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# Workshop on Flower Development

Organized by
H. Saedler, J. P. Beltrán and J. Paz-Ares

P. Albersheim
J. P. Beltrán
E. Coen
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# I N D E X

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## PROGRAMME

1. **Session on signals affecting floral induction and flower development**

| | |
|---|---|
| G. Bernier | - Signalling in the whole plant during the flowering process. |
| J.M. Martínez Zapater | - Genetic analysis of floral induction in Arabidopsis. |
| V.K. Sawhney | - Development and physiology of floral mutants in tomato. |
| P. Albersheim | - Oligosaccharins can regulate growth and organogenesis in plants. |
| K. Tran Thanh Van | - Control of morphogenesis in plant: Molecular markers of flower differentiation in in vitro and in vivo systems. |

2. **Session on self-incompatibility and male sterility in plant**

| | |
|---|---|
| C.P. Scutt | - Characterization of S-gene sequences in Brassica oleracea. |
| R.D. Thompson | - The role of major pistil proteins in self-incompatibility (SI) in Solanum tuberosum. |
| J. Leemans | - Anther-specific gene expression and male sterility in plants. |
| J.P. Beltrán | - Molecular analysis of three stament-specific genes, tap 1, tap 2, and fil 1, that are putative genes of deficiens in Antirrhinum majus. |

3. **Session on flower homeotic mutants as a tool to understand flower development**

| | |
|---|---|
| G.W. Haughn | - Genitic analyses of FLO 10 and LEAFY: two homeotic genes which control floral development in Arabidopsis thaliana. |
| E.M. Meyerowitz | - Genetic analysis and molecular cloning of the homeotic genes that direct Arabidopsis flower development. |
| H. Sommer | - Analysis of homeotic genes controlling floral organogenesis in Antirrhinum majus. |

E. Coen - Homeotic genes controlling flower development in Antirrhinum.

E. Lifschitz - Flower development in tomato: Identification and characterization of molecular markers and regulatory genes.

SHORT ORAL PRESENTATIONS: **C.J. Rossbach, B. Veit, D. Ye, V.E. Franklin-Tong, O.Vicente, N. Harris.**

**4. Session on flowers as a tool to study gene expression in plants**

R. Jorgensen - Homology-based trans interactions and developmental control of petal pigmentation.

J. Paz-Ares - Molecular analysis of the petunia gene family related to the maize regulatory C1 gene and to animal myb proto-oncogenes.

C. Martin - The control of gene expression in Antirrhinum flowers.

H. Saedler - Methylation and development.

# INTRODUCTION

H. SAEDLER
J. P. BELTRAN
J. PAZ-ARES

Flower induction and development has been a matter of study since a long time ago. In fact, at the beginning of the century, when genetics was developing as a science, numerous mutants displaying alterations in flower development were isolated. Subsequently, most work was restricted to physiological aspects of these processes. This has led to an impressive amount of information on how environmental factors such as light and temperature as well as plant growth regulator applications influence flowering and flower development. However, only very recently, the introduction of the molecular approach combined with genetic analysis has already started to shed light on the mechanisms underlying flower development. Moreover, it is possible to think that all the information available can be combined to start to provide an integral picture of flower development. At this point, the organizers thought the time has come to bring together, for the first time, scientists using all different approaches in a forum of discussion to analyze progress in the field and to propose directions for future research. Fortunately, this initiative was taken by the Fundación Juan March which provided us its excellent facilities and expertise highly contributing to the success of the meeting.

# FIRST SESSION

G. BERNIER
J. M. MARTINEZ-ZAPATER
V. K. SAWHNEY
P. ALBERSHEIM
K. TRAN THANH VAN

# SIGNALLING IN THE WHOLE PLANT DURING THE FLOWERING PROCESS

**Georges BERNIER**

Laboratory of Plant Physiology, University of Liège,

B22, Sart Tilman, B4000 Liège, Belgium.

This talk will summarize our physiological analysis of the long-distance signals transmitted in whole plants of the long-day (LD) species *Sinapis alba* (the mustard, a close relative of *Arabidopsis thaliana*) induced to flower by exposure to (a) a single LD or (b) a single displaced short day (DSD). The first flower primordium of the raceme is initiated by the shoot apical meristem at 55-60 h after start of the LD or the DSD. Additional flower primordia are successively initiated at later times. After a LD, 4.0 and 9.7 flower primordia are present in the apical bud at 72 and 120 h after start of induction, respectively.

