fong and J. D. Smith, Soil & Crop Sciences Dept., Texas A&M Univ. College Station, TX 77843

CPTA is a water soluble inhibitor of cyclization reactions in carotenoid biosynthesis during kernel development. CPTA, topically applied at 9,11,and 13 days after pollination resulted in red colored seeds which accumulated lycopene, instead of β-carotene and xanthophylls as the major colored carotenoid. When treated kernels were allowed to mature in the field a high percentage of vivipary resulted, 39,% 62%, and 84%, in 3 separate ears. The abscisic acid content of 15 DAP embryos was reduced by 50% in CPTA-treated kernels. These results in conjunction with earlier studies with fluridone, a lipophyllic inhibitor of carotenoid desaturation reactions, and with vp mutants (vp-2, vp-5, vp-7, w-3) provide further evidence that carotenoids may be converted to abscisic acid in developing maize kernels.

F58 FUNCTIONAL ANALYSIS OF THE T-DNA ONC GENES, Dirk Inzé, Anders Follin, Harry Van Onckelen*, Patrick Rüdelsheim*, Marc Van Montagu and Jeff Schell, Laboratorium voor Genetica, RUG, B-9000 Gent; *Dept. voor Biologie, UIA, B-2610 Wilrijk (Belgium)

Agrobacterium tumefaciens causes crown gall tumor formation by the introduction of a well defined DNA segment, called T-DNA, in the plant nuclear genome. The combined activities of the T-DNA genes 1 and 2 have an auxin-like effect. We have isolated Nicotiana tabacum plants transformed with either gene 1 (called rG1) or gene 2 (called rG2), as the only T-DNA gene. Both rG1 and rG2 plants grow and develop in a normal fashion. These observations lead to the following conclusions. First, normal plant cells do not contain a compound, such as indole-3-acetamide (IAM), which can be converted to a biologically active auxin by the gene 2 product. Second, Nicotiana tabacum cannot convert the auxin precursor synthesized by the gene 1 product into IAA at a physiologically significant rate. The IAM content of rG1 plants was about 500-1000 times higher (about 1500 pmol/g fresh wt) compared to an untransformed control plant. These data lead to the conclusion that the DNA gene 1 is responsible for the accumulation of IAM in transformed plants. A similar auxin synthesis pathway (tryptophan → IAM → IAA) has been described for the plant pathogenic bacterium, Pseudomonas savastanoi. We could show that the P. savastanoi iaaM gene (encoding tryptophan-2-monooxygenase) can mimic the phenotypic effect of gene 1. This was demonstrated by infecting rG2 plants, with an E. coli strain expressing the iaaM gene of P. savastanoi. In all infections we performed, we found large outgrowths which sprouted numerous roots. No such proliferations were observed when the same E. coli strain was inoculated on rG1 or on normal tobacco plants.

F59 IMMUNOCYTOCHEMICAL LOCALIZATION OF THE AUXIN TRANSPORT CARRIER USING MONOCLONAL ANTIBODIES, Mark Jacobs, Department of Biology, Swarthmore College, Swarthmore, PA 19081

Monoclonal antibodies (Abs) can be obtained against the protein that binds the auxin transport inhibitor naphthylphthalamic acid (NPA) in pea stem tissue. The Abs can be used to demonstrate the distribution of that protein within stem cells and to partially purify and begin to characterize it. Since the solubilized NPA binding protein also binds auxin it is likely that it is the same protein as, or co-solubilizing and interacting with, the auxin anion efflux carrier operating during polar auxin transport. The techniques and inherent problems of : (a) screening for monoclonal Abs using hormone binding assays, (b) indirect immunofluorescence localization in plant stem tissue, (c) immunoprecipitation experiments and Western blot immunoassays using monoclonal Abs will be discussed.

F60 REGULATORY PROCESSES INVOLVED IN THE SWITCH FROM A DEVELOPMENTAL TO A GERMINATIVE PROGRAMME.

Allison R. Kermode and J. Derek Bewley. Department of Botany, University of Guelph, Guelph, Ontario, N1G 2W1, Canada.

Seeds removed from the capsule at 50 days after pollination (DAP) and later will germinate when placed in water, whereas developing seeds at 25-45 DAP require a desiccation period before germination can occur. This switch from development to germination elicited by premature drying is mirrored by a redirection in metabolism within the endosperm of the castor bean seed. Isolated endosperms of 30 and 40 DAP seeds, which have not completed all developmental events, are tolerant of premature drying and will respond upon subsequent rehydration in a manner which is characteristic of an endosperm from a germinating mature seed. In particular, the pattern of soluble and insoluble protein synthesis upon rehydration of dried 30 and 40 DAP seeds is identical to that of hydrated mature endosperms: proteins characteristic of development cease to be synthesized while those associated with germination and growth are then produced. The induction of enzymes essential to the post-germinative (growth) phase of seedling development occurs as a consequence of premature drying. Such changes may occur because drying affects the hormonal balance of the seed: ABA breakdown may be enhanced by the natural drying process. Or, drying could decrease the sensitivity of the seed (embryo) to ABA, resulting in a loss in competence to respond to this hormone (perhaps because of a change in receptor levels or conformation).

F61 ISOLATION OF AUXIN MUTANTS IN VITRO, Patrick J. King, Anne D. Blonstein, Jurg Oetiker, Friedrich Miescher-Institut, Postfach 2543, 4002 Basel, Switzerland.

Cell clones arising from mutagen-treated haploid leaf protoplasts were tested for an induced auxin requirement. One temperature-sensitive auxin auxotroph was found amongst 60'000 clones of Hyoscyamus muticus. Regenerated shoots have the same phenotype. The trait is recessive in fusion hybrids with other ts and amino-acid auxotrophs. This variant is rescued by IAA but analysis with HPLC/RIA has shown that variant cells can synthesise and accumulate IAA to wildtype levels. Five non-ts auxin auxotrophs found by testing 56'000 clones of Nicotiana plumbaginifolia are stable, have an absolute requirement for auxin and are not cross-fed by wildtype cells. Regenerated shoots are abnormal. Complementation studies by protoplast fusion are in progress.

F62 **Regulation of Gene Expression by ABA in Barley Aleurone Layers**. Liang-Shiou Lin and Tuan-Hua David Ho, Department of Biology, Washinton University, St. Louis, MO

The plant hormone abscissic acid (ABA) plays an important role in the development and germination of higher plant seeds. In barley aleurone layers, it is well documented that ABA inhibits the GA-enhanced synthesis of α-amylase and other proteins. In addition to its inhibitory role, ABA also induces a new set of proteins. It has been suggested that some of these proteins may be involved in the self-induced conversion of ABA to phaseic acid which is probably the active component of this hormone in aleurone layers. Here we report the time course, the dosage response, and cellular localization of these ABA-induced proteins.

There are at least 4 induced proteins on one-dimensinal SDS gel, and considerably more on a two-dimensinal gel. The most abundant one is the 27 kD protein which is also synthesized in tissue incubated without ABA. These proteins start to show enhanced synthesis as early as 2 h after ABA treatment, and the synthesis continues up to 48 h, although different proteins peak at different times. *In vivo* synthesis of the 27 kD protein declines as ABA concentration decreases, with 10^{-9}M ABA approaching control level. *In vitro* translation using total RNA isolated from ABA treated aleurone layers indicate that translatable mRNA levels more or less parallel protein levels both in time couse and in dosage response.

Three of the ABA induced proteins, including the 27 kD one, are found in 1,000 g centrifugation pellet, suggesting that they are associated with nucleus or protein bodies. One protein is found in the 80,000 g supernatant, and is probably a soluble protein. The 27 kD protein appears to be sensitive to sulfhydryl type proteases because it is protected from degredation by leupeptin and bromate but not by PMSF. As expected, the induction of these proteins by ABA is supressed by GA. Work is underway to purify the 27 kD protein and to determine whether it is a barley lectin which has a similar molecular weight.

