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Lecture Course on
Approaches to Plant Development

Organized by
P. Puigdoménech and T. Nelson

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251 Lecture Course on Approaches to Plant Development

Fundación Juan March

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I N D E X

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GENERAL PROGRAM OF THE LECTURE COURSE

February 12

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T. Nelson (Chairman)

I. Sussex - Organization of plant apical meristems.
R.S. Poethig - Genetic regulation of phase change in maize.

Afternoon: SECOND SESSION

F. Salamini (Chairman)

M. Delseny - Structure of plant nuclear genome.
M. Freeling - Mutants analysis supports the model that cell fates in the maize leaf to age in a developmental chronology.
S.C. de Vries - Developmental mutants and extracellular proteins in carrot somatic embryogenesis.

February 13

Morning: THIRD SESSION

I. Sussex (Chairman)

J.H. Rothman - Genetic control of cell lineage and development in **caenorhabditis elegans.**
J. Modolell - Development of the pattern of sensory organs in **drosophila.**

POSTER DISCUSSION

M. Delseny

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F. Salamini - Developmental muntants of barley.
M.A. Estelle - Characterization of hormone-resistant mutants of arabidopsis.
J.M.Martínez Zapater - Floral mutations in **arabidopsis thaliana.**

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M.A. Estelle (Chairman)

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R.S. Poethig (Chairman)

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INTRODUCTION

P. Puigdoménech

Centro de Investigación y Desarrollo

Departamento de Genética Molecular

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Barcelona (Spain)

INTRODUCTION

Plant development is becoming a subject that interests an increasing number of research groups. The reasons are both based in its intrinsic interest and in the need to understand plant regeneration, an essential step in many plant transformation techniques. Therefore plant development is a matter studied for both basic and applied research groups. The first ones would like to know how the genetic information of a plant species directs the growth of the individuals, the second ones would like to predict conditions allowing any plant species to produce the desired plants from tissue cultures.

The use of recombinant DNA technology has opened a wealth of new possibilities. It allows to use DNA probes as markers for developmental events in plants, to identify genes responsible for the regulation of specific steps in plant development and to transfer engineered genes to an increasing number of plant species. The results obtained with the use of these methodologies have greatly increased the knowledge available on the basic mechanisms of plant growth and they opened the way for the introduction of new genes into plant species and therefore calling the attention of plant breeders.

The Course held in Fundación Juan March tried to convene scientists working in plant development from different approaches. The molecular aspects are certainly essential to understand the mechanisms underlying the phenomenon. However when what happened in the animal field is considered, it is clear that information coming from genetics and cell biology may be decisive to interpret the results and to find ways towards the central pathways that control plant development. In this sense groups working in the plant field have the advantage (and the disadvantage) to contrast what they are doing with what happens in the animal systems which are in general much more advanced than in plants.

The course was designed to bring together people working in plant development from different experiences. One is the genetic approach to plant development, where groups are working trying to identify new mutations and to analyze them, to understand

cell types and lineages. The use of transposons to detect and to clone such mutations is particularly useful in plants. Another one is the molecular approach to identify and analyze genes that regulate plant growth or that may be useful to unravel some developmental stage. The general structure of the plant genome and the use of parasites (mostly those of the *Agrobacterium* genus) to understand how hormone action takes part in the control of plant growth have also been reviewed. Two main species are being used for these studies: cereals (mostly maize) and *Arabidopsis thaliana*, for different reasons. In order to confront the results in plants with those of the animal world speakers were invited to review some aspects of the developmental work that is being carried out in the two best known animal species: *C. elegans* and *Drosophila*. The aim of the course was to promote discussion among participants and to allow an overview of the subject in a relaxed atmosphere.

FIRST SESSION

I. SUSSEX

R.S. POETHIG

ORGANIZATION OF PLANT APICAL MERISTEMS.

Ian Sussex.
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Lineages of cells initiated in the apical meristems differentiate as the tissues and organs of the plant. By generating plants that are genetic chimeras, in which sectors of cells are marked by phenotypically identifiable markers, it is possible to determine how cells of the meristems are partitioned out to tissues and organs during development. For example, the number of cells that are recruited for leaf formation, or formation of the leaf blade, or for formation of nodes and internodes can be accurately assessed, as can the time when a subpopulation of meristem cells becomes determined for reproductive development

A second type of chimera, a periclinal chimera in which one layer of cells in the meristem is genetically different from the others, is usually stable throughout development and can provide information on longterm or repetitive developmental events. For example, periclinal chimeras can be used to identify internal tissue boundaries and to distinguish between lineage dependent and position dependent differentiation. These chimeras also provide information on how developmental coordination between the meristem layers is achieved during formation of lateral organs

Genetic regulation of phase change in maize

R.S.Poethig, A.Bassiri and M.Dudley, Department of Biology, University of Pennsylvania, Philadelphia, PA 19104-6018.

Several dominant, gain-of-function mutations in maize(Teopod 1,Tp2, Tp3 and Corngrass) cause the prolonged expression of juvenile vegetative traits. Although these mutations increase the duration of vegetative growth, they do not increase the duration of shoot growth or affect the time at which the shoot becomes determinate. Furthermore, the morphology of mutant plants is intermediate in character, rather than being purely juvenile. These results suggest that juvenile development is regulated independently of the transition to an adult/reproductive phase of shoot growth. We propose that phase change is regulated in a combinatorial fashion by the interaction of several more-or-less independent developmental programs.

SECOND SESSION

M. DELSENY

M. FREELING

S.C. DE VRIES

STRUCTURE OF PLANT NUCLEAR GENOME

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The plant nuclear genome is rather complex and contains various types of sequences which have been recognized during the last fifteen years. A detailed knowledge of the plant nuclear genome is necessary for the Plant breeder to evaluate the genetic distance between two species and (or) varieties to be crossed. He needs genetic markers to analyse the progeny of crosses and to estimate the recombination capacity of two genomes. Several of this problems can now be approached because of recent progress in our knowledge of the plant nuclear genome at the molecular level.

The numerous and complementary strategies to investigate the plant genome will be briefly analyzed: cytogenetics and cytophotometry, renaturation kinetics, cloning individual genes or DNA sequences, genetic mapping, RFLP and long range mapping. The type of informations given by these techniques will be discussed with respect to the evolution of homologous chromosomes in different species.

Particular emphasis will focus on the various types of repeated sequences and their arrangement with low copy number sequences. Many repeated sequences are species specific and it is likely that they play a major role in the speciation phenomena, limiting out-crossing with different species. Analysis of their sequence very often reveals interesting features which suggest hypothesis for their origin and evolution. Data on yeast indicate that such repeated sequences located at strategic positions (centromere and telomere)play a major role in stabilizing chromosomes. Attempts to isolate similar elements from higher plants chromosomes will be described. Repeated sequences are sources of trouble in chromosome walking strategies various ways to overcome these difficulties will be discussed as well as the possibility to use polymorphic variants to map several loci at the same time.

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MUTANTS ANALYSIS SUPPORTS THE MODEL THAT CELL FATES IN THE MAIZE LEAF TO AGE IN A DEVELOPMENTAL CHRONOLOGY

Michael Freeling, Phillip Becraft, Ralph Bertrand, Kelly Dawe, John Fowler, Barbara Lane and Anne Sylvester (alphabetically), Department of Plant Biology, University of California, Berkeley, CA 94720.

The maize leaf has been nurtured as a system to the point where questions of plant development may actually get answered (1). My laboratory is describing several leaf mutants that specify organ transformation that may disconnect age from position. The maize leaf is a gradient of developmental age from the tip of the blade to the base of the sheath. Between younger sheath and older blade is the auricle-ligule.

9 loci are defined by mutants that transform blade to sheath but none transform sheath to blade. This fact may be explained if blade cells (oldest) necessarily passed through sheath (youngest) and auricle maturation stages.

Histology, mutant descriptions, and the analysis of genetic mosaics for liguleless mutant tissue implies that a specific "make auricle and ligule" signal is generated at a specific time at the midrib and is somehow propagated toward the margin. The product of the Lgl wildtype allele is to encode a product necessary to both make ligule and to propagate the signal. This signal overrides the default cell division programs that we have also been describing for leaf growth.

Our continuing studies of the **Knotted-1** (**Kn1**) mutants lead to a working hypothesis predicting that the relatively undifferentiated tissue that surrounds the lateral veins of Knotted leaves is actually "young for its age" and is, thus, out-of-place sheath. SEM examination of epidermal cuticle supports this prediction. I will present evidence that cells in the primordial leaf undergo "default" cell division and maturation programs through which they acquire developmental age, and that various age along the way correspond to particular competences to "hear" signals that pass over the leaf at various moments. Leaf developmental mutants have phenotypes that fit this notion; these are being characterized, transposon-tagged, and will eventually be used to identify meaningful molecules. (As we build on the maize leaf as a system, certain generalizations emerge: leaf primordia do have preferred lineage-cell fate correlations; the epidermis may not send important developmental signals; compression-stress has been excluded as an important factor in coordinating leaf cell division rates; and the anther is a determinant meristem, not a leaf homolog.)