The levels of 3 different kinds of signals were determined in saps : carbohydrates, cytokinins and $Ca^{2+}$. Saps were collected at 3 different locations : (a) root exudate collected at the stem base and believed to be xylem sap, (b) leaf exudate collected by the EDTA technique of King & Zeevaart (1974) at the petiole base of mature leaves and believed to be phloem sap, (c) apical exudate collected at the top of the stem (just below the apex) and believed to be the phloem sap reaching the apex.

Carbohydrates. The major carbohydrate in leaf and apical exudates was sucrose. Its level in both saps increased dramatically in plants exposed to either a LD or a DSD (Lejeune

*et al.*, 1991; P. Lejeune, unpublished results). These increases started very early after start of induction, i.e. 10 h after start of the LD or immediately after start of the DSD. They resulted in an early rise of the sucrose level in the apex (Bodson & Outlaw, 1985).

Cytokinins. The major cytokinin in the root exudate was zeatin riboside (ZR) whereas in leaf and apical exudates isopentenyladenine and its riboside were most abundant (Lejeune *et al.*, in preparation). An increase in the level of ZR was detected in root exudate quite early after induction, i.e. at 9 h after start of the LD, that is only one h within the photo-extension period of the LD (Bernier *et al.*, 1990). This increase was correlated with a decrease in the cytokinin level in roots (P. Lejeune, unpublished results), and could thus be attributed to an enhanced export of cytokinins out of the root system. These observations implied the production by leaves exposed to a LD of a signal of unknown nature and the movement of this signal from leaves to roots within the first h of the photo-extension period (Bernier *et al.*, 1990). Interruption of the movement of this signal by bark ringing at the stem base reduced flowering (Lejeune *et al.*, in preparation).

The cytokinin activity in leaf and apical exudates started to increase several h later, i.e. about 16 h after start of the LD (Bernier *et al.*, 1990; Lejeune *et al.*, in preparation). This resulted in an increase of the total cytokinin level in the apex at 16 h (Sotta *et al.*, 1990). There is also evidence that the total auxin level in the apex decreased at the same time, so that the auxin : cytokinin ratio was considerably reduced at 16

h (Sotta *et al.*, 1990). So far, it is not known whether this decrease in auxin level is due to an increased movement of this regulator out of the apex or to other reasons.

Ca$^{2+}$. The $Ca^{2+}$ level was increased in both the root and apical exudates, but not in the leaf exudate (A. Havelange, unpublished results). The increases in $Ca^{2+}$ were recorded after induction by either a LD or a DSD. They were relatively late, e.g. $Ca^{2+}$ level was increased at 20 and 32 h after start of the LD in the root exudate and apical exudate, respectively. As a result the total $Ca^{2+}$ level increased in the apex but at relatively late times (Havelange, 1989).

Conclusions. Although well ordered in time and space, the changes of the signalling system in the whole plant during the early steps of the flowering process are complex. All plant parts participate in the exchange of signals and some signals are transported very early, so that all parts are very rapidly instructed about the changes of the environmental conditions, in this case a change of the photoperiodic regime to which the leaves are exposed. No doubt that chemical signals other than those studied here will also be found to participate in the signalling system of the early steps of flowering (Bernier, 1988). There is evidence that the situation during the further steps, i.e. flower and inflorescence development, is not less complex. Perhaps, it is even more complex since new parts (organs) are then present and more interactions are possible (Kinet *et al.*, 1985).

In the study of flowering, the physiological dissection at the whole plant level, described here, is complementary to the dissection at a lower level of organization (molecular and cellular) performed on mutants and using the techniques of molecular genetics.

References

Bernier, G. 1988. Ann. Rev. Plant Physiol. Plant Mol. Biol. 39 : 175.

Bernier, G., Lejeune, P., Jacqmard, A. & Kinet, J.-M. 1990. In Plant Growth Substances 1988 (Pharis, R.P. & Rood, S.B., eds). Springer-Verlag, Berlin, p. 486.

Bodson, M. & Outlaw, W.H., Jr. 1985. Plant Physiol. 79 : 420.

Havelange, A. 1989. Plant Cell Physiol. 30 : 351.

Kinet, J.-M., Sachs, R.M. & Bernier, G. 1985. The Physiology of Flowering. Vol. III. The Development of Flowers, chap. 6. CRC Press, Boca Raton, Fl.