F63 SIGNAL TRANSDUCTION ACROSS PLASMA MEMBRANES OF CULTURED CELLS, Philip S. Low and Peter F. Heinstein, Purdue University, West Lafayette, IN 47907

Cultured cotton cells (Gossypium arboreum) produce phytoalexins in response to stimulation by autoclaved extracts of verticillium cell walls; however, the mechanism of elicitor signal transduction across the plant cell's plasma membrane has not been explored. In order to determine whether ion gating might be involved in the transmembrane signalling, fluorescent probes of membrane potential and pH were incubated with the cultured cells and their fluorescence intensities were monitored following stimulation with the verticillium elicitor. In all cases, the fluorescence remained constant for a brief "lag period", after which a dramatic change in fluorescence intensity was observed. The length of the lag period was found to be logarithmically related to the amount of elicitor added, while the rate of the fluorescence change reached a maximum at intermediate elicitor concentrations and decreased gradually at either higher or lower concentrations. Ionophores which depolarized the cell membrane mimmicked the effect of the elicitors in the fluorescence assay, suggesting the verticillium extract promoted a decrease in the cell's membrane potential. This change in membrane potential is readily explained if the elicitor stimulates ion gating across the plant's plasma membrane. Importantly, the rate of fluorescence change correlated directly with the phytoalexin content of the cultured cells assayed 24 hrs. after stimulation, suggesting the procedure can be used to quantitatively assay phytoalexin elicitors in cultured cells.

F64 MUTATIONAL AND MOLECULAR ANALYSIS OF POLYAMINE SYNTHESIS IN TOBACCO, Russell L. Malmberg, Botany Department, University of Georgia, Athens, Georgia 30602

We have used somatic cell genetic techniques to isolate mutants that have lesions in the polyamine synthesis pathway. Specific inhibitors exists for 4 of the 6 enzymes that lead to putrescine, spermidine, and spermine. We have isolated mutants that are resistant to DFMO, an ornithine decarboxylase inhibitor, and ones that are resistant to MGBG, a SAM decarboxylase inhibitor. We also have isolated a temperature sensitive mutant. Plants regenerated from the mutants display a variey of growth defects, including short internodes and developmental switches in the flower. Most of the mutants are completely sterile, but genetic analysis of two mutants indicates they are nuclear dominant, and that the floral abnormalities co-segregate with the polyamine lesion. Ornithine decarboxylase is a floral specific enzyme in tobacco. Two independent variants with near zero levels of enzyme activity do not flower, but a revertant that restores ODC activity does flower. This suggests that ODC is required for flowering. Mutants that affect the levels or structure of SAM-DC display switches in the flower, such as stigmoid anthers, petaloid anthers, leafy sepals, stamenoid ovules, petaloid sepals, and extra petals, as well as more complex rearrangements. --- We have obtained a cDNA clone that may encode the enzyme argnine decarboxylase, by differential hybridization to mRNA from tissues that are induced or repressed for the enzyme. The cDNA clone is regulated by stress, by exogenous polyamines, and is differentially expressed in leaves vs. flowers.

F65 THE MESSENGER RNA POPULATIONS IN THE EMBRYONIC AXES OF PHASEOLUS VULGARIS DURING DEVELOPMENT AND FOLLOWING GERMINATION. Santosh Misra and J. Derek Bewley*. 1985. Department of Biology, University of Calgary, Calgary, AB T2N 1N4 Canada
*Dept. of Botany, University of Guelph, Guelph, ON N1G 2W1 Canada.

Messenger RNAs were extracted from young, mid-maturation, late maturation, mature dry and 20 h germinated embryonic axes of Phaseolus vulgaris var. Taylor's Horticultural. They were translated in vitro in a rabbit reticulocyte lysate protein synthesizing system. Analysis of the translation products using two-dimensional polyacrylamide gel electrophoresis indicated that there were substantial changes in the messenger RNA population of the developing and germinating axes. The number of polypeptides synthesized increased sharply at 20 - 22 days after pollination and then declined. There was a parallel increase and decrease in the Poly(A)$^+$ content of the seed axes. The analysis showed that certain messages were present throughout development and were stored in mature dry seed. Some messages appeared during mid-maturation but declined during later stages of development and were absent from the mature seed. Using a recombinant probe for phaseolin messenger RNA, we find that levels of β-phaseolin mRNA is high in mid-maturation stages, but declines in abundance as the seed matures. In the germinating seed a set of messages unique to germination appeared.

F66 IMMUNOAFFINITY PURIFICATION OF PLANT HORMONES AS AN ANALYTICAL TOOL, Roy O. Morris and Elizabeth M.S. MacDonald, Department of Agricultural Chemistry, Oregon State University, Corvallis, OR 97331

Polyclonal and monoclonal antibodies to a number of cytokinins have been raised and used to construct column matrix materials useful for single-pass isolation of cytokinins from heavily contaminated plant or bacterial extracts (1,2). Individual antibodies have different affinities for different cytokinins, but may be combined to prepare columns of the desired specificity. Recoveries are generally excellent (>70%) and extensive purification of cytokinins is achieved from materials as diverse as bacterial culture filtrates (Agrobacterium tumefaciens and Pseudomonas savastanoi), aquatic monocots (Lemna gibba), developing wheat spikes and wheat grain, and Douglas-fir vegetative and cone-bearing apices. The antibodies also form the basis of a rapid and effective method for the assay of the prenyl transferase from Agrobacterium tumefaciens responsible for the synthesis of iPA in crown gall tumor tissue (3).

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F67 EFFECTS OF ABA ON THE EXPRESSION OF α-AMYLASE ISOZYMES IN BARLEY ALEURONE LAYERS. Randall C. Nolan, L.S. Lin and Tuan-hua David Ho. Dept. of Biology, Washington University, St. Louis, MO, USA 63130

The addition of gibberellic acid(GA) to barley aleurone layers results in the synthesis of two groups of α-amylase isozymes, which have different pI's. Addition of abscisic acid(ABA) at the same time as GA inhibits the synthesis of both groups of isozymes. However, ABA selectively inhibits high pI synthesis when it is added 12 hrs or more after GA. This mid-course inhibition is detectable within 2 hrs of ABA addition. Inhibitors of transcription and translation block the late-addition effects of ABA. Addition of ABA has no effect on the secretion of previously synthesized α-amylase isozymes. Northern analysis was performed using 2 α-amylase cDNA's, pM/C and clone E , which represent messages for the high and low pI groups respectively. Subclones of these 2 cDNA's containing 95 and 93 bp. sequences that are nonhomologous between the 2 cDNA's were also used. Hybridization results of both pM/C and its subclone paralleled the protein synthesis results for the high pI isozymes. Hybridization of the high pI probes to aleurone RNA decreased drastically after mid-course addition of ABA. However, hybridization to the low pI probes was not greatly diminished by mid-course ABA addition. These data show that ABA inhibits α-amylase expression at the mRNA level rather than by translational control. The data also indicate that mid-course ABA addition decreases α-amylase mRNA stability.

F68 CHARACTERISATION OF A TEMPERATURE-SENSITIVE, AUXIN-AUXOTROPHIC MUTANT OF HYOSCYAMUS MUTICUS. Juerg Oetiker and Patrick J.King, Friedrich Miescher-Institut, P.O. Box 2543, 4002 Basel, Switzerland

A tissue culture line of H. muticus has been isolated after MNNG treatment of haploid protoplasts which fails to grow at temperatures above 29°C unless supplied with an auxin. Shoots regenerated from this line bleach and die after transfer to restrictive temperature. This trait is recessive in protoplast fusions. The line was recloned from protoplasts and ca. 10^6 colonies examined for reversion. No revertants were detected.The addition of indoleacetic acid-ethylester at 0.25μM is sufficient to give 50% of normal growth at the restrictive temperature. But this line could not be rescued by other medium supplements such as cytokinin, amino acids, purines and pyrimidines, vitamins or elevated concentrations of ammonium-nitrate and sucrose. Current experiments involve:

a) Analysis of the metabolism of indoleacetic acid (IAA) in the mutant.
   Tracer experiments feeding (2-^{14}C)-IAA to the mutant and analysis of its metabolites by HPLC revealed no differences between the wild-type and the mutant to which its temperature-sensitive phenotype could be causally ascribed.
b) Analysis of the metabolism of L-(5-^{3}H)-Tryptophan and (2-^{14}C)-Indole in the mutant.
c) Analysis of the levels of endogenous IAA in the mutant.
   The pool size of free IAA in the mutant after transfer to restrictive temperature was determined by RIA after subsequent partitioning and purification of the extracts by HPLC. The IAA content (5-60 pmoles/g fresh weight) did not vary significantly from the wild-type.
d) Transformation of mutant cells with genes 1 & 2 of the T-DNA of A. tumefaciens.