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DEVELOPMENTAL MUTANTS AND EXTRACELLULAR PROTEINS IN CARROT SOMATIC EMBRYOGENESIS

Sacco C. de Vries, Hilbert Booij, Jan Cordewener, Fred A. van Engelen, Anke de Jong, Ab van Kammen, Fiorella Lo Schiavo[1], Gerard Schellekens, Peter Sterk and Mario Terzi[1].
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Somatic embryogenesis in carrot suspension cultures is a widely accepted model system to study the molecular mechanisms underlying plant embryogenesis (1). Secreted medium glycoproteins appear to be important in several steps in the process of somatic embryogenesis since a) high molecular-weight conditioned medium components promote the formation of embryogenic cell clusters (2), b) one or more secreted glycoproteins are able to rescue somatic embryogenesis in cultures inhibited by the glycosylation inhibitor tunicamycin (3), and c) somatic embryogenesis can be rescued in the temperature-sensitive emb$^-$ cell line ts11 (4) by the addition of secreted medium glycoproteins. The aim of the present study was to identify the proteins responsible for the complementation by addition of separated medium glycoproteins to tunicamycin-inhibited wild-type embryo cultures and ts11 embryo cultures at the non-permissive temperature of 32°C.

So far three individual proteins have been purified that promote somatic embryogenesis at different developmental stages. a) Somatic embryogenesis in tunicamycin-inhibited cultures can be restored by the addition of a 38 kDa isoperoxidase. This peroxidase appears highly specific in its activity in the inhibition-complementation assay, since it was purified from a mixture of at least 8 other isoperoxidases with identical molecular weight and only slight variations in i.e.p. The activity of the complementing peroxidase is detectable in embryo cultures only, from 1 day after embryo initiation onwards. Based on morphological observations the complementing peroxidase is apparently involved in very early stages of somatic embryo development, before any detectable embryo forms have appeared. b) The temperature-sensitive mutant ts11 can be rescued with 60 to 70 % efficiency at the non-permissive temperature by addition of a purified 30 kDa fucosylated secreted glycoprotein from a wild-type culture. Since ts11 is blocked in the transition of globular to heart somatic embryos, the complementing effect of this protein suggests that also in later stages of embryo development secreted medium proteins are essential. The 30 kDa secreted glycoprotein is probably dependent on correct fucosylation of its glycans for proper complementing activity.

c) A secreted medium glycoprotein of 46 kDa was purified from wild-type cultures that resulted in an 8 to 10 fold increase in the number of globular embryos formed in a ts11 culture. All of the additionally formed globular embryos retained the mutant ts11 phenotype.

All three purified embryo-promoting proteins exhibited a very narrow concentration range in their activity, with optima between 0.1 and 0.5 nM. In addition to these three proteins a fourth secreted

medium protein of 52/54 kDa was identified that inhibited somatic embryogenesis in a concentration-dependent fashion in wild-type embryo cultures. Analysis of a specific antiserum raised against a λgt11 52/54 kDa - lacZ fusion protein revealed that the protein is present in cell walls of single suspension cells of all cultures investigated and in basal parts of the root and stem of seedlings. In contrast, a second clone corresponding to a secreted glycoprotein of unknown function, also isolated by expression screening, was shown to be present in embryogenic cultures and somatic embryos and in the shoot apical meristem and young flower meristems of carrot plants.

In conclusion, we have obtained evidence that carrot somatic embryogenesis depends on a delicate balance of promoting as well as inhibiting secreted glycoproteins of which the specificity is, at least in part, located in the sugar moiety. In addition, we have shown that genes encoding secreted glycoproteins of embryogenic carrot suspension cultures exhibit strikingly tissue-specific expression patterns in the intact plant.

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THIRD SESSION

J. H. ROTHMAN

J. MODOLELL

GENETIC CONTROL OF CELL LINEAGE AND DEVELOPMENT IN *CAENORHABDITIS ELEGANS* Joel H. Rothman, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH England

The nematode *Caenorhabditis elegans* has proven to be an ideal experimental organism for studying the genetic control of cell lineage, pattern formation, and morphogenesis. Its short generation time and large brood size make genetic studies straightforward (1, 2). Mutations can be be homozygosed and propagated by self-fertilization of hermaphrodites, and genetic crosses can be performed with males. Hundreds of genes, mapped to its six chromosomes, include those involved in development, physiology, behavior, and morphology. *C. elegans* is arguably the most completely understood metazoan in many regards. Its small cell number, transparency, and stereotyped cell lineage have made it possible to determine the entire cellular anatomy and nervous system connectivity by electron microscopic reconstruction (3), and to describe its development fully in the form of a lineage chart of all somatic nuclear divisions (4, 5, 6). This knowledge allows genetic or physical perturbations in development to be investigated at single-cell resolution. A physical map of the nematode genome is nearly complete and has greatly simplified molecular analyses (7, 8).

A number of principles have emerged from the determination of the cell lineage. With few exceptions the lineage is invariant. It is parcelled into modules of cell divisions that occur within each of 6 developmental stages: the embryo, four larval stages, and the adult. Often a stereotyped lineage pattern or "sublineage" occurs at multiple positions throughout the lineage suggesting that a particular combination of genes specifies a given sublineage by cell-intrinsic mechanisms; identification of mutations that perturb a particular type of sublineage at several points in the lineage support this notion. Direct comparison of the lineage in related nematode species illustrates ways it can be modified throughout evolution (9, 10). For example, the number of times that a sublineage is sequentially repeated, the polarity of a particular cell division, and the partitioning of different cell fate potential to one product of a division *versus* its sister differ among nematode species, indicating that one or a few genes control each of these processes in particular sublineages. In fact, mutations altering these parameters of the lineage have been identified (11).

A primary goal of analyzing the lineage genetically is to understand how genes control developmental decisions at each cell division. A large number of viable mutations causing aberrant lineages have been identified; in general, such mutations affect only particular portions of the lineage (11). Many produce homeotic transformations, resulting in substitution of one sublineage for another and often profound changes in gross morphology. Among the most conspicuous of these are mutants defective in development of the vulva (12). The "MuV" mutations lead to multiple vulva-like cell groups along the ventral hypodermis in addition to a vulva at its normal ventral position. The vulvaless ("Vul") mutations transform the sublineages that normally generate the vulva to those that result in non-vulval fates. The Vul and MuV genes have been ordered into a genetic pathway that defines sequential stages in generation and determination of vulval precursor cells and expression of vulval-specific lineages. Certain lineage mutants show temporal rather than spatial transformations in lineage; mutations in four such "heterochronic" genes, which show either premature or delayed expression of cell lineages and fates, have been ordered genetically into a linear pathway of action (13). Thus, heterochronic genes regulate the timing of execution of particular cell fates within the overall context of worm development. Some of the lineage genes encode nuclear proteins, and some contain homeobox motifs, implying that at least some of these genes control lineage decisions by altering expression of other genes (14, 15, 16).

The invariance of the lineage does not reflect strict cell-autonomous determination of cell fates; cellular interactions are important at several stages. The precursors of the vulva constitute an "equivalence group" -- although they retain the capacity to express identical fates, they normally express different fates as the result of interactions with an underlying gonadal cell and between each other (17). The gene *lin-12* participates in these interactions (18). Two different cellular interactions, regulation of the switch from mitotic proliferation of gamete precursors to meiotic division, and an interaction between cells in the early embryo required for normal development of the pharynx, both require the *glp-1* gene (19, 20). *glp-1* and *lin-12* are tightly linked genes whose products share strong sequence identity with each other, and with the product of *Notch*, a gene involved in a particular cellular interaction in *Drosophila* (21, 22). These three genes appear to encode transmembrane receptors that receive signals from nearby cells and transduce the signals into changes in the fates of the receiving cells.

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DEVELOPMENT OF THE PATTERN OF SENSORY ORGANS IN DROSOPHILA.

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Centro de Biología Molecular, CSIC and UAM, 28049 Madrid, Spain.

The larval and adult cuticles of Drosophila contain many sensory organs (SOs) with mechano- or chemoreceptor function (hairs and bristles and sensilla of other classes). The type and position of these SOs form a stereotyped pattern which can be modified by mutations at many genes. Several of these genes have been extensively analyzed both at the genetic and molecular level. This work has shown that the patterning of SOs constitutes an excellent system to study the mechanisms that specify position at the individual cell level (Ghysen and Dambly-Chaudière, 1989, review).

Classical work by Stern (1954) suggested that SOs develop in specific positions because SO-inducing genes are locally activated in response to an underlying "prepattern" of positional cues. The pioneering work of Wigglesworth (1940) demonstrated that cell interactions, like lateral inhibition, also play a most important role in refining the pattern by, for instance, controlling the spacing between SOs. Moreover, computer simulations by Ghysen and Richelle (1979) indicated that lateral inhibition allows SOs to form at reproducible locations even if the regional control of SO-inducing genes is not very precise.

The best characterized SO-inducing genes are those clustered in the achaete-scute complex (AS-C) (García-Bellido, 1981; Ghysen and Dambly-Chaudière, 1987, review). The molecular analysis of this complex has shown that it contains four genes which encode putative transcriptional regulators. These probably activate genes effecting SO differentiation. As predicted by the genetic and developmental studies, the expression of AS-C genes is spatially restricted to many separate groups of cells (Romani et al., 1989). Within a group, one or several cells will become SO precursors. At least for one of the genes (scute), the pattern of expression seems controlled by a set of cis-acting enhancer-like elements scattered over 40 kb of non transcribed AS-C DNA (Ruiz-Gómez and Modolell, 1987). Each element would interpret appropriate combinations of spatial cues (prepattern) and activate scute expression in one or a few groups of cells. Within each group of expressing cells, the number of SO precursors is limited by cell interactions (lateral inhibition) mediated by genes like the neurogenic loci, which encode transmembrane proteins (Campos-Ortega, 1988, review), and shaggy (Simpson and Carteret, 1989). It is also limited by the products of the hairy and extramacrochaetae genes, which seem to sequester the AS-C proteins in complexes inneffective in promoting neurogenesis (Garrell and Modolell, submitted).