King, R.W. & Zeevaart, J.A.D. 1974. Plant Physiol. 53 : 96.

Lejeune, P., Bernier, G. & Kinet, J.-M. 1991. Plant Physiol. Biochem. 29, in press.

Sotta, B., Lejeune, P., Maldiney, R., Kinet, J.-M., Miginiac, E. & Bernier, G. 1990. International Symposium on Physiology and Biochemistry of Cytokinins in Plants, Liblice, Czechoslovakia, 10-14 September, Abstracts, p. 55.

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## GENETC ANALYSIS OF FLORAL INDUCTION IN *Arabidopsis*

Martinez-Zapater, J.M. and J.A. Jarillo.

Dpto. de Protección Vegetal CIT-INIA, Ctra. de La Coruña km 7, 28040 Madrid, Spain.

Floral induction is a crucial step in plant development. Reproductive success depends largely on flowering under optimal conditions and this is probably the reason for the complex floral induction processes found in different plant species. Although the enviromental factors responsible for floral induction are generally known for most of the cultivated species (1), the molecular mechanisms underlying the process remain mostly uncovered (2).

As for other developmental processes of unknown biochemistry, a genetic analysis of the floral induction process can give information about the identity and function of the genes involved. The available data on the genetic control of floral induction in a few species, have been obtained from the segregation analysis of the floral phenotype in crosses between cultivars or ecotypes with different flowering times (3,4). A systematic mutant analysis of floral induction has only been possible in *Arabidopsis*, which small size and short life cycle has allowed the identification and characterization of a large number of mutant plants with an altered floral phenotype (5, 6).

Flowering is promoted in *Arabidopsis* by long day photoperiods and cold temperatures. Different ecotypes can be classified as early or late ecotypes depending on their flowering time when grown under long days. Exposure of late ecotypes to cold temperatures, during the seedling or vegetable stages, drastically reduces their flowering time (4). Mutagenesis of early flowering ecotypes has resulted in the isolation of mutants that are delayed in flowering time under floral inductive conditions (5). The genetic and physiological analysis of these late flowering mutants is permitting the understanding of the function of the loci involved (7). Moreover, localization of those loci on the genetic map of *Arabidopsis* allows the use of molecular genetic approaches to achieve their cloning and molecular characterization (8). We will discuss the progress in our understanding of the floral induction process in *Arabidopsis*.

REFERENCES

1.- Halevy, A.H. (1990) Handbook of flowering. Vols I, II, III, IV. CRC Press, Boca Raton, FL.
2.- Bernier, G. (1988) Annu. Rev. Plant Physiol. 39:175-219.
3.- Murfet, I.C. (1977) Annu. Rev. PLant Physiol. 28:253-278.
4.- Napp-Zinn, K. (1987) In: JG Atherton, ed. Manipulation of flowering. Butterworths, London.
5.- Finkelstein, R.; Estelle, M.A.; Martinez-Zapater, J.M.; Somerville, C.R. (1988) In: D.P.S. Verma, R.B. Goldberg, eds. Temporal and Spatial Regulation of Plant Genes, Plant Gene Research Vol 5. Sringer-Verlag, New York.
6.- Koornneef, M. (1990) In: S.J. O'Brien ed. Genetic Maps. Cold Spring Harbor.
7.- Martinez-Zapater, J.M.; Somerville, C.R. (1990) Plant Physiol. 92:770-776
8.- Meyerowitz E.M. (1989) Cell, 56:263-269

# DEVELOPMENT AND PHYSIOLOGY OF FLORAL MUTANTS IN TOMATO

V.K. Sawhney

Department of Biology

University of Saskatchewan

Saskatoon, SK, S7N OWO, Canada

Single gene, homozygous recessive, floral mutants of tomato (Lycopersicon esculentum) are used to analyse the developmental and physiological mechanisms in flower development.