F69 THE USE OF THE TRANSPOSABLE ELEMENT, ROBERTSON'S MUTATOR (MU), TO ISOLATE GENES CONTROLLING DWARFISM IN MAIZE. Bernard O. Phinney and Clive Spray. Department of Biology, University of California, Los Angeles 90024.

In maize the five nonallelic dwarfing genes, d1, d2, d3, d5 and an1, control specific and different steps in the GA biosynthetic pathway leading to the key bioactive gibberellin, GA_1. At maturity mutants are 1/3 to 1/5 the height of normal plants; mutant phenotypes are expressed from the early seedling to maturity; both in the dark and in the light. Addition of exogenous GA results in normal growth to give plants indistinguishable from normals. Such mutants provide powerful tools for the analysis of hormone-dependent growth and differentiation.

We(Phinney, Robertson, Freeling, 1985) have recovered five GA-responding dwarf mutants from Robertson Mutator (Mu) lines. The dwarf mutants are presumably the products of the insertion of Mu into a region of the genome associated with GA biosynthesis. These mutants are now being used to define their relative positions in blocking specific steps in the GA biosynthetic pathway. They are also being used to isolate the dwarfing genes. We have used Mu1(cloned by Bennetzen et al, PNAS 81:4125, 1984) as a probe to locate sequences in our material homologous to Mu1. Southern blot analsis show Mu1 copy number in our material to be fewer than 20 per genome. One (Mu)dwarf plant was found to have only three copies. We are now constructing a genomic library as a first step in the isolation of specific dwarfing genes.

F70 PURIFICATION AND CHARACTERIZATION OF ACC SYNTHASE FROM WOUNDED TOMATO PERICARP, Laura Stein Privalle, CIBA-GEIGY Biotechnology Research, Research Triangle Park, NC 27709

ACC (1-aminocyclopropyl-1-carboxylic acid) synthase catalyzes the rate limiting step in the biosynthesis of the plant growth regulator, ethylene. This enzyme is present in low quantities and to date has proven very difficult to purify to homogeneity. We report significant advances in the purification of ACC synthase from wounded pink tomato pericarp. Techniques utilized included: ammonium sulfate fractionation, DEAE-cellulose ion exchange chromotagraphy, phenyl sepharose hydrophobic chromatography, gel permeation, reverse phase HPLC and affinity chromatography. Preparative isoelectric focusing, although not useful as a purification step indicated an isoelectric point around neutrality. The highest specific activity, obtained after phenyl sepharose, was 391 nmoles ACC/hr.mg protein. This represents a 400 fold purification. Further purification is achieved at the expense of specific activity with approximately 2% of the original activity being recovered. Kinetic pH variation studies indicated that lysine (with an apparent pK of 9.0) is the catalytically important amino acid. Assay conditions were optimized with respect to temperature, duration and pH. A Q_{10} (between 25 and 35°C) of 2.5 was found.

F71 AN ENZYME-IMMUNOASSAY FOR QUANTITATIVE ANALYSIS OF ABSCISIC ACID IN WHEAT Natasha V. Raikhel, D. Wayne Hughes, Barry A. Palevitz and Glenn A. Galau, Botany Department, University of Georgia, Athens, GA 30602, U.S.A.

Wheat germ agglutinin (WGA) has been identified as a wheat embryo-specific protein, and its synthesis and localization during grain development appears to be under abscisic acid (ABA) control. ABA enhances the accumulation of WGA in adult plants and the lectin level can be decreased if plants are treated with Fluridone (Raikhel et al., 1985, Pl. Physiol. in press), an herbicide which reduces endogenous ABA in maize embryos (Fong et al., 1983, Pl. Physiol. 73:899). Consequently, we wished to determine if Fluridone reduces the level of endogenous ABA in wheat embryos, and perhaps thereby reduces WGA levels.

We developed ELISA assays using procedures of Weiler (1982, Physiol. Pl. 54:510). It is based on competitive binding between free and alkaline phosphatase-labeled ABA with monoclonal (Idetek, Inc.) or polyclonal (Miles, Inc.) antibodies. The monoclonal antibody is directed against (±)ABA(C-4)BSA (Clone C5; Mertens et al., FEBS Let. 160:269), and should see free (+)ABA. The polyclonal antibody is directed against (±)ABA(C-1')HSA, and should see predominantly free and bound (-)ABA (Walton et al., 1979, Planta 146:139; Weiler, 1980, Planta 148:262). As predicted, in our hands the monoclonal antibody sees essentially only (+)ABA, and the polyclonal antibody sees essentially only (-)ABA. The detection limit of the monoclonal-based assay is 16 pg (60 fmole) free (+)ABA (160 pg/ml).

Partially purified ABA from wheat extracts behaved similarly to (+)ABA and (±)ABA standards. Seedlings grown on nutritional medium have about 300 ng (+)ABA/g fresh weight in the shoot-base region. Treatment with 10ppm Fluridone reduces (+)ABA to one-half this level. The estimates of free (+)ABA obtained with ELISA were virtually identical with those using gas chromatography with a Tracor 550 gas chromatograph and a ^{63}Ni electron detector, an assay which does not distinguish (-) and (+)ABA.

We are using ELISA to correlate levels of endogenous ABA and WGA during wheat embryogenesis, as well as during similar ABA-regulated events in cotton embryogeneis.

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F72 ISOELECTRIC FOCUSING OF OAT ROOT MEMBRANES

Richard G. Stout, Montana State University, Dept. of Biology, Bozeman, MT 59717

Experiments have been performed to study the feasibility of purifying plasma membranes from oat roots on the basis of their surface charge properties. We have used isoelectric focusing (IEF) to separate subcellular membrane particles on the basis of their isoelectric points (pI).

Membranes from oat (Avena sativa L. cv Garry) root homogenates were first fractionated using discontinuous sucrose density gradient centrifugation and then electrophoresed using a microanalytical IEF column. The column contained either a broad-range (pH 3 to 9) or narrow-range (pH 3 to 5) pH gradient stabilized by a 5 to 15% (w/v) Ficoll gradient. Results from the broad-range columns confirmed that the isoelectric points of the membrane particles were in the acidic range, with pI's ranging from 3.9 to 5.2. Using narrow-range pH gradients, it was possible to further fractionate the oat root membranes obtained from the sucrose density gradient and to determine more precisely their pI values. We had no success at fractionating crude membrane preparations from oat roots. Experiments with narrow-range pH gradients were much more successful when we used commercial ampholytes to generate the gradient as opposed to gradients generated using acetate/acetic acid mixtures. Our results indicate that IEF may be more useful as an analytical, rather than a preparative, technique for the purification of plant cell membranes.

Supported by grants from the Research Corporation and MONTS.

F73 A GENETIC MODEL FOR THE INTERACTION OF TWO GENES INFLUENCING HORMONE SENSITIVITY.

Virginia M. Ursin and Kent J. Bradford, Dept. of Vegetable Crops, University of California, Davis, CA, USA 95616. A recessive mutation at the diageotropica (dgt) locus in the tomato (Lycopersicon esculentum Mill.) renders the tissue highly insensitive to applied auxin (see Kelly and Bradford, these abstracts) and results in a phenotype of horizontal growth, an absence of lateral roots, thin stems and hyponastic leaves. We report a second, partially dominant mutation, Epinastic leaf (Epi), which results in epinastic leaves, thickened stems and petioles and short, highly branched roots. Both the dgt and Epi loci behave as single, independent genes when crossed to wild type plants. However, crosses between the dgt and Epi homozygotes result in an abnormal F_1 progeny. The phenotype of this double heterozygote (E+D+), termed 'mottled' is twisted and highly branched with mottled, misshapen leaves. F_2 and F_3 family analyses have revealed that the heterozygous condition at the dgt locus combined with one or two mutated alleles at the Epi locus produces the mottled phenotype. Other gene dosage combinations give distinct, predictable phenotypes, without evidence of disruptive intragenic interactions. Thus, the mutant Epi gene apparently interacts with the heterozygous condition of dgt to result in the abnormal, mottled growth. We propose that these observations can be explained by a model in which the dgt gene codes for a dimeric molecule. The wild type plant would have a homodimer of normal subunits (++), while the dgt phenotype would result from a mutated homodimer (DD). The dgt heterozygote would have 50% heterodimers (+D), which apparently are sensitive to the influence of the mutated Epi gene, resulting in the mottled phenotype. If the dgt gene does code for an auxin receptor, this genetic evidence suggests that the active molecule may be dimeric.