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FOURTH SESSION

F. SALAMINI

M. A. ESTELLE

J.M. MARTINEZ ZAPATER

31

DEVELOPMENTAL MUTANTS OF BARLEY

F. SALAMINI

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Developmental mutations of barley are discussed in the frame of a model of plant developed for the **Gramineae.** Relevant issues proper to the definition of this model are:

—the concept of the phytomer
—the phytomeric structure of the plant
—homology of organs
—phytomer type 1 and 2
—phytomer type 1 and morphogenesis
—continuous embryology and recurrent embryogenesis

Developmental mutants are classified, according to the model introduced, as inducing transitions (the phytomer in a region of the plant assumes characteristics proper of a contiguous or not contiguous region), organ modifications, addition and reduction of phytomers.

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LIST OF MUTANTS

ari (lk) = short awn (breviaristatum)
awl = awnless
abr (lb) = accordion basal rachis internode
acr = accordion rachis internode
als (abs) = absent lower laterals
adp = awned palea
be = branched ear
bh = bushy heart (= branched 5)
lb = long basal rachis internode
bir (brc) = branching rachilla (similar to branched 5 = bh = compositum = com)
br = brachytic, dwarf
bra (trd) = bracteatum
bra-cd = semibracteatum
cal (sk) = calcaroides
cud = curly dwarf
D = dwarf (sterile)
De = lax spike
dwf = vegetative dwarf
e (lep) = elongated outer glume
flo = extra floret
K (K^e) = hooded
int-l81 = intermediate (modified ear)
lax-a8 = laxatum
Lc (L) = lax spike
Lfb = leafy bract
Lfl = leafless
li = liguleless
lnt = low number of tillers
lo = small lodicules
lr = lateral lemma appendix reduced
mgl = many glumes on laterals
mlt (mul2) = multiflorous
mn = many noded
mnb (den6) = multi noded branched
mo = multiovary
mo 1-5 = stamen --> ovary
mo 5 = lodicules to leaf
nld = narrow leaf dwarf
op = opposite spikelets
ov1 = ovaryless
rin = low number of rachis internode
sid = single internoded dwarf
tr (Tr) = triple awned lemma
tri = triaristatum (awned palea)
u4 = unbranched style
uc2 = uniculm
uz = uzu-semibrachytic
v = deficiens
viv4 = viviparoides

CHARACTERIZATION OF HORMONE-RESISTANT MUTANTS OF ARABIDOPSIS. Cynthia Lincoln,F.Bryan Pickett,Candace Timpte,Jocelyn C.Turner,Allison K.Wilson and Mark A. Estelle. Dept. of Biology, Indiana University, Bloomington IN.47405.

We are interested in the role of the plant hormones IAA and ethylene during plant growth and development. In order to understand the function of these regulatory molecules at the tissue or whole plant level it will be necessary to understand the mode of hormone action as well as the mechanisms which regulate hormone levels throughout the plant. Our approach to these problems has been to identify genes involved in hormone action by isolating mutants of **Arabidopsis** which are resistant to exogenous application of either auxin or ethylene. Because disruption of any of a number of functions may confer resistance (uptake or transport proteins, receptors, signal transduction components), our initial goal has been to use screens for hormone-resistance to identify as many interesting genes as possible. M2 seedlings were screened for resistance to auxin on agar media containing inhibiting concentrations of either IAA, 2,4-D or I-NAA.

Ethylene-resistant mutants were isolated in a similar way using the ethylene precursor ACC as a selective agent. A number of mutants which offer resistance to either auxin or ethylene have now been recovered .Genetic analysis has demonstrated that we have identified at least 3 genes which confer resistance to auxin and at least 2 which result in ethylene resistance. In addition to hormone resistance, mutations at each gene confer a distinctive morphological phenotype. As a prelude to cloning we have positioned two of the auxin-resistant loci (**axr1** and **axr2**) on an RFLP map generated by Chang et al. (1). Both genes appear to lie within approximately 2 map units of the closest RFLP. The results of our genetic studies as well as the characterization of the mutant phenotypes will be presented.

1)Chang et al. Proc.Natl.Acad.Sci.85:6856-6860

FLORAL MUTATIONS IN Arabidopsis thaliana

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In contrast to the important role of genetics in our understanding of animal development, genetic analysis has not been a widely used approach in plant development studies. One plant, Arabidopsis thaliana, is particularly well suited for studying the molecular genetics of plant development. The small size and short generation time of this species allows for the rapid isolation and characterization of mutants. Moreover, its small genome size may allow Arabidopsis genes to be cloned only on the basis of their genetic identification.

The transition from vegetative to reproductive growth is the most conspicuous change in plant development. Two different processes, floral induction and flower development at the apical meristem, can be distinguished. In Arabidopsis, flowering is induced by both long-day photoperiod and low temperature applied at the seedling or vegetative stages. Physiological factors should also be important since plants flower even under non-inductive conditions once a certain developmental stage is reached. By selecting for mutants that are delayed in flowering time when grown under inductive conditions, a total of 11 loci have been identified which are probably involved in floral induction. Physiological analyses of these mutants indicate that several pathways are implicated in floral induction in Arabidopsis.

Floral induction produces profound changes in the pattern of growth and differentiation of the apical meristem: i) growth becomes determinate, all meristematic cells differentiate and no axillary meristems are produced; ii) internode elongation is strongly inhibited; iii) four different flower organs (sepals, petals, stamens and carpels) differentiate in place of leaves. Mutations that affect each one of these processes have been isolated in Arabidopsis and more than 10 loci required for normal flower development have been identified. Morphological characterization of different mutant alleles at the same loci and the analyses of double mutant phenotypes indicate that some of these loci are responsible for the control of flower organ identity.

We will summarize the results of the genetic analyses of floral induction and development in Arabidopsis and discuss the progress towards the molecular cloning of those loci.

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FIFTH SESSION

A. SPENA

P. J. J. HOOYKAAS

Plant pathogens and genetic engineering: an approach to the study of plant development.

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Pathological conditions are sometime exageration, sometime deficiency, but always alteration of certain processes which take place in a healthy organism. Similarly developmental anomalies are deviations from the normal pathway of developmental events. Consequently knowledge of the genesis of developmental anomalies can help us to understand also normal developmental events. For this reason we have used plant genetic engineering tecniques to construct transgenic plants altered in their morphology and growth habit. Genes able to alter plant morphogenesis and growth can, of course, be cloned from the genome of plants. However plant pathogens can be an alternative and useful source of morphogenetic genes. In this respect I will describe the use of the *ipt* gene of *Agrobacterium tumefaciens* and of the *rolA, B,* and *C* genes from *Agrobacterium rhizogenes* to generate developmental alterations in transgenic plants.
Plant genetic engineering allows one to introduce a gene into the plant genome and by providing the gene of interest with appropriate regulatory sequences, one can control its expression. It will be presented a novel approach for studying gene action in plants, which could be particularly useful for the study of morphogenetic genes. It is well known that transposable elements are able to selectively activate gene expression in clonal populations of cells. Consequently they can be used as switches to control gene expression. Genetic mosaics, caused by transposon excision and consequent reactivation of a transposon-splitted gene, could be used to address the cell autonomous aspects of gene action and to circumvent regeneration problems due to the expression of morphogenetic genes in plant cells. In this respect, the used of transposon-splitted constructions to address the cell-autonomous behaviour of the *rolC* gene will be presented.
Altered phenotypes obtained by plant genetic engineering could be used in the same way as mutant phenotypes obtained by classical genetics to correlate morphological and physiological alterations with hormonal content. In this respect hormonal analysis in transgenic plant material will be presented and discussed.

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Agrobacterium as a developmental parasite.

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The bacterium Agrobacterium tumefaciens induces tumours called crown galls on dicotyledonous plant species. During tumorigenesis agrobacteria transfer a segment of their tumour inducing (Ti) plasmid to plant cells at the infection site. Expression of onc-genes located in the T (Transferred)-DNA leads to tumour formation. The transfer apparatus is determined by vir-genes located next to the T-DNA on the Ti plasmid and by chv-genes located in the bacterial chromosome. One of the early steps in tumorigenesis involves the induction of the expression of the vir-genes by phenolic plant compounds such as acetosyringone, sinapic acid and coniferyl alcohol. The regulatory genes virA and virG determine a transmembrane receptor protein and an activator protein, which together form a twofactor regulatory system. The induced Vir-proteins mediate the formation of single stranded copies of the T-region (T-strands), which are probably intermediates in the T-DNA transfer process.