The stamenless-2 (sl-2/sl-2) mutant produces flowers in which stamens produce microspores in the distal part of the anther and ovules near the base. Thus, in the same organ, both the male and female characteristics are expressed. The development of mutant stamens can be regulated by temperature conditions as well as by plant growth regulators (PGRs). Mutant plants grown in relatively low temperatures, or treated with gibberellic acid ($GA_3$) produce phenocopies of normal flowers. In contrast, plants grown in high temperatures, or treated with indole-3-acetic acid (IAA) produce carpel-like structures in place of stamens. Analyses of various PGRs showed that mutant plants contain lower levels of various gibberellins, e.g., $GA_1$, $GA_3$, $GA_{4+9}$, $GA_8$, $GA_{20}$, than normal at low and high temperatures, and at low temperatures the gibberellin content was greater than at high temperatures in the two lines. In contrast, there was a 10-20 fold increase in IAA levels in the mutant in comparison to the normal, at both low and high temperatures. The level of polyamines, i.e. putrescine, spermidine and spermine, and their biosynthetic enzymes, was also greater in the mutant than normal at high, but not at low temperatures. It appears, therefore, that the sl-2 gene affects the levels

of a number of PGRs which in turn regulate stamen and carpel morphogenesis in the mutant.

The solanifolia (sf/sf) mutation in tomato affects both the leaf shape and flower structure. The mutant flowers possess separate floral organs unlike the fused organs of normal flowers. Develomental studies revealed that the separation of floral organs in the mutant was related to one or more of the following factors; limited lateral growth of organs, small size of organ primordia, greater distance between primordia than in the normal, and large size of the floral apex. Gibberellic acid induced the separation of stamens and carpels in normal flowers, whereas 2-chloroethyltrimethyl ammonium chloride (CCC) - an inhibitor of gibberellin biosynthesis - promoted the fusion of these organs in the mutant. It is proposed that sf/sf mutation through its effect on endogenous gibberellins, affects the meristematic activity in the floral apex causing an increase in apex size, organ number and resulting ultimately in non-fusion of stamens and carpels.

OLIGOSACCHARINS CAN REGULATE GROWTH AND ORGANOGENESIS IN PLANTS. P. Albersheim, A.G. Darvill, V. Marfà, S. Eberhard, D. Mohnen, and C. Augur. Complex Carbohydrate Research Center and the Department of Biochemistry, The University of Georgia, 220 Riverbend Road, Athens, GA 30602 USA; F. Cervone and G. De Lorenzo, Dipartimento di Biologia Vegetale, Universita di Roma "La Sapienza", Rome, ITALY.

Two seemingly unrelated lines of research--one, elucidating the walls of growing plant cells, and the other, studying how plants defend themselves against disease--have provided evidence for the functioning in plants of carbohydrate regulatory molecules. The combined results of these projects established that oligosaccharins, structurally defined fragments of plant and microbial cell wall polysaccharides, can function in plants as chemical messages with specific regulatory properties. Oligosaccharins can trigger plant defense responses against pathogens and other types of stress and are also able to regulate growth and organogenesis of plant tissues. This lecture will describe the biological assays, purification, structural characterization, and initial studies of the mode of action of several oligosaccharins involved with regulation of growth and development, including the formation of flowers. Acknowledgments: This work is supported by U.S. Department of Energy grant DE-FG09-85ER13425, and by DE-FG09-87ER13810 as part of the USDA/DOE/NSF Plant Science Centers Program.

CONTROL OF MORPHOGENESIS IN PLANT:
MOLECULAR MARKERS OF FLOWER DIFFERENTIATION IN *in vitro* AND *in vivo* SYSTEMS.

Tran Thanh Van, K.; Richard, L. and C. Gendy

Laboratoire Physiology Végétale. Centre National de la Recherche Scientifique. 91190 GIF sur YVETTE. France

Flowering is a complex event in plant development. The mechanisms of its triggering are not well understood due to the lack, until recently of mutants of floral induction. The determination to flowering is not one step process. It results from a progressive transition from a vegetative pattern which in turn is modulated by organ/tissue interactions in response to multiple stimuli from enviromental factors. At the molecular level, it was shown that mRNA populations of vegetative and floral organs are highly homologous (1). Genes involved in a specific developmental stage of an entire plan (*in vivo*) are difficult to be identified (2).