F74 MONOCLONAL ANTIBODIES TO PLANT GROWTH REGULATORS: PRODUCTION, CHARACTERIZATION AND USE, Elmar W. Weiler, Pflanzenphysiologie, Fachbereich Biologie/Chemie, Universität Osnabrück, Postfach 4469, D-4500 Osnabrück, West Germany

A panel of high affinity mouse monoclonal antibodies (MAB) directed against the plant growth regulators (PGR) abscisic acid, indole-3-acetic acid, cytokinins of the trans-zeatin, dihydrozeatin and isopentenyladenosine type, and gibberellins will be presented. These MAB form the backbone of a set of enzyme-linked immunosorbent assays (ELISA) which allow a facile quantitation of picogram amounts of the respective PGR. MAB technology is also used to purify PGR by immunoaffinity chromatography prior to immunological or physico-chemical assay. Future applications of MAB may involve immunocytochemistry of PGR and tracing of PGR receptors. Thus, MAB emerge as a powerful tool in the study of plant growth regulation. It is the aim of this contribution to provide a critical assessment of the potential and limitations of the immunological analysis of PGR and to outline an integral system of PGR extraction, purification and analysis - exploiting the separating power of MAB - and specifically designed for ELISA or ELISA-HPLC analysis.

F75 CHARACTERIZATION OF THREE SINGLE GENE DWARF MUTANTS OF TOMATO, Jan A. D. Zeevaart, MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824

Three single gene dwarf mutants (ga-1, ga-2, and ga-3) of tomato were obtained from Dr. J.H. van der Veen, Agricultural University, Wageningen, to determine whether these mutants are deficient in gibberellins (GAs), and if so, which sites in the biosynthetic pathway are blocked. The ga-3 mutant has a semi-dwarf growth habit, whereas in ga-1 and ga-2 stem growth is less than 10% of that of the wild type. The latter two mutants not only require GA for stem elongation, but also for germination, leaf expansion, and flower bud development. When treated repeatedly with GA, the mutants grow as the wild type, flower, and produce fruits with viable seeds.

The ga-3 mutant contains very low levels of GAs as compared to the wild type, whereas no GAs are detectable in fruits and shoots of ga-1 and ga-2. Results of feeding studies with various GAs and precursors indicate that ga-1 has a block in the GA biosynthetic pathway before ent-kaurene, and ga-2 between ent-kaurenoic acid and GA_{12}; ga-3 appears to be a leaky mutant with a block prior to ent-kaurene. Thus, all three tomato dwarfs are GA mutants.
Supported by U.S.-D.O.E. under Contract #DE-AC02-76ER01338.

Hormone Receptors and Second Messengers

F76 Inhibitors of Ethylene Action. F. B. Abeles, USDA, Appalachian Fruit Research Station, Route 2 Box 45, Kearneysville, WV 25430
The action of five purported inhibitors of ethylene action were tested for their ability to block a number of ethylene mediated processes. The inhibitors used in this study include silver ions in the form of silver thiosulfate, 2,5-norbornadiene (NBA), carbon dioxide, 3,5-diiodo-4-hydroxybenzoic acid (DIHB), and 5-methyl-7-chloro-4-ethoxycarbonylmethoxy-2, 1,3-benzothiadiazole (MCEB). The ethylene regulated systems studied included ripening, lettuce seed germination, lettuce seedling root growth, and cucumber cotyledon senescence. Only silver ions and NBA were found to block ethylene action without causing toxic side effects. CO_2 was also an inhibitor but it had additional effects which limits its usefulness in these studies. DIHB and MCEB were not active as antiethylene compounds in the systems studied. These latter two compounds had toxic side effects which prevent their use in physiological studies.

F77 PARTIAL SEQUENCE AND BINDING SITE STUDIES ON A WHEAT EMBRYO CYTOKININ-BINDING PROTEIN, A.C. Brinegar and J.E. Fox, ARCO Plant Cell Res. Inst., Dublin, CA 94568
Cytokinin-binding factor-1 (CBF-1) is an embryo-specific protein from wheat which has a high affinity for cytokinin-active N^6-substituted purines. The native protein is thought to be composed of three identical 54 kD subunits which bind only one cytokinin molecule between them. A ^{14}C-labeled photoaffinity probe (2-azido-6-benzyladenine) has been used to covalently label the binding residues in CBF-1. After proteolysis, labeled fragments were separated by reverse phase HPLC. Work is underway to sequence the labeled peptides and identify the residue(s) involved in cytokinin binding. An 800 base pair cDNA selected from a phage expression vector library with antibodies specific for CBF-1 has been sequenced. The coding sequence represents 40% of the protein, and like the protein is rich in glutamate/glutamine, glycine, and arginine. Protein sequencing of CBF-1 fragments is underway to confirm the identity of the cDNA.

F78 GIBBERELLIC ACID INDUCES THE TRANSFER OF LIPASE FROM PROTEIN BODIES TO LIPID BODIES IN BARLEY ALEURONE LAYERS, Donna E. Fernandez and L. Andrew Staehelin, Dept. of MCD Biology, University of Colorado, Boulder, CO 80309-0347

Exposure of aleurone layers in cereal seeds to gibberellic acid (GA_3) leads to a series of metabolic and structural changes related to the mobilization of storage materials. However, it is not clear whether changes in physiology precede changes in gene expression. The following structural and biochemical findings suggest that the physiological alterations come first. In particular, our freeze-fracture analysis of barley aleurone cells that have been treated with GA_3 indicates: (1) lipid bodies become associated with the protein bodies, (2) the monolayers of the lipid bodies fuse with the outer leaflets of the bilayers of the protein bodies, and (3) membrane complexes are transferred from the protein bodies to the lipid bodies. Some of these membrane complexes appear to be lipases. We find that, within one hour after exposing layers to GA_3, lipase activity is transferred from the membranes of protein bodies into lipid bodies. When lipid bodies are isolated on sucrose gradients, those from untreated layers contain 20-25% of the total lipase activity while those from layers treated with GA_3 contain 75-80% of the total activity. Conversely, lipase activity in pellets containing the membranes of protein bodies decreases from 75-80% (untreated) to 20-25% (GA_3-treated). As a consequence of the transfer of lipases, lipid bodies are rapidly digested and the free fatty acids which are released can be degraded to provide energy for synthetic activities or used as building blocks for membrane phospholipids. Supported by NIH grant GM 18639.

F79 SOME STUDIES OF THE DISTRIBUTION OF NPA BINDING PROTEINS IN ORGANS, Richard D Firn, Department of Biology, University of York, York, England, YO1 5DD, UK.

NPA is a potent inhibitor of basipetal auxin transport and of plant tropistic responses. Although it has often been assumed that the latter effect is a consequence of the former, there are reasons to doubt this explanation. Some interesting questions about the relationship between these two actions can now asked because information about the NPA receptor is becoming available. For instance, Jacobs & Gilbert (1) suggested by immunofluorescent methods that the NPA receptors in pea epicotyls were located in only in cells near the starch sheath. Does this imply that polar auxin transport occurs exclusively in those cells? Why should NPA action in those cells inhibit tropistic response? Two approaches are being adopted in an attempt to answer these questions. First attempts have been made to identify in which cell types basipetal auxin transport can occur. Second, using simple organ fractionation methods, the distribution of NPA binding proteins in hypocotyls is being studied.

(1) Jacobs,M. & Gilbert,S.F. (1983) Science 220:1297-1300.

F80 NAA BINDING PROTEINS IN COLEOPTILES AND HYPOCOTYLS. Richard D Firn, Department of Biology, University of York, Heslington, York, England, YO1 5DD, UK.