The T-DNA from the Ti plasmid contains several genes that are expressed in the transformed plant cells. Three of these are onc-genes involved in the production of the auxin indole acetic acid (aux-genes) and the cytokinin isopentenyl-AMP (cyt-gene). The functions of a fourth T-DNA onc-gene, which codes for mRNA 6^b, is not known, but this onc-gene is by itself able to provoke tumour formation in certain plant species. The presence of the complete T-DNA in plant cells usually prevents their regeneration of specialized tissues such as roots and shoots. However, upon deletion of either or both of the aux genes shoot regeneration becomes possible. The regenerated shoots do not form roots, however and thus they can grow out into flowering plants only after grafting onto normal understems of the same plant species. Similar results have been obtained with plant cells transformed with the cyt-gene alone. Apparently, the internal overproduction of a cytokinin due to the presence of the cyt gene converts normal plant cells into cells which cannot differentiate into roots. Two other properties in which T-cyt containing plantlets differ from normal plants are that they do not form fertile pollen (male sterilitiy) and that they do not respond to infection with tumorigenic Agrobacterium strains with tumour formation. It can thus be concluded that already the introduction of a single T-DNA gene can have a profound effect on the growth and development of plants. In the lecture a review will be given of the current knowledge on the Ti T-DNA onc-genes, on their molecular mechanism of action and on their use for specific purposes.

SIXTH SESSION

T. NELSON
P. PUIGDOMENECH
M. PAGES

PATTERNS OF DEVELOPMENT IN C4 LEAVES. Timothy Nelson and Jane A.Landgale, Yale Biology Department, P.O.Box 6666, New Haven, CT 06511

In plants utilizing the C4 scheme for carbon fixation, the vascular system is the framework around which the two required photosynthetic cell types are arranged. Each vein is surrounded by a photosynthetic bundle sheath (BS). Photosynthetic mesophyll (M) cell and air spaces occupy the remaining mesophyll space. The operation of the C4 cycle relies on the presence of one set of photosynthetic enzimes exclusively in BS cells (e.g., RuBPCase, NADP-ME) and a complementary set in M cells (eg., PEPCase, PPdK, NADP-MDH).BS and M cells are interconnected by abundant plasmodesmata, through which the intermediates of the C4 cycle pass. We propose that the distinct patterns of differentiation shown by mature BS and M cells in C4 leaves represent an extreme state of a regulatory system capable of gene expression patterns permitting C3-type, C4-type, or combined modes of carbon fixation.

In situ hybridization studies suggest that C4 genes are controlled in a radial fashion around developing vascular centers, such that BS genes are activated at one radius and M genes at a greater radius (1). This pattern is evident before BS and M cells are morphologically distinct (2). Leaves such as husk leaves contain M cells that differentiate at up to 10-cells distance from the nearest vein, in contrast to the 2-cell maximal distance found in developing foliar leaves (3). C4 development occurs adjacent to veins in both foliar and husk leaf types under equivalent illumination, but beyond a several-cell radius, M-cells develop as conventional C3 chlorenchyma. Along with physiological data, these experiments suggest that positional control of M-cell development acts locally within a small radius of each vein and that C3-type photosynthetic development is the default scheme (3).

Once BS and M cells take on a pattern C4 pattern of gene expression, it is relatively stable, even under "C3" conditions of low light. However, the C3 pattern is rapidly shifted to a C4 pattern with additional illumination. We found that these patterns are correlated with distinct patterns of methylation near or within individual C4 genes. We suggest that methylation events "lock in" the distinct gene expression patterns for BS an M cells early in their differentiation under illumination, but not in darkness.

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PLANT CELL WALL PROTEIN GENES AS DEVELOPMENTAL MARKERS

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Cell wall is one of the essential and characteristic components of plant cells. Since the identification of extensin, one of the main hydroxyproline-rich glycoproteins extracted from dicot tissues, and the cloning of its corresponding cDNA and genes (see refs. 1-3 for reviews), they have shown to be interesting systems to study gene regulation in plants. They are also useful developmental markers. Extensin genes appear to be preferentially expressed in defined plant organs, to be induced in transgenic plants overexpressing Agrobacterium genes coding for cytokinin genes and to take part in the defense mechanisms of the plant. In particular they may be induced by fungal infection and elicitors. Extensins may be coded by multigene families having differential patterns of expression.

Other cell wall components have been identified marking defined cell types or tissues. These include a group of glycine-rich protein genes preferentially expressed in the vascular system of dicots (4), a group of proline-rich protein genes from soybean that show complex patterns of expression (5), a gene taking part in early events of lateral root formatin (6) and nodulin-75, one of the genes induced by nodulation in legumes (7). Finally, monoclonal antibodies have been obtained marking cell wall components defining positional events in embryo development (8). In monocot species only a gene coding for a proline-rich protein containing a highly repetitive sequence has been identified (9). The protein seems to be coded by a small number of genes (one or two) expressed in tissues rich in dividing cells, induced by wounding of young leaves and marking the formation of the vascular system (10).

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GENES INDUCED BY ABSCISIC ACID AND WATER STRESS IN MAIZE

Montserrat Pagès Departamento Genética Molecular. C.I.D. (C.S.I.C.) Jorge Girona 18-26. 08034 Barcelona. Spain.

In earlier studies on the regulation of gene expression during embryogenesis in Zea mays we have shown the expression of a set of specific polypeptides which were rapidly induced by ABA in young embryos upon hormone treatment (1,2). These polypeptides also appear during normal embryogenesis when the development of the embryo is progressing to the maturation stage, coinciding with the period where the endogenous level of ABA attains the maximum peak. After accumulating in dry embryos they disappear during the first hours of germination. cDNAs and genomic clones corresponding to these proteins were obtained. Northern blot hybridization showed that these genes are involved in generalized plant ABA responses as they accumulate not only during dehydration of the embryo and in ABA-treated tissues but also in leaves under conditions of water-deficit (3,4,5).

The function of the proteins encoded by ABA-regulated genes is unknown.The sequence of the maize 16 kDa ABA induced protein (AIP-16) deduced from the sequence of the cDNA (pMAH9), predicts that is an RNA-binding protein since it possesses a ribonucleoprotein consensus sequence (RNP-CS)-type RNA binding domain (CS-RBD) and contains the RNP-CS (RGFGFVTF) and RNP2 (CFVGGL) sequence motifs (3,6). Like several animal RNA-binding proteins, AIP-16 also has a glycine-rich carboxyl-terminal domain (7). AIP-16 is the first RNP-CS plant protein to be described. By means of ribohomopolymer binding assays and using antibodies raised against a synthetic peptide from the predicted sequence, we demonstrated that AIP-16 is a "bona fide" RNA-binding protein with preference for guanosine-rich sequences. These findings establish a role for an inducible RNA-binding protein in the response of plants to the hormone abscisic acid in embryogenesis and dehydration, and suggest possible functions for the AIP-16.

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POSTER SESSIONS

L. BALCELLS
P. BARCELO
J. L. CAMPOS
J. CARBONELL
P. CAROL
C. DIAZ-SALA
F. GARCIA-MAROTO
J. A. GODOY
L. GOMEZ
J. JORDANO
A. LEITE
M. LEPETIT
E. LOPEZ-JUEZ
J. PAZ-ARES
A. M. PELACHO
A. PIQUERAS CASTILLO
K. J. RAKOWSKI
M. SAHRAWY B.
P. SANCHEZ DE LA HOZ
M. A. SANCHEZ-PINA
R. SIMON
G. SPANGENBERG
B. STEINITZ
J. VEUSKENS

DEVELOPMENT OF A TRANSPOSON TAGGING SYSTEM IN ARABIDOPSIS THALIANA BASED ON THE AC ELEMENT OF MAIZE.

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We are developing a transposon tagging system (1) using the maize transposable element *Ac* (2) in *Arabidopsis thaliana*. *Ac* transposes with a low frequency in *Arabidopsis* and gives rise to potentially unstable mutations. To overcome these drawbacks we are using a two-component system: a) *Ac* with an internal deletion that makes the transposase gene inactive (*Ds*) and b) the *Ac* transposase gene under the control of different promoters, more active than the original one. The transposase gene fusions are unable to transpose, because they lack one of the termini of the *Ac* element. However, the *Ds* element can transpose in the presence of the transposase.

Most mutations caused by the insertion of transposable elements show occasional revertants among the progeny, which correspond to excision events that restore the activity of the mutated gene. Thus, in our system we will be able to distinguish mutations caused by the *Ds* element from those due to somaclonal variation, by identifying revertants. Any mutation caused by a *Ds* element can be made stable by selecting a plant that lacks the *Ac* transposase gene, using Mendelian segregation.

To transform *A. thaliana*, we use the method of Valvekens et al. (3), which involves infecting root explants with *Agrobacterium tumefaciens* and selecting the transformed calli by means of resistance to an antibiotic. We have obtained transformed plants (T_0), using constructs containing a *Ds* element or the *Ac* transposase gene. We plan to cross T_1 plants homozygous for the construct (F_0), in order to obtain transposition in the F_1 generation. If the Ds element inserts in a gene or regulatory region, the mutation will become apparent in the F_2 or F_3 generation. The plants with interesting mutant phenotypes will be used to clone the tagged gene.

Our ultimate aim is to charaterise genes involved in the control of flowering time. Once our transposon tagging system is developed, we will use it to clone such genes.

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CALLUS PROLIFERATION, PLANT REGENERATION AND PROTOPLAST ISOLATION FROM INFLORESCENCE SHEATH LEAVES OF MATURE TRITORDEUM AND WHEAT PLANTS.