Using high specific activity ^{3}H-NAA further attempts have been made to find saturable auxin binding in organs known to be capable of showing a good, short term growth promotion in response to auxin. While membrane associated NAA binding proteins are readily found in all types of coleoptile (maize, wheat, rye, oat) such binding has not been found in any dicotyledonous organ (Firn & Kearns, 1983). The conditions employed in the binding assay have been varied very widely in studies of dicotyledonous organs without success. The distribution of the NAA binding proteins in coleoptiles has been studied in an attempt to probe the relationship between the presence of the binding proteins in various cells and their responsiveness to NAA.

(1) Firn,R.D & A.W.Kearns (1983) Plant Growth Substances 1982. Ed.P.F.Wareing. Academic Press. pp 385-393.

F81 INSENSITIVITY OF THE TOMATO MUTANT, DIAGEOTROPICA, TO AUXIN. Maureen Kelly and Kent J. Bradford, Department of Vegetable Crops, University of California, Davis, CA 95616

Phenotypic characteristics of the single-gene mutant of tomato (Lycopersicon esculentum Mill.,), diageotropica (dgt), include horizontal growth, absence of lateral roots, and hyponastic leaves. Features of this phenotype can be normalized by application of low concentrations of ethylene. Dgt has thus been termed the "ethylene-requiring" mutant. Ethylene production by dgt tissues is highly insensitive to stimulation by IAA relative to its parent line, VFN8, and it has been suggested that the primary genetic lesion of the mutant results in failure of IAA to induce ethylene synthesis. However, the dgt mutation could result in general insensitivity to auxin, and lack of stimulation of ethylene synthesis by auxin may be only one manifestation of the defect. To distinguish between these possibilities, the sensitivities of VFN8 and dgt hypocotyls to auxin were tested in a hypocotyl growth assay which was demonstrated to be specific for active auxins and independent of ethylene and polar auxin transport. Hypocotyls of VFN8 seedlings showed an approximately log-linear growth response from 0.1 to 100 μM IAA, while no significant growth occurred in dgt hypocotyls in response to less than 500 μM IAA. Fusicoccin (1μM), however, caused greater growth and ethylene production in dgt than VFN8 hypocotyls.

These data indicate that the dgt locus controls tissue sensitivity to auxin. Growth of dgt hypocotyls in response to high levels of applied auxin or low concentrations of fusicoccin suggest defective perception rather than response. The dgt gene may code for an auxin receptor, which,when mutated, has reduced affinity for auxin. If so, dgt may provide a useful genetic tool for the study of auxin receptors.

F82 IN VITRO GIBBERELLIN A_1 BINDING TO A SOLUBLE FRACTION FROM DWARF PEA EPICOTYLS Coralie C. Lashbrook, Brian Keith and Lawrence Rappaport, Plant Growth Laboratory, Department of Vegetable Crops, University of California, Davis, CA 95616, USA.

A 100,000 x g cytosol was prepared from the epicotyls of 4 day-old dark grown dwarf peas ('Progress No. 9'). Incubation of cytosol with 10^{-7}M [^{3}H]GA_1±10^{-5}M unlabelled GA_1 resulted in low levels of specific binding as measured by Sephadex G-100 chromatography. Further purification of the cytosol using G-200 gel filtration was required in order to produce a fraction capable of high levels of specific binding. Binding of [^{3}H]GA_1 to the G-200 fraction was rapid, saturable and pH sensitive. Labelled hormone was completely exchangeable with the biologically active GA_1 but not with the biologically inactive GA_{17}.

F83 IN VITRO BINDING OF GIBBERELLIN A_4 IN EPICOTYLS OF DWARF PEA AND TALL PEA, Zin-Huang Liu and Lalit M. Srivastava, Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6

In vitro gibberellin (GA) binding properties of a cytosol fraction from epicotyls of Dwarf pea (Pisum sativum L. cv. Progress No. 9) and Tall pea (Pisum sativum L. cv. Alaska) were investigated using [^{3}H]GA_4 in a DEAE filter paper assay at 0-3°C. The binding obtained is saturable, reversible, temperature labile in Dwarf pea, and has a half-life of dissociation of 5-6 min. By varying the concentration of [^{3}H]GA_4 in the incubation medium the Kd was estimated to be 1.1 x 10^{-7} M in Dwarf pea and 6.9 x 10^{-8} M in Tall pea. The number of binding sites was estimated to be 0.5 and 0.4 P mole mg^{-1} soluble protein in Dwarf pea and Tall pea, respectively. Biologically active GAs, such as GA_3 and GA_4 could reduce the level of [^{3}H]GA_4 binding much more than the biologically inactive GA_4 methyl ester. Specific binding was enhanced by ammonium sulfate fractionation followed by desalting in a Sephadex G25 column.

F84 IDENTIFICATION OF AN AUXIN RECEPTOR AS THE ELECTROGENIC AUXIN UPTAKE CARRIER, Terri L. Lomax, Rainer Hertel and Winslow R. Briggs, Carnegie Inst. of Wash., Stanford, CA 94305 and Inst. f. Biol. III,

The "site III" type binding of auxin exhibits a specificity which indicates that it is involved in the polar transport of auxin through plant tissue. We have shown that, in osmotically-sealed membrane vesicles prepared from zucchini hypocotyls, pH-driven uptake of IAA occurs via those binding sites again with a specificity for auxin analogs which matches in vivo auxin transport. The uptake carrier can be demonstrated in both dicots and monocots and has been found only in those tissues which actively transport auxin, indicating tight developmental regulation. Only those membrane vesicles of plasma membrane origin will specifically transport IAA, and the accumulation of IAA within the vesicles can be increased by blocking efflux carriers with auxin transport inhibitors such as TIBA and NPA. Auxin uptake by the vesicles can be blocked by covalent linkage of azido auxins which specifically bind to the transport site. Ionophores and electron spin resonance determinations of sealed vesicle volume and pH gradient magnitude demonstrated that the uptake of auxin by these carriers can be driven electrogenically, which we propose to occur via a two proton cotransport mechanism. These studies represent the first assignment of a physiological function for any of the putative auxin receptors.

F85 LOCATION OF CARRIERS FOR IAA TRANSPORT IN MAIZE ROOTS, Hilary V. Martin and Paul-Emile Pilet, Inst. Plant Biol. and Physiol., University, 1015 Lausanne, Switzerland

The uptake of indol-3yl-acetic acid (IAA) by maize root segments was found to have a non-saturable component and a saturable part with (at pH 5.0) an apparent K_m of 0.285 µM and V of 55.0 pmol per gram fresh mass per minute. These results are consistent with those which might be expected for a saturable carrier capable of regulating IAA levels.
The degree of saturable uptake was compared for different root parts. Within the 2-10 mm region (counting from the root tip) saturable uptake was greatest for the 2-4 mm zone and declined for more basal zones. When isolated stelar and cortical segments were compared, a higher degree of uptake was observed for stelar segments and this was attributable to differences in uptake by both saturable and non-saturable processes.
The location of the carrier for IAA efflux in different parts of the root, as assessed by the sensitivity of IAA efflux to NPA, will also be discussed. The location of both uptake and efflux carriers may be important for IAA movement and levels in the root tip. These processes could, at least partly, control root growth and gravireaction.

F86 AUXIN, AUXIN ANTAGONISTS, AND CALCIUM MODIFY ACTIVE GLUCOSE UPTAKE INTO T-DNA TRANSFORMED TOBACCO CELLS, Thomas Rausch, Botanisches Insitut, J.W.Goethe Universität D-6000-Frankfurt, F.R.G.

The drastic inhibition of 14C-O-methylglucose and 14C-labelled amino acid uptake by several auxin antagonists in primary crown gall tumors from potato has been recently described (Rausch T, Hilgenberg W, Kahl G 1984 Plant Phys. 76:335-339). It has been proposed that the T-DNA encoded auxin formation increases the uptake of glucose by stimulation of the H+ - glucose symport.
These experiments have now been extended to bacteria-free T-DNA transformed cells of tobacco. Again by variing the exogenous auxin or auxin antagonist concentrations the glucose uptake may be influenced significantly. In other experiments the intracellular Ca2+ concentration has been modified by a) 10 µM A 23187 in the presence of 100 µM Ca2+, b) 1 - 10 µM verapamil, c) 10 µM A 23187 plus 1 mM EGTA pretreatment in the absence of Ca2+, with subsequent addition of different Ca2+ concentrations in the presence of A 23187. The results show that there exists a threshhold level for endogenous Ca2+ for optimal glucose uptake. Thus a modulation of intracellular Ca2+ concentration by IAA may be responsible for the effect on glucose uptake.
Isolation of microsomal membrane vesicles allowed to study the kinetics of ATP-dependent glucose transport in more detail. Evidence is presented for two transport systems: 1) a H+ - glucose symport at the plasma membrane, 2) a H+ - glucose antiport (or OH- - glucose symport) at the tonoplast. The relevance of these results for the growth of crown gall cells will be discussed.

F87 PARTIAL PURIFICATION OF A GIBBERELLIN BINDING PROTEIN FROM CUCUMBER, Nasser Yalpani and Lalit M. Srivastava, Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6

Proteins which reversibly and specifically bind gibberellins (GAs) with high affinity have been identified in cucumber, pea, and corn. Using (^{3}H)GA_4 and a series of selected GAs in an in vitro assay we have partially characterized the GA - binding site of a putative GA receptor from the cytosol of cucumber hypocotyls (Yalpani and Srivastava. 1985. Plant Physiol. in press). For further purification of the protein, fractions eluted from molecular sieve and ion exchange chromatography columns were assayed for specific binding using biologically active and inactive GAs. The GA-binding protein has an apparent MW between 45,000 and 80,000 daltons and elutes with 0.22 to 0.27 M KCl in 20 mM tris-phosphate pH 7.0 from a DEAE cellulose column. Nondenaturing PAGE suggests that fractions enriched in specific binding contain about 10 proteins. Efforts are underway to determine which of these bands is specific for (^{3}H)GA_4 binding.

F88 MOLECULAR AND GENETIC ANALYSIS OF FACTORS CONTROLLING HOST RANGE IN AGROBACTERIUM TUMEFACIENS, Martin Yanofsky, Brenda Lowe and Eugene Nester, University of Washington, Seattle, WA 98195

We have investigated the factors which contribute to the host specificity of a tumor-inducing plasmid of Agrobacterium, pTiAg162, which confers a narrow host range. Determinants both within the T-DNA and virulence regions contribute to host specificity. Within the T-DNA a defective cytokinin biosynthetic gene limits host range. Nucleotide sequence analysis revealed a large deletion in the 5' coding region of this gene when compared with the homologous gene from the wide host range tumor-inducing plasmid, pTiA6. Introduction of the wide host range cytokinin biosynthetic gene into the T-DNA of the limited host range strain expanded the host range and suppressed the rooty morphology of tumors incited by the limited host range strain. Two genes from the virulence region of the wide host range plasmid, designated virA and virC, must also be introduced into the limited host range strain in order to restore a wide host range phenotype. The wide host range strain is avirulent on some cultivars of Vitis plants on which the limited host range strain induces tumors. This avirulence is apparently due to a hypersensitive response in which infected plant cells are killed at the site of inoculation. Mutations within the virC locus of the wide host range plasmid prevented the hypersensitive response and allowed the formation of tumors by the wide host range strain.

Light and Pathogen Regulation

F89 RED LIGHT AND CALCIUM MEDIATED INHIBITION OF RAPID GROWTH IN PEA EPICOTYL, Grant M. Barkley, Laura L. Coe and Teri R. Smith, Department of Biological Sciences and Laboratory for Physiological Botany, Kent State University, Warren, Ohio 44483

Red light has been shown to cause a number of physiological and developmental effects which require calcium in plant tissue. It has also been known for some time that red light and calcium inhibit growth in intact etiolated pea epicotyl. In this work we show that red light and calcium have similar effects in reducing tissue sensitivity to auxin and H+ ions and that red light inhibition of growth is mediated by higher levels of tissue calcium.

In the present study we have employed 4 or 5-day-old abraded etiolated pea epicotyl tissue (Pisum sativum var. Alaska) imbibed or grown in calcium solutions (1-100mM) to achieve higher tissue calcium levels and similar tissue treated with red light (650nm) for various times.

Tissue grown in calcium show typical inhibition of auxin promoted growth in long-term growth studies, with a shift in auxin optimal from 10^{-6} to 10^{-4} M. This inhibition and shift in auxin optimal is similar with greening tissue given red light for several hours. Transducer 'rapid growth' measurements of auxin and H+ promoted growth, with calcium treated tissue, show typical initial kinetics followed by a rapidly decaying growth rate. This decline in responsiveness is also demonstrated with red light treated tissue and is paralleled by greening of tissue and a dramatic increase in level of tissue calcium. The effect of the calcium channel blocking agents verapamil and diltiazem will also be demonstrated.

It is suggested that the effect of red light on epicotyl elongation is mediated by an influx of calcium ions, which in turn regulates wall materials or enzymes for growth.

F90 THE EFFECT OF PHOTOPERIOD ON ENZYME ACTIVITIES OF THE GIBBERELLIN BIOSYNTHETIC PATHWAY IN SPINACH LEAVES, Sarah J. Gilmour, Jan A. D. Zeevaart, Jan E. Graebe and Ludger Schwenen, MSU-DOE Plant Research Lab., Michigan State University, E. Lansing, MI 48824; Pflanzenphysiologisches Institut der Universität, 3400 Göttingen, F.R.G.

Stem elongation in spinach (Spinacia oleracea L.), a long day (LD) rosette plant, is photoperiodically controlled. This photoperiodic control is mediated by gibberellins (GAs). The aim of this project is to study at the biochemical level the mechanism by which the photoperiod affects stem length.

Spinach leaves possess the early 13-hydroxylated GA biosynthetic pathway:

$$[\longrightarrow GA_{53} \longrightarrow GA_{44} \longrightarrow GA_{19} \longrightarrow GA_{20} \longrightarrow GA_{29}]$$

In crude preparations from leaves of plants grown in short days (SD) enzyme activities for the conversions of GA_{53} to GA_{44}, and GA_{19} to GA_{20} were absent or low. When plants were placed in LD, however, these two enzyme activities were much increased. The activity of the GA_{44} to GA_{19} step was not affected by photoperiod. Thus light turns on the GA biosynthetic pathway by causing an increase in the activities of two of the enzymes of the pathway. Therefore, in LD an active GA is produced, which in turn causes stem elongation.

Results of preliminary experiments indicate that the GA_{44}-oxidizing enzyme can be partially purified by ion exchange HPLC. This, and other techniques are being investigated with a view to purifying the enzymes of the GA biosynthetic pathway. Also experiments to determine if light causes de novo synthesis of the two regulated enzymes are under way.

Supported by the N.S.F. grant No. PCM 83-04229 and by the Deutsche Forschungsgemeinschaft.

F91 PROLYL HYDROXYLASE FROM THE GREEN ALGA, CHLAMYDOMONAS REINHARDII: HYDROXYLATION REACTION AND REGULATION DURING CELL WALL SYNTHESIS, Deborah D. Kaska, Volkmar Günzler, Kari I. Kivirikko and Raili Myllylä, University of California, Santa Barbara, CA 93106, and Collagen Research Institute, University of Oulu, SF 90220 Oulu 22, Finland

Synthesis of the extracellular matrix of plant and animal cells is characterized by post-translational modification of proline catalyzed by prolyl-4-hydroxylase. The enzyme, isolated from plants, shows similarities to the vertebrate prolyl-4-hydroxylase, yet recognizes a distinctly different peptidyl substrate, the poly-l-proline II helix. The hydroxyproline rich glycoproteins found in plant cell walls play a role in development, cell cell recognition, growth and defense. Hydroxylation of proline may be an important element of these processes. In order to explore the hydroxylation reaction in plants and the regulation of activity during cell wall biosynthesis, we have studied the prolyl hydroxylase of the unicellular green alga, Chlamydomonas reinhardii. The algal enzyme has an apparent M_r of 40000 compared with M_r values of 240000 and 300000 for the vertebrate and higher plant enzymes respectively. In spite of this difference in size, analysis of the enzymatic active site responsible for binding the co-substrate 2-oxoglutarate reveals that the structure of this site in the algal enzyme is remarkably similar to the complex site found in the collagen hydroxylases. By studying wall regeneration and a wall-less mutant, we determined that the level of prolyl hydroxylase is not altered by signals which recognize the presence or absence of cell walls. This suggests that regulation of prolyl hydroxylase in higher plants may result from more specific inducers generated by infection or wounding.