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With the final aim of using leaves as a source tissue for protoplast isolation, cell proliferation and plant regeneration capability of the inflorescence sheath leaves of mature plants of wheat and Tritordeum (amphiploid barley-wheat) were studied.
Leaf segments of plants with flag leaf lengths ranging from 0.2 - 30 cm (final mature flag leaf length) were cultured independently. Sixty 1 mm segments up from the final node were cultured and maintained on LM1 medium with 2 mg/l 2,4-D.
In both wheat and Tritordeum all inflorescence sheath leaves showed the ability for callus formation, with the maximum response up to 5.5 cm for wheat and 4 cm for Tritordeum.
For plant regeneration calli were transferred to MS medium with 0.5 mg/l 2,4-D and 1 mg/l BAP. After one month green plants were obtained from some cultures.
Protoplasts have been isolated from leaves identified as being able to proliferate. Data from these experiments will be presented.

MOLECULAR EFFECTS OF UV-C IRRADIATION ON PLANT DEVELOPMENT

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The retardation of growth and flowering after UV-C (254nm) irradiation was previously reported in Nicotiana rustica (Tiburcio et al 1985). The irradiation with UV-C induced an accumulation of carotenoids with a simultaneous decrease in the chlorophyll (Chl) content. Analysis of putrescine-derived alkaloid levels revealed the existence of a "simulatory" effect induced by the UV-C treatment. In addition irradiated leaves showed specific changes in the protein pattern (Tiburcio et al 1985).

In current experiments, we are investigating the effect of UV-C irradiation in Arabidopsis thaliana. The irradiated plants show a decrease (about 2-fold) in fresh weight. This effect can be correlated with the decrease in the expression of α tubulin gene. Analysis of Chl content reveals a decrease in Chl b (about 3-fold), without significant changes in Chl a. In contrast, carotenoid levels increase about 2.5 fold. The increase in the carotenoid content is correlated with the induction of HMG-CoA reductase gene, thought to play an important regulatory role in plant isoprenoid biosynthesis (Caelles et al. 1989). The UV-C treated plants also show a dramatic increase in the polyamine contents, especially the free and the hydroxycinnamic acid conjugated forms of spermidine and putrescine. We are also trying to correlate this effect with changes in the expression of the phenylpropanoid pathway genes (PAL,4Cl,CHS). Preliminary analysis of total proteins of leaves reveals changes in the banding pattern with respect to control leaves.

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DEVELOPMENT AND SENESCENCE IN PEA OVARIES. PROTEOLYTIC ACTIVITIES AND PLANT HORMONES.

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Unpollinated pea (Pisum sativum L., cv Alaska) ovaries have been used to study comparatively development and senescence processes. Unpollinated ovaries stop growing at about two days post anthesis and one day later initiate a senescence process characterized by: a) degenerative changes in the structure of the endocarp and mesocarp cells, b) increased spermine levels, c) the presence of new neutral proteolytic activities, and d) the loss of the ability to develop in response to gibberellic acid (GA_3) treatment. However the senescence program of the ovaries can be changed to a development program either by natural pollination (producing fruits with seeds) or by the addition of plant growth regulators (producing parthenocarpic fruits, i. e., without seeds) to the ovaries. Fruits obtained with GA_3 are apparently identical in morpholgy and structure to those obtained by pollination so have been used to study fruit development. Development is characterized by a) differentiation of endocarp and enlargement of mesocarp cells, b) low spermine levels, and c) the absence of the neutral proteolytic activities observed in senescent ovaries. Proteolytic activity in pea ovaries have been studied by the hydrolysis of endogenous (ribulose-1,5-bisphosphate carboxylase oxygenase) and exogenous (gelatin, azocasein and others) substrates. Gelatin films have also been used for localization experiments. Increase in some proteolytic activities, mainly endopeptidases, and the presence of new proteolytic activities in senescent ovaries (one of them localized in the endocarp) and the absence of these events after GA_3-treatment of the unpollinated ovaries suggest that control of proteolytic activity plays a key role in pea ovaries during fruit set and that plant hormones are involved in that control.

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Identification of a plastid ribosomal protein wich shows no homology with any bacterial ribosomal protein.

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The chloroplast possesses it's own translational apparatus that translates plastidial messenger RNAs.

Ribosomal RNA (rRNA) and one third of plastidial ribosomal proteins are encoded on the plastidial genome while the remaining proteins are encoded by the nuclear genome, synthesized in the cytoplasm and imported into the plastid.

The bicompartimental origin of the plastidial ribosomal components implies the tuning of their accumulation. A recent study showed that the accumulation of the plastid ribosomal components start early during seed germination in a non-synchronous way. It is probable that the nuclear genes coding for plastidial ribosomal proteins are under a developmental rather than a light regulated control.

Although nuclear encoded, most of these genes are clearly related to procaryotic (such as E. coli) genes, illustrating the procaryotic origin of plastids.

We present here the cDNA coding for a spinach plastidial ribosomal protein named CS-LA. The protein is associated with ribosomal large subunit, has been purificated by HPLC and characterised by N-terminal sequencing. The sequence shows no homology to any E. coli or B. stearotermophilus known ribosomal protein and present a homology with a ribosomal protein also identified in pea.

A comparison between spinach and pea cDNA of CS-LA shows a relative high divergence in sequence. A hybrophobic cluster analysis shows the conservation of the secondary structure suggesting that this ribosomal protein is involved in the general architecture of plastid ribosomes. The CS-LA transit peptide is more closely related to it's homologue in the pea than to the transit peptide of other spinach plastidial ribosomal proteins.

The relatively high divergence between the homologous proteins in pea and spinach suggests a high evolutionary rate which could explain the lack of homology with distant organisms such as E. coli.

To investigate the possible role of the protein CS-LA in plastid ribosomes, transgenic plants deregulated in the production of CS-LA, tobacco plants overproducing the protein or, producing an anti-sense RNA, A. thaliana producing an antisense RNA, are to be constructed.

We assume that plants which are affected in the expression of one plastidial ribosomal protein will show an abberrant development especially during seed germination and plastid differenciation.

MOLECULAR CHARACTERIZATION OF "IN VIVO" AND "IN VITRO" REJUVENATION IN FILBERT

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Maturation and aging seem to be responsible of the morphogenic potencial decline founded for the most of the woody species. As is common, micropropagation pathways founded in filbert (Corylus avellana L.) are linked to the aging and phase-change; situations that may be, at least, exogenously manipulated and, probably, reversed. The fact that "in vitro" subcultures and severe prunning of trees may favour rejuvenation, resulting in an increase in micropropagation success, was analyzed based on the characterization of "in vitro" morphogenic capacities and molecular differences among mature clones, embryonic clones, "in vitro" plantlets from mature clones after several subcultures and mature clones after several prunning. The current status of micropropagation pathways of filbert can be summarized as follows:
a.- **Embryonic clones** were able to yield a good rate of shoot proliferation, rooting rates and callus induction in a defined media. By changing the cytokinin/auxin ratio embryoid induction and plantlet regeneration was achieved. b.- With **mature clones** a reduction in the morphogenic pathways was always present. The impact of high concentrations of cytokinin during the first subcultures favoured the establishment of cycloclonal lines. Subsequent subcultures on complex media may favour shoot proliferation and rooting rates in mature tissues. c.- **"In vitro" plantlets from mature clones after several subcultures** may behave quite similarly to those derived from seedlings. d.- With **mature clones after two time severe prunning** a decrease of culture period and cytokinin concentration were necessary for culture establishment.

Total proteins electrophoresis in all the material assayed reveals the existence of specific polipeptides that can be correlated with the chronological stage of the plant material. Polyamine titers may also be in relation with the variation of the morphogenic capacities. DNA purification and their methylation patterns may also help to explain the "in vitro" benaviour of different plant tissues.

CLONING OF cDNA AND CHROMOSOMAL LOCATION OF GENES ENCODING THE THREE TYPES OF SUBUNITS OF THE WHEAT TETRAMERIC INHIBITOR OF INSECT α-AMILASE

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We have characterized three cDNA clones corresponding to proteins CM1, CM3 and CM16, which represent the three types of subunits of the wheat tetrameric inhibitor of insect α-amilases. The deduced amino acid sequences of the mature polypeptides are homologous to those of the dimeric and monomeric α-amilase inhibitors and of the trypsin inhibitors. The mature polypeptides are preceded by typical signal peptides. Southern-blot analysis of appropriate aneuploids, using the cloned cDNAs as probes, has led to the location of genes for subunits of the CM3 and of the CM16 type within a few kb of each other in chromosomes 4A, 4B and 4D, and those for the CM1 type of subunit in chromosomes 7A, 7B and 7D. Known subunits of the tetrameric inhibitor corresponding to genes from the B and D genomes have been previously characterized. No proteins of this class have been found to be encoded by the A genome in hexaploid wheat (genomes AA,BB,DD) or in diploid wheats (AA) and no anti α-amilase activity has been detected in the latter, so that the A-genome genes must be either silent (pseudogenes) or expressed at a much lower level.

A TOMATO cDNA CLONE INDUCED BY ABSCISIC ACID AND OSMOTIC STRESS. J.A. Godoy, S. Torres-Schumann, O. del Pozo y J.A. Pintor-Toro. Inst. de Recursos Naturales y Agrobiología. CSIC. Avda. Reina Mercedes, s/n. Aptdo. 1052. 41080 Sevilla.