F92 PHYTOCHROME REGULATION OF A WHEAT GENE ENCODING THE LIGHT-HARVESTING CHLOROPHYLL A/B POLYPEPTIDE IN WHEAT AND TRANSGENIC TOBACCO. Steve A. Kay, Ferenc Nagy and Nam-Hai Chua, Laboratory of Plant Molecular Biology, The Rockefeller University, New York, New York 10021-6399, U.S.A.

We have reported previously (Lamppa et al., Nature 316, 750-752, 1985) that a wheat gene (whAB1.6) encoding the major chlorophyll a/b binding protein (Cab) of the light-harvesting complex retains regulation by white light when introduced into the genome of tobacco via a Ti-mediated gene transfer system. We have demonstrated that the ability to respond to light/dark as well as organ-specific regulation resides in DNA sequences 1.6 kb upstream from the 5' mRNA terminus. When this region of DNA is fused to the bacterial gene encoding chloramphenicol acetyltransferase (CAT) and the hybrid gene is transferred to tobacco, CAT activity is expressed correctly in a photoregulated and leaf-specific manner. Northern hybridizations using a coding sequence probe show that the wheat Cab gene family is regulated by phytochrome in etiolated wheat seedlings. By using a gene specific probe in S_1 nuclease protection experiments, we have been able to show that whAB1.6 exhibits a highly sensitive phytochrome response in both etiolated wheat and transgenic tobacco, whereas the small subunit gene family is induced quantitatively less by red light. The endogenous tobacco Cab and small subunit genes are also regulated by phytochrome in etiolated leaves. We will present data on the photoregulation of whAB1.6 expression in mature green leaves, which show a marked difference in regulation to that of etiolated tissue. The role of cis-acting DNA sequences in mediating the phytochrome response will be discussed.

F93 LOCALIZED METAL ACCUMULATION PRECEDING ASYMMETRIC CELL DIVISION AND CELL DIFFERENTIATION. Jane L. Kotenko, John H. Miller & Alix I. Robinson. Dept. of Biology, Syracuse University & Dept. of Microbiology, SUNY Upstate Medical Center, Syracuse, NY.

Cell differentiation often is initiated by an asymmetrical cell division. In Onoclea sensibilis gametophytes and Vittaria graminifolia gemmae the resultant small cells give rise to antheridia in the presence of antheridiogen Pt or gibberellic acid respectively. In the absence of these growth factors, the small cells differentiate into rhizoids or vegetative outgrowths. In all cases, nuclear migration precedes asymmetric division. Cytochemical and fluorescent methods reveal an accumulation of metal ions at the differentiation site prior to nuclear migration, regardless of the developmental fate of the small cell. The metals are found at the inner cell wall and plasmalemma. Evidence strongly suggests that calcium is the principal metal. Caffeine reversibly inhibits both nuclear migration and asymmetric division and eliminates localized metal accumulation, suggesting that the accumulation is essential in directing nuclear migration. Cytochalasin b (CB) alters nuclear migration but not asymmetric division, resulting in an abnormally postioned small cell. Effects of CB on metal accumulation are currently being examined.

F94 IN VITRO [^{3}H]GA_1 BINDING BY MACROMOLECULAR CONSTITUENTS OF GA-SENSITIVE DWARF MAIZE MUTANTS. Lawrence Rappaport and Brian Keith. Plant Growth Laboratory, Department of Vegetable Crops, University of California, Davis, CA 95616, USA.

A 100,000 x g supernatant fraction was prepared from the first and second leaf sheaths of light grown Zea mays L. cv. Golden Jubilee. [^{3}H]GA_1 binding to a high molecular weight (HMW) fraction (>500 Kdaltons) was demonstrated at 4°C using Sephadex G-200 chromatography. The HMW component was shown to be a protein and the [^{3}H] activity bound to this protein was [^{3}H]GA_1 and not a metabolite.

The binding properties of an intermediate molecular weight (IMW) fraction will also be described. The specificity and partial purification of both HMW and IMW binding components will be described for 'Golden Jubilee' and some dwarf, single-gene mutants of corn that are either GA-sensitive (d_1, d_2, d_3 and d_5) or GA-insensitive (D_8).

F95 PHYTOCHROME-REGULATED ATPase ACTIVITY IN GUARD CELLS. N. Roth-Bejerano, A. Najidat and Ch. Itai, Ben-Gurion University, Beer-Sheva, Israel.

Stomatal opening in Commulina communis is driven by K^+ uptake. It is an active process which is triggered by phytochrome. Ion transport in various plant tissues is believed to be related to ATPase activity. ATPase activity in guard cells and its regulation by phytochrome were therefore studied. This was done in microsomal fraction obtained from isolated guard cell protoplasts. The protoplasts and the microsomal fraction were isolated in light, and the enzyme was assayed in darkness in a dim safe light.

ATPase activity was evident within a broad pH range, with two small peaks at pH 6.5 and 7.5. It seems that at pH 7.5 the tonoplast ATPase dominated, while at pH 6.5 the plasmalemma one, as judged by the response to KNO_3 and to vanadate. The activity at pH 6.5 was unsensitive to oligomycin indicating the lack of mitochondrial ATPase activity. This ATPase activity was Mg^{2+} dependent, and KCl stimulated. The response to KCl was modulated by phytochrome. A short preirradiation with far red light, which converted the Pfr to Pr, reduced the response to KCl, comparatively to preirradiation with red light. Moreover, the stimulatory effect of red light was reversible by far red one.

F96 **ANTIBODY LOCALIZATION OF EXTENSIN IN CARROT CELL WALLS**, Joel P. Stafstrom and L. Andrew Staehelin, MCD Biology, University of Colorado, Boulder, CO 80309.

Extensin monomers are secreted into the plant cell wall and covalently crosslinked to each other to form a rigid matrix. Expression of the extensin matrix has been correlated with the inhibition of cell elongation during normal development and resistance to pathogens, which suggests that the major function of the matrix may be to strengthen cell walls. We have begun to study the structure of the extensin matrix in situ using immuno-electron microscopy. A rabbit antiserum was raised against glycosylated extensin isolated from aerated carrot root tissue. Semi-quantitative dot blots indicate that glycosylated extensin is recognized by the antiserum about 50-fold better than deglycosylated extensin and about 10-fold better than extensin-2, a second extensin-like hydroxyproline-rich glycoprotein isolated from carrots. Other data suggests that the major epitope recognized by this antiserum includes the non-reducing terminal of hydroxyproline tetra-arabinoside.

For electron microscopy, carrot root tissue was fixed in glutaraldehyde and osmium and embedded in Spurr's resin. Extensin antibodies were detected using a ferritin-conjugated secondary antibody. Labeling is quite heavy and uniform across the entire wall between two cells but is absent in the expanded middle lamella at the junction between three cells. Small domains lack extensin label, probably because they are rich in other wall polymers. Clusters of label occur in all orientations. Since cells from carrot root tips contain very little label, we suggest that extensin is incorporated into a the cell wall after cellulose has been laid down. An second antiserum raised against deglycosylated extensin recognized this antigen on blots but does not label sections. Supported by GM 18639.

F97 MOLECULAR CHARACTERIZATION OF AN UPSTREAM ENHANCER ELEMENT INVOLVED IN rbcS GENE PHOTOREGULATION, Michael P. Timko and Anthony R. Cashmore, Laboratory of Cell Biology, Rockefeller University, New York, NY 10021

The nuclear genes encoding the small subunit polypeptide of ribulose-1,5-bisphosphate carboxylase (rbcS) are expressed in a light-regulated, tissue-specific manner in pea (Pisum sativum). In order to define the nucleotide sequence requirement for photo-regulated expression a chimaeric gene consisting of a 5'-noncoding fragment (-4 to -973 bp upstream of the cap site) from the rbcS 3.6 gene was fused to the coding sequence of the bacterial cat gene and its expression studied in tobacco callus. These studies demonstrated that nucleotide sequences within 973 bp of the rbcS cap site contain sufficient information required for the photoregulated expression of the cat gene. Subsequent deletion analysis has shown that sequences at least 722 bp 5' to the cap site are required for high levels of photoregulated expression (80 % of wild-type 973 promoter level) and that sequences within 92 bp of the cap site are capable of directing low levels (5% of wild-type) of photoregulated expression. Furthermore, sequences directing high levels of photoregulated expression and contained within a fragment extending -90 to -973 function independently of orientation when fused to their homologous promoter. These same sequences also confer high levels of photoregulated expression when fused to a normally non-photoresponsive heterologous promoter in an orientation independent manner. These data suggest that rbcS photoregulated gene expression involve a genetic element with some of the properties attributed to animal viral and cellular enhancers. A more detailed analysis of the rbcS enhancer sequences is presently underway.

F98 T-DNA controlled hormone metabolism. H. VAN ONCKELEN [1]*, P. RUDELSHEIM [1], M. VAN LIJSEBETTENS [2], D. INZE, (2), M. VAN MONTAGU [2] and J. DE GREEF [1], (1) University of Antwerpen, UIA, B-2610 Wilrijk, Belgium; (2) University of Ghent, RUG, B-9000 Gent, Belgium.

Kinetics of the endogenous levels of indolyl-3-acetic acid (IAA), indolyl-3-acetamide (IAM) and cytokinin during the growth cycle of cloned tobacco crown gall tissues were analysed and compared with untransformed control tissue. Infection was achieved by co-cultivation of Nicotaniana tabacum L. cv. Petit Havanna SR I either with the nop$^+$ Agrobacterium tumefaciens C 58 wild type (pTi C 58) or with pGV 3845 (1$^-$), pGV 3123 (2$^-$) and pGV 4025 (4$^-$) mutants.

Data presented in this report proved the Trp → AM → IAA biosynthetic pathway to be controlled by the prokaryotic T-DNA genes 1 and 2, gene 1 encoding a specific tryptophane monooxygenase. Mutations in gene 1 or gene 2 not only reduced the endogenous IAA levels, although not drastically, but increased significantly the endogenous cytokinin levels. The cytokinin dependent pGV 4025 (4$^-$) tissue showed not only,as could be expected,a low endogenous cytokinin level but also a remarkably low endogenous IAA level. When cultivated on exogenously applied BAP the endogenous IAA level in the 4$^-$ tissue increased dramatically. Besides the direct control of the prokaryotic T-DNA genes (1,2 and 4) on the IAA and cytokinin levels, a pronounced mutual interaction of both hormones was observed in the different tobacco crown gall types analysed.

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F99 **IN VITRO PHOSPHORYLATION OF PHYTOCHROME AS A PROBE OF LIGHT-INDUCED CONFORMATIONAL CHANGES,** Yum-Shing Wong*, Heung-Chin Cheng**, Donal A. Walsh** and J. Clark Lagarias, Departments of Biochemistry and Biophysics* and Biological Chemistry**, University of California, Davis, CA 95616

Phytochrome mediates a wide range of light-controlled growth and developmental responses in plants. Although the molecular mechanism for the signal transduction pathway is not well understood, recent in vitro studies have provided support for a mechanism involving light-induced conformational changes in the phytochrome photoreceptor. In the present study, we have utilized two mammalian protein kinases, cAMP-dependent protein kinase (catalytic subunit) from rabbit skeletal muscle and protein kinase C from rabbit brain, and a protein kinase preparation from etiolated Avena seedlings to probe light-induced conformational changes in Avena phytochrome in vitro. These studies show that all three protein kinase preparations phosphorylate the native photoreceptor and that the sites of phosphorylation differ for the P_r and P_{fr} forms. Limited proteolysis of phosphorylated phytochrome has been undertaken to determine the localization of the phosphorylation sites within the 124 kDa subunit.

F100 CALMODULIN mRNA IN BARLEY: APPARENT REGULATION BY LIGHT AND CELL PROLIFERATION, Raymond E. Zielinski, University of Illinois, Urbana, IL 61801

Calmodulin (CaM) mRNA has been identified in barley and pea by a combination of in vitro translation and blot hybridization experiments. In both plants, CaM is an approximately 600-nucleotide molecule that accounts for between 0.02 and 0.1% of the total translatable mRNA. Two- to three-fold higher steady-state levels of CaM mRNA and protein are found in the meristematic region of barley leaves, compared with the levels found in non-dividing leaf tissues. During light-induced development in barley leaves, the level of translatable CaM mRNA declines two- to four-fold. This apparent, light-dependent decrease is detectable in as little as 30 min after the onset of illumination; and it occurs with a t 1/2 of about 1 hr. In addition, cytoplasmic mRNAs that may encode CaM-like proteins are also detected in barley and pea. The levels of several of these putative Ca^{2+}-binding protein messengers are modulated during the course of barley leaf cell development. The identities of these mRNAs and the polypeptides they encode, however, are not yet known.

Late Additions

F101 BINDING SITE STUDIES ON A WHEAT EMBRYO CYTOKININ-BINDING PROTEIN J. E. Fox and A. Chris Brinegar, ARCO Plant Cell Research Institute, 6560 Trinity Court, Dublin CA 94568

Cytokinin-binding factor-1 (CBF-1) is an embryo-specific protein from wheat which has a high affinity for cytokinin-active 6-substituted purines. We have shown that CBF-1 is a trimer composed of three identical subunits and that only one cytokinin binding site exists per holoprotein. Preliminary results indicate that only a limited number of fragments are labeled after proteolysis of CBF-1 which had been treated with (^{14}C)-2-azido-6-benzyladenine, a photoaffinity probe. Monoclonal antibodies against CBF-1 have been produced and will be screened for their ability to prevent binding of benzyladenine to CBF-1 or to react with proteolytic fragments labeled with the photoaffinity probe. An 800 base pair cDNA clone has been selected from a phage expression vector library using anti-CBF-1 IgG. The coding sequence represents 40% of the protein and is being confirmed by protein sequencing. Our goal is to identify the sequence(s) and amino acid residues involved in cytokinin binding and to localize the binding site within the structure of the protein.

F102 REGULATION OF RIBOSOMAL DNA EXPRESSION IN THE YEAST SACCHAROMYCES CEREVISIAE.

R. Mestel, M. Yip, J. Holland, E. Wang and M. Holland, University of California, Davis, CA 95616.

Ribosomal DNA (rDNA) cistrons are tandemly organized in the yeast genome. The 18, 25 and 5.8S ribosomal RNAs are derived from the processing of a 35S precursor. A spacer region separates each unit of 35S information.

An in vitro RNA polymerase I-dependent transcription system has been developed. In this system, transcription initiates within the spacer, 2.2 kilobases upstream of the 35S start site. The same region of the spacer contains sequences which enhance the level of 35S expression 15-20 fold in vivo. The sequences responsible for the enhancing effect are being localized by deletion analysis. They appear to include sequences required for the in vitro-defined promoter activity.

F103 CHLOROPLAST DIFFERENTIATION: ROLE OF CYTOKININ AND LIGHT, B. Parthier, J. Lehmann, S. Lerbs, W. Lerbs, R. Wollgiehn, Institute of Plant Biochemistry, Acad. Sci., DDR-4020 Halle (S.), G.D.R.

In excised, etiolated Cucurbita cotyledons chloroplast development, as visualized by chlorophyll accumulation and increase in activity and amount of RuBPCase, is controlled by the interaction of light and cytokinin (benzyladenine, BA). If white light has been varied in intensity and applied with BA in saturating amounts, the influence of BA appears to become weaker with increasing light intensity, but the effect of BA is much more pronounced at low light intensities. - Light as well as BA induce or enhance the expression of certain nuclear genes coding for chloroplast proteins, among them the SSU of RuBPCase and the apoprotein of LHCP, as shown by increased mRNA availability. However, the biosynthesis of these major plastid polypeptides are controlled primarily by light, whereas only few mRNAs for not yet identified polypeptides are specifically induced by BA in darkness (2-D separation of in vitro translation products). After illumination of etiolated spinach leaves chloroplast RNA polymerase subunits, which are separated and identified by antibody-linked polymerase assay, increase in amount except a 33 Kd subunit that disappears. - Although the results favor a regulation by light and cytokinin at the transcription level, control of chloroplast differentiation at the post-transcription levels are not excluded.