A cDNA clone (TAS15) was isolated by differential hybridization of a cDNA library constructed from NaCl- and abscisic acid- (ABA) treated tomato seedlings. TAS15 mRNA is accumulated in seeds and in ABA-, NaCl- and manitol-treated seedlings, while it is not present in cold-treated, wounded or non-stressed seedlings. Hydroponically grown plants also accumulate TAS15 mRNA in roots, stems and leaves upon addition of NaCl or ABA to the nutrient solution. The TAS15 nucleotide sequence shows an open reading frame that codes for a 14 Kd, glycin-rich and highly hydrophilic polypeptide. The aminoacid sequence predicted for TAS15 product shows similarities with rice RAB21, cotton LeaD11 and some barley dehydrins genes products. The "in vitro" translation product of TAS15 hybridization-selected RNA shows a mobility in IEF/SDS PAGE similar to the non-phosphorylated form of a previously observed ABA- and NaCl-induced phosphorylated polypeptide.

WHEAT MONOMERIC INHIBITORS OF INSECT ALPHA-AMYLASES

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A major fraction of the albumins and globulins of wheat and barley endosperms is represented by a protein family which includes inhibitors of heterologous alpha-amylases and of trypsin. This family is receiving an increasing attention because of i) its possible role in plant protection, ii) its relationship with flour technological properties, iii) its allergenic effect in baker's asthma disease and iv) its potential as a model to investigate structure/activity relationships in an enzyme/inhibitor system.

Wheat inhibitors of heterologous alpha-amylases can be classified into monomeric, dimeric and tetrameric forms based on their degree of aggregation. Although considerable information is available concerning the last two classes, only one monomeric inhibitor has been so far characterized.

In this communication we report the isolation and characterization of the four major monomeric inhibitors from hexaploid wheat as well as the chromosomal location of their corresponding genes. We also report the "in vitro" effect of the most abundant inhibitor on amylase activities from diverse agricultural insect pests.

"Expression of *Lycopersicon esculentum* TAS 15 gene in abscisic acid- responsive protoplasts from tomato".

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TAS15 is a cDNA expressed in tomato plants in response to abscisic acid (ABA) and to salt (NaCl) and osmotic (i.e. manitol) stress. In addition, TAS15 is expressed in tomato dry seed, and the sequence of the putative protein coded by TAS 15 shows similarities with the rice rab21 (Mundy and Chua, 1988) and other maize (Gómez *et al.* 1988; M. Pages, personal communication) cotton (Baker *et al.* 1988) and barley (Close *et al.* 1989) Late Embryogenesis Abundant (LEA) plant genes (Godoy *et al* in preparation).

We report here results from preliminary experiments showing the that TAS15 gene is expressed in response to ABA in protoplasts prepared from tomato calli. In addition we have started setting the conditions for transient expression of chimeric bacterial ß-glucuronidase (GUS) gene fusions in these protoplasts. Once fully developed, this experimental system will allow us the functional dissection of the TAS15 hormone response control mechanisms.

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THE STORAGE PROTEINS OF Coix lacryma-jobi.

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The endosperm of seed of **Coix lacryma-jobi** var. Adlay contains ca. 20% protein. Amino acid analysis revealed that protein fractions from **Coix** endosperm has the typical composition of **Panicoideae** endosperm. The major component is a prolamin known as coixin. It is rich in proline and leucine and poor in lysine. The **Coix** protein fraction obtained by extraction with 90% isopropanol consists of four protein classes with molecular weights of 27(C1, 8%), 25(C2, 56%), 17(C4, 4%), and 15(C5, 12%) kDa. Since these four coixin bands have the same solubility properties as α-zeins, they are referred to as α-coixins. The fraction extracted with 60% isopropanol containing 1% 2-ME, γ-coixin, contains one major 22kDa protein (C3) corresponding to 15% of total coixin and a minor component of 15kDa. This coixin fraction has similar solubility properties to γ-zein. Isoelectric focusing (IEF) of coixin resolved seven major protein bands. Analysis of the individual IEF bands by SDS-PAGE and immunoblotting, using rabbit antibodies raised against C2 and C3,indicate an even greater complexity with a total of 17 proteins being detected. Polyclonal antibodies raised against C1 recognize C1 and C2 and cross-react strongly with the 22kDa α-zein, as did C4 and C5 antisera. The antiserum against γ-coixin showed strong cross-reaction with γ-zein. The **Coix** genome shows complex cross-hybridization sequences with the 22kDa α-zein cDNA, while no cross-hybridization was observed with the 19kDa cDNA clone. The cDNA representing the 28kDa γ-zein cross-hybridizes with only one band of **Coix** genomic DNA, in contrast to the tree bands observed in maize. This same **Coix** sequence also cross-hybridizes with the cDNA clone representing the 16kDa γ-zein. cDNA clones coding for α-coixin, were isolated from a cDNA library constructed from protein body associated mRNAs.

FUNCTIONAL STUDIES OF PLANT HISTONE GENE PROMOTERS

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The promoter regions of five genes encoding histone H3 or H4 of maize (H3C4, H3C3, H3C2, H4C7, H4C14) and *Arabidopsis* (H4A748, H4A777, H3A725, H3A713) have been studied by transient expression of a reporter gene in protoplasts.

Vectors containing the 35S-CAT gene as internal control and the GUS gene under control of the histone promoter were introduced into tobacco protoplasts by PEG mediated direct transfer.

In tobacco protoplasts, *Arabidopsis* promoters very actively drive the expression of the GUS reporter gene. Among the maize histone gene promoters, some are less active than the *Arabidopsis* promoters and one of them was found to be inactive.

These results compared with the expression of the same genes in the plant show that :

- histone promoters can be active in maize but not during transient expression in tobacco
- histone genes can be non-expressed in the original plant but promoters can be active during transient expression in tobacco
- most of histone promoters are active both in the original plant and during transient expression in tobacco.

Different regions of the H4C7 (from maize) and H4A748 (from *Arabidopsis*) promoters were deleted and the efficiency of the truncated promoters was tested by transient expression in tobacco protoplasts. Some regions seem to play a regulatory role, one as a silencer and the second as an activator. To confirm this model, experiments using site-directed-mutagenised promoter are in progress.

Experiments are carried out to study histone promoter activity in transgenic tobacco and *Arabidopsis*.

ANALYSING THE ROLES OF PHYTOCHROME IN PLANT DEVELOPMENT

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Phytochrome, a plant photoreceptor, controls many aspects of plant development. It is involved in gene expression related to greening. It is capable of perceiving shade-light quality, and evoking the shade-avoidance reaction of growth.

As it is known that photosynthesis is drastically modified under shade-light, we have checked the possible participation of phytochrome in these responses. Induction of a shade-type phytochrome status (low phytochrome photoequilibrium) results in change in growth pattern in clover plants, but photosynthesis is not strongly altered. This might mean that phytochrome has separate functions during chloroplast biosynthesis, and during chloroplast adaptation to shade light (lack of control in this second case). We have examined that possibility using photomorphogenetic mutants.

A tomato au mutant, defficient in light-labile or type I phytochrome, shows problems for greening throughout plant life, but fully exhibits a growth response to altered phytochrome photoequilibrium. On the other hand a cucumber lh mutant, probably defficient in light-stable or type II phytochrome, has a normal type of photosynthetic machinery, but is unable to respond to altered photoequilibrium (it behaves as in permanent null photoequilibrium).

We therefore propose separate roles for both phytochrome forms, type I being active in gene expression and greening, and type II evoking the shade avoidance reaction. Nevertheless, results obtained by others on plants with transgenic phytochrome, indicate that both types might interact.

Petunia hybrida GENES RELATED TO THE MAIZE *C1* REGULATORY GENE AND TO ANIMAL *Myb* PROTO-ONCOGENES: MOLECULAR CLONING (cDNA) AND CHARACTERIZATION.

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The *C1* gene of *Zea Mays* is a regulatory gene of the anthocyanin pathway which is part of a multigene family characterized by encoding proteins with homology to the DNA-binding domain of animal *myb* proto-oncoproteins. The presence of this family of *myb*-related plant genes has only been reported thus far in maize and barley monocotiledonous plants.

The aim of this study was to search for and characterize *myb*-related genes of *Petunia hybrida*, a dicotiledonous plant which can be readily transformed and regenerated. For this purpose, a flower specific cDNA library was screened using as a probe a mixture of 20-mer oligonucleotides corresponding to a strech of seven amino acids totally conserved in all known MYB-related plant proteins. three different petunia cDNAs were isolated. Sequence analysis of these cDNAs indicate that they correspond to genes encoding proteins (MYB.Ph1, MYB.Ph2 and MYB.Ph3) which include a MYB-homologous domain at their N-terminal regions. No additional homology regions with any other MYB-related protein were encountered. However, acidics domains present towards the C-terminal part of all MYB-related monocot proteins are observed at similar positions in MYB.Ph2 and MYB.Ph3 indicating further structural similarities among all these proteins. Acidic domains at the C-terminus of MYB.Ph1 protein have not been encountered yet, perhaps due the the fact that the available sequence is incomplete at this region of the protein. Protein analysis after subcellular fractionation and "in situ" inmunolocalization experiments with MYB.Ph3 specific antibodies indicate that MYB.Ph3 is a nuclear protein.

Structural organization of all known MYB-related plant proteins, containing a putative DNA-binding MYB-homologous domain and an acidic putatively activator domain, resembles that of transcriptional activators. This fact suggests that *myb*-related plant genes play a regulatory role at the transciptional level, as it has been shown for the maize *C1* regulatory gene of the anthocyanin pathway. The nuclear location of MYB. Ph3 protein further supports this posibility. However, due to the divergence in sequence among the different members it can not be anticipated whether or not all they will regulate the same biosynthetic pathway. The clones described here may shed light on this point.

EARLY KINETIN INDUCED IN VITRO POTATO TUBERIZATION MARKERS

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To study tuber initiation we chose an in vitro stolon tuberizing system which can be triggered on by addition of 2.5 mg/l kinetin to the culture medium, in the dark. This system produces physiologically normal tubers. First swellings are visible after 14 days in culture.

As a mean to monitor early stages of kinetin induced tuber initiation, attention was focused on the appearance of cytological and biochemical parameters, which can be used as specific markers.

Starch synthesis and cell division were detected on the fourth day. Patatin and proteinase inhibitor II were detected on the eleventh day by labelled ssDNA hybridization from field tuber cDNA recovered pUC8 clons after subapical region RNA Northern blotting. This further establishes the biochemical normality of the in vitro, kinetin induced, tubers.

SOMATIC EMBRYOGENESIS IN CITRUS LIMONUM.A SIMPLE REPRODUCIBLE MODEL FOR DEVELOPMENTAL STUDIES IN WOODY PLANTS.

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Embryogenic cultures were initiated from nucellar tissues of Citrus limonum on a basal medium supplemented with the auxin 2,4-D and two carbon sources galactose and sucrose. Culture conditions in ilumination and in the dark were also tested.
Nucellar tissues were exposed to several levels of 2,4-D and sucrose as carbon source for an induction period of four weeks half of the tissues were kept in the dark followed by a second culture period in ilumination but with sucrose or galactose to test embryo production.Callus surface and % of embryiogenesis were estimated.
Differences were observed between levels of 2,4-D and control medium, being somatic embryogenesis inhibited by levels of 2,4-D higher than 0.1 mg/l and the use of sucrose instead of galactose in control medium. Embryogenesis was increased when galactose was used in absence of 2,4-D as carbon source.
For plant regeneration somatic embryos were transfered to basal medium supplemented with 1.0 mg/l of GA3.
This nucellar tissue -somatic embryo-plant model is simple and easy to reproduce and controll at several levels of plant tissue diferentiaton and it is because of that we think it could be a valuable modell for studies on woody plants developmental Biology.

PROTEOLYTIC ACTIVITY IN THE STEM CAMBIAL REGION OF *PINUS SILVESTRIS* L. - A CONTRIBUTION TO THE SPECIFIC DIFFERENTIATION OF SECONDARY XYLEM AND PHLOEM.

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ABSTRACT.

Activity of autolytic proteases was demonstrated in fresh tissue extract from the differentiating secondary xylem of stems of 7-12 year-old *Pinus silvestris* trees. The proteolytic activity was absent in tissues of the cambial region during winter dormancy. It was initiated with the onset of cambial activity in spring and subsisted during subsequent stages of xylem differentiation. Acid protease activity in phloem could be detected only after release from inhibition by a macromolecular inhibitor found in the extract. Thus, synthesis of autolytic proteases with optimum of activity at pH 4.0 occurs in both the cambial derivative vascular tissues. Their identity, however, remains uncertain, because a different electophoretic mobility of the proteins concerned was demonstrated. On the other hand, synthesis of inhibitor protein of autolytic acid proteases may prove specific for the developmental program of secondary phloem-conducting system.

Proteolytic activity in the extract from differentiating secondary xylem was found to be totally reduced in decapitated 2-3 year-old segments of the main stem of 4-7 year-old pine trees after a few weeks. Simultaneous application of auxin (IAA) in lanolin paste prevented this reduction. Proteolytic activity was restored within 2 days when auxin was applied later, after the original activity had ceased following decapitation. Analogous responses to decapitation reduced and auxin subsequently restored cambial xylem production and extractable protein found in differentiating xylem were observed. The latter effects were not correlated in time with the effects upon the activity of proteases. The results suggest dependence of proteolytic system on the shoot apical control.

Isolation of the coding sequence for cytosolic fructose-1,6-bisphosphatase of plants

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In plants two FBPase isoenzymes are necessary for photosynthesis to take place. One form, localised in the cytosol, is involved in sucrose synthesis from triose phosphates exported from the chloroplasts. The other, found within chloroplasts, takes part in the regeneration of ribulose bisphosphate in the photosynthetic carbon reduction cycle. These enzymes have potentially regulatory roles as their estimated activities *in vitro* are little more than sufficient to account for the observed rates of CO_2 fixation and sucrose biosynthesis. Also, both catalyse a reaction which is essentially irreversible. The chloroplastic FBPase cDNA of wheat has been isolated and sequenced. The deduced amino acid sequence of this FBPase shows considerable homology to the known FBPase sequences of yeast, mammals and bacteria.

In order to get an amplified DNA fragment with coding sequence for cytosolic enzyme, mixed oligonucleotide primers corresponding to two conserved regions of the amino acid sequence were used in conjunction with wheat and potato cDNA as templates in the polymerase chain reaction (**PCR**). Gel electrophoresis of the amplified sequences shows that several oligomers are synthesized, with two or three of the expected size of around 500bp. Cloning and sequencing these amplified DNA fragments will allow the determination of which oligomer has the cytosolic FBPase sequence, and this will then used as a probe to find the complete coding sequence of the cytosolic enzyme in a cDNA library. In addition a cDNA epression library in gt11 will be screened using polyclonal antibodies raised against the cytosolic FBPase.

MOLECULAR CHARACTERIZATION OF SUCROSE SYNTHASE GENES IN WHEAT AND BARLEY AND ITS EXPRESSION IN RESPONSE TO ENVIRONMENTAL FACTORS

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The enzyme sucrose synthase (EC 2.4.1.13) catalyzes the reversible cleavage of sucrose into UDP-glucose and D-fructose. This is a key enzyme in starch biosynthesis in cereal kernels and consequently plays an important role in the yields of these important crops.

cDNA clones corresponding to two types of sucrose synthase genes have been isolated and characterized from developing wheat endosperm library. Using the inserts of these clones as probes in Southern blot analysis of the appropriate genetic stocks, linked genes of the two types have been assigned to the short arms of chromosomes 7A, 7D and possibly 7B in wheat, and to chromosome 7H in barley.

Using the wheat cDNA clones as heterologous probes, both a cDNA and a genomic library from barley have been screened and the isolated clones are being characterized. The barley cDNA clones have been classified also into two types on the basis of their relative ability to hybridize with the probes and their restriction maps. A genomic clone corresponding to the Ss1 type, is actually being sequenced with a special emphasis placed in the promoter characterization.

The expression of the two types of sucrose synthase genes, Ss1 and Ss2, in hexaploid wheat (Triticum aestivum, L.), has been investigated using type-specific probes corresponding to the 250-270 bp C-terminal portions of the respective cDNA clones. Both types of genes are highly expressed in developing endosperm. Expression of Ss genes is lower in etiolated leaves and in roots, where Ss1 mRNA is much more abundant than Ss2. In these tissues, Ss1 mRNA sharply increases in response to anaerobiosis and to cold shock (6°C), while the level of Ss2 mRNA is not significantly affected. Upon illumination of etiolated leaves, Ss1 mRNA decreases significantly while Ss2 mRNA increases.

IMMUNOLOCALIZATION OF PLANT NUCLEAR PROTEINS DURING SOMATIC EMBRYOGENESIS AND POLLEN GRAIN DEVELOPMENT

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Somatic embryogenesis and pollen grain development are two ideal developmental processes to study the nuclear proteins due to the striking nuclear activity changes taking place during them.

Nuclear proteins, as molecules involved in the regulation of differential gene expression, play a main role in nuclear differentiation. Despite that, they are scarcely known, especially in plant cells.

We have done an immunocytochemical study at the L.M. and E.M. levels to detect three kind of nuclear proteins: Small nuclear ribonucleoproteins (snRNPs), nuclear matrix proteins and nucleolar proteins using different monoclonal and polyclonal antibodies. The material used were somatic embryos of *Daucus carota L.* and anthers of *Scilla peruviana L.* and *Capsicum annum L.* after cryoprotection, cryofixation in liquid propane and cryosectioning at -110ºC.

The protein localization pattern after immunogold labeling can be very well correlated with the immunofluorescence one in both experimental systems. SnRNPs and nuclear matrix proteins are localized in the nucleus by immunofluorescence. Gold labelling form like a network inside the nucleus for the snRNPs antibody, while it is localized over the condensed chromatin for the nuclear matrix antibody. Nucleoli show immunofluorescence in the case of the nucleolar antigens, at the EM level they are localized in both dense fibrillar and granular components but not in the fibrillar centres.

The immunolocalization of these antigens in such different plant systems as well as in animal and human cells indicates the high level of conservation of some epitopes in nuclear proteins throughout the evolution of eukaryots which should mean that they have very basic and important roles in nuclear functions.

MOLECULAR APPROACHES TO TOMATO DEVELOPMENT

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Isolation of Apex-specific Genes

In the shoot apex of a higher plant new pathways of development like flower- or leaf-formation are initiated and sets of formerly silent genes are activated during this process. We have tried to isolate apex-specific genes by differential cDNA-hybridization techniques and by comparison of 2D-Protein gels. By transforming tomato and tobacco plants with vectors that express isolated cDNA-clones either in sense or in antisense orientation we try to obtain mutant plant phenotypes that should be valuable for understanding the function of the genes. The tissue specificity of the genes is investigated by RNA-in-situ hybridization and by plant transformation experiments with promoter-marker gene fusions.

Gene tagging in tomato

A gene tagging system in tomato was set up to isolate molecularly genes of known developmental mutants. As no endogenous transposable elements are known in tomato we employed th Ac/Ds system of maize. By T-DNA mediated transformation various Ds-elements are introduced into the tomato genome. The genomic DNA sequences flanking the T-DNA insertion are isolated by the inverted polymerase chain reaction. These probes are then used in RFLP-mapping experiments to determine the integration sites of the (inactive) Ds-elements in the chromosomes . Tomato lines that carry a Ds-insertion in proximity to a known locus are crossed to plants harbouring a transactivating Ac that may now enable the Ds-element to transpose to a closely linked site. Plants that have the Ds-element integrated into the gene of interest will exhibit a mutant phenotype after selfing or crossing to an already existing mutant plant. The presence of Ds sequences allows the fast and easy cloning of the tagged gene.

FLORAL DEVELOPMENT AND TRANSMISSION GENETICS IN SOMATIC HYBRIDS OF NICOTIANA OBTAINED BY MICROFUSION OF DEFINED PROTOPLASTS FROM MALE FERTILE AND MALE STERILE FORMS

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Somatic hybrid/cybrid plants produced by one-to-one electrofusion of defined selected protoplast + protoplast and protoplast + cytoplast pairs (**microfusion**) of male fertile N. tabacum and cytoplasmic male sterile tobacco alloplasmic lines and microculture of the fusion products were analyzed for their organellar and nuclear composition.

The fate of chloroplasts was assessed by streptomycin resistance/sensitivity assay using somatic leaf tissue (R_0 generation) and R_1 seedlings. For the analysis of mitochondrial (mt) DNA, species specific patterns were generated by Southern hybridization of restriction endonuclease digests of mtDNA and total DNA with DNA probes of N. sylvestris mitochondrial origin.

Nuclear fate was characterized by Southern hybridization analysis as well as expression assays for the functionality of selectable marker genes: npt II (Km resistance) and hph (Hm resistance) previously introduced by direct gene transfer into the parental nuclear genomes.

In addition, flower morphology from independent regenerants was analyzed by developmental histology and SEM studies on floral bud formation and ontogeny of floral organs.

In this way, transmission genetic analysis concerning organellar and nuclear fate as well as effects of nuclear-cytoplasmic interactions on flower ontogeny in somatic hybrids/cybrids obtained by microfusion of defined preselected protoplasts is reported for the first time.

CONTROL OF GLADIOLUS CORM FORMATION IN TISSUE CULTURE

Benjamin Steinitz, Department of Plant Breeding and Genetics, A.R.O., The Volcani Center, Bet Dagan 50 250, Israel.

Gladiolus propagates vegetatively by virtue of corm formation at the base of the shoot and by cormel differentiation on tips of stolons. The precocious generation of both organs was accomplished in vitro on plantlets incubated for one month in a liquid shake culture supplemented with paclobutrazol. Paclobutrazol-dependent corm and cormel development could be prevented by addition of GA_3 to a paclobutrazol-containing medium, thereby indicating that paclobutrazol mediates its influence by reducing the endogenous GA level. BA was found to either promote or inhibit corm formation; the mode of response depends on the plants' developmental stage and on the cytokinin concentration. The dose-response relations for the influence of paclobutrazol, BA and GA_3 on corm development will be presented, and the role of the different growth regulators in differentiation of the propagation organs will be discussed.

MELANDRIUM ALBUM, A MODEL SYSTEM FOR THE SEX DETERMINATION IN HIGHER PLANTS.

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Melandrium album (2n= 24), a dioecious species, shows a clear-cut correlation between the phenotypic sex and the presence of a pair of heteromorphic sex chromosomes (male : 24,XY and female : 24,XX).

Morphologically, the chromosomes were either metacentrics or submetacentrics. They were classified into three distinct groups : group A comprising 6 pairs of autosomal metacentrics, group B comprising 5 pairs of autosomal submetacentrics and the sex chromosomes X and Y. the X chromosome is metacentric (R=1.44) which accounts for more than 14% of the genome. The Y chromosome is metacentric with virtually, equal arms (R=1.09) and accounts for 21% of the genome, being the largest of the complement. The largest autosome accounts for only 9% of the genome.

For the production of haploid plants out of anthers (separation of the X and Y sex chromosomes), different parameters such as genotype, pollen stage, cold treatment and culture media compounds, were essential for a reproducible yield of embryos. The procedure merely increased the number of responsive anthers intead of the number of responsive microspores per anther. Most likely, the experimental system allows the recovery of "competent" microspores, and this on a medium containing either an auxin or a cytokinin.

Flow cytometric measurements of the anther-derived plants indicated that 90% of the population were haploids.

Also they expressed a female phenotypic sex and whenever analysed cytogenetically, the plants exhibited the corresponding female genetic sex (one or two X chromosomes).

These results are the first ones in a series attempting to study at molecular, cellular and phenotypic level, the developmental aspects of sex determination.

SUMMARY

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SUMMARY

The study of plant development has recently been enriched by an infusion of new techniques from genetics and molecular biology. At the same time, a new generation of scientists has become intrigued with understanding how patterns of molecules, cells, and organs are formed into plants. With new scientific tools, the beauty of the process of development becomes more accessible to our appreciation and analysis.

The lecture course Approaches to Plant Development was envisaged as a means of presenting in a concentrated format a representative selection of the best in current studies of plant development. Formal lectures were interspersed with generous informal discussion time, during which the international mixture of students and lectures exchanged ideas, asked questions, and generally became better acquainted.

On the first day, I. Sussex (New Haven, USA) gave an overview of the development of the shoot and root systems from apical meristems. He explained the advantages of various types of genetic chimeras for the study of meristem derivatives and cell-cell interactions. S. Poethig (Philadelphia, USA) and M. Freeling (Berkeley, USA) both illustrated sophisticated applications of such genetic chimeras. Poethig described his work with **Teopod (Tp)** mutants of maize that shift the relative juvenile and adult phases of development. He used chimeras in clonal analyses to show the **Tp2** gene acts in a cell-autonomous manner, the **Tpl** gene not. Freeling described his model for the action of **liguleless** genes, which normally appear to induce a ligule (joint) in the maize leaf in cells of a particular age through a signal diffusing from the midrib to the leaf margin. If a mutant sector interrupts this diffusion, the ligule in the distal region is displaced. M. Delseny (Perpignan, France) presented a useful overview of the distinguishing features of plant genomes, including their unfortunate propensity to maintain large blocks of repetitive DNA. S. de Vries (Wageningen, The Netherlands) summarized his work with glycoproteins released into the culture medium by embryogenic carrot cell cultures. He described several of these, including a peroxidase, which are also present in normally developing plants in locations suggesting roles in normal development.

The following day, J. Rothman (Cambridge, UK) and J. Modollel (Madrid) offered outstanding summaries of research on the developmental biology of the nematode **Caenorhabditis elegans** and the fruitfly **Drosophila melanogaster**, respectively. The elegance of the molecular genetic approaches described in these two talks stimulated much discussion. F. Salamini (Köln, FRG) presented a thought-and discussion- provoking model for

the phytomeric structure of grasses, in which all vegetative and floral parts of the plant are interpreted as variations of basic repeating units. He suggested that various barley morphological mutants could be interpreted as transitions, modifications, additions, or reductions of the phytomer units he proposed. J.M.Martínez Zapater (Madrid,Spain) introduced the advantages of the **Arabidopsis** system, and gave examples from his genetic analysis of flowering control pathways. T.Nelson (New Haven, USA) returned to the maize system and presented an analysis of cell differentiation in leaves using in situ and physiological methods.

The meeting resumed the following afternoon, after an excursion to beautiful Toledo. A. Spena (Köln,FRG) described a means of inducing sectors of tissue with altered hormone levels. His approach relies on the activation of a hormone gene in identifiable clones of cells by transposable element excision at defined times in development. The enormous potential of this approach generated many ideas for new experiments during the discussion. P. Hooykas (Leiden, The Netherlands) reviewed the advantages of the **Agrobacterium** system that is the standard for generating transgenic plants for developmental studies. M. Estelle (Bloomington, USA) described the developmental phenotypes of hormone-resistant mutants of **Arabidopsis**. The ease of isolating mutants from **Arabidopsis** (once a screen is devised) is one of the great attractions of this system. P. Puigdoménech (Barcelona,Spain) presented a study of genes encoding a special class of cell wall proteins (HPRGs) in maize. The HPRG genes are especially active near developing vascular centers and after wounding. M.Pagés (Barcelona,Spain) closed the course with a tour-de-force analysis of genes and proteins induced by the hormone abscisic acid and by water stress. Her study was remarkable in its combination of biochemical and molecular biology approaches.

The overall perspective that emerged from this lecture course is that only biologists armed with skills and appreciation of both classical approaches (developmental morphology and genetics) and newer ones (molecular genetics, plant transformation) can hope to answer questions of significance in plant development. The clear enthusiasm for this interdisciplinary point of wiew at the course promises to make the field of plant development very exciting in the next few years.

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