Several mutants in floral organ pattern found in *Arabidopsis* and *Antirrhinum* have led to the isolation of genes involved in *floral organ*(sepal, petal, anther, carpel) *differentiation*. Genes involved in the *transition* from a vegetative pattern to a floral pattern (in *in vivo* systems) need, for their identification, that rare gene transcripts be identified. In contrast, the *direct differentiation* of *pure* organogenic *programs* in Thin Cell Layer (TLC) systems especially pure and direct flower program versus pure vegetative program in response to well defined factors (kinetin or dihydrozeatin versus zeatin) (3) - has allowed several research groups to identify molecular markers of morphogenesis and of flowering on *Nicotiana tabacum* TCL. Specific peroxydase isozymes (4,5) and specific polyamins, spermidine (6,7) versus putrescine (8) were found in Flower-induced and Root-induced TCL respectively. A unique protein found to bind to spermidine in Flower-induced TCL (9), is similar to the one bound covalently to spermidine during sea urchin embryos development (10), a post-translational modification which indicates a possible role of this protein in the developmental process. Five gene families expressed early, at the onset of cell division of the subepidermal layer leading to the direct differentiation of floral meristems, were isolated from Flower-induced TCL (2). Thaumatin-like (Thl) proteins of 46,41 and 27 kD were exclusively detected in floral TCL. They were localized in the cytoplasm and were different from the ones synthesized under stress situations and accumulated in the vacuole in tobacco cell suspension or root. Similar Thl proteins of 42, 31 and 27 kD were also detected in floral meristems *in vivo*. The purification of these Thl proteins and the identification of the corresponding genes will allow the study of their possible function in floral differentiation.

REFERENCES

(1) Kamalay JC, Goldberg RB 1980. Regulation of structural gene expression in tobacco. Cell 19: 935-946.

(2) Meeks-Wagner DR, Dennis ES, Tran Thanh Van K, Peacock WJ 1989. Tobacco genes expessed during in vitro floral initiation and their expression during normal plant development. The Plant Cell 1: 25-35.

(3) Tran Thanh Van K 1990. Molecular aspects of in vitro floral initiation. In Genetic and Breeding of Ornamental Species. Eds. J. Mol, J. Harding and F. Sing. Kluver Academic (in press).

(4) Thorpe T, Tran Thanh Van K, Gaspard T 1979. Isoperoxydases in epidermal layers of tobacco and changes during organ formation in vitro. Physiol. Plant. 44: 388-394.

(5) Kay LE, Basile DV 1987. Specific peroxydase isozymes are correlated with organogenesis . Plant Physiol. 84: 89-94.

(6) Kaur-Sawhney R, Tiburcio AF, Galston AW 1988. Spermidine and flower-but differentiation in thin-layer explants of tobacco . Planta 173: 282-284.

(7) Kaur-Sawhney R, Geeta K, Mc Gonigle B, Galston A 1990. Further experiments on spermidine mediated flower buds formation in thin cell layer explants of W38 tobacco. Planta 181: 212-215.

(8) Tiburcio AF, Gendy C,Tran Thanh Van K 1989. Morphogenesis in tobacco subepidermal cells: putrescine as marker of root differentiation. Plant Cell Tissue and Organ Culture 19: 46-54.

(9) Appelbaum A, Canellakis ZN, Applewhite PB, Kaur-Sawhney R, Galstone A 1988. Binding of spermidine to a unique protein in thin layer tobacco tissue culture. Plant Physiol. 88: 996-999.

(10) Canellakis ZN, Bondy PK, Infanpe AA 1985. Spermidine is bound to a unique protein in early sea urchin embryos. PNAS 82: 7613-7615.

# SECOND SESSION

C. P. SCUTT
R. D. THOMPSON
J. LEEMANS
J. P. BELTRAN

ABSTRACT - JUAN MARCH FOUNDATION, MADRID (MARCH 11, 1991)

Charles P. Scutt, Department of Biological Sciences, Durham University, England.

Characterisation of S-Gene Sequences in *Brassica oleracea*

Self-incompatibility in *Brassica oleracea* is controlled by a single S-locus with approximately fifty allelic forms. Control of the self-incompatibility reaction in pollen resides with the parent sporophyte; in this case the S-gene is thought to be expressed in the tapetum which lines the anther loculus. A range of dominance and codominance relationships exist between pairs of S-alleles in *Brassica*. These relationships in certain cases differ in pollen and stigma.

Molecular analysis has identified an S-linked gene, designated SLG, which appears to encode a stigma-expressed glycoprotein. This gene putatively controls the pollen-stigma self-recognition process, at least from the female side of the interaction. No evidence has been published to demonstrate either expression of the SLG gene in anther tissue, or the presence of its glycoprotein product in pollen. Southern blot hybridisation and molecular cloning have revealed further SLG-related sequences, one of which, designated SLR1, has been characterised independently in several laboratories.

The results presented here include evidence of a third class of expressed, S-gene-related sequence in a single line of *B. oleracea* plants. Oligonucleotide probe hybridisations of gene- and allele-specific probes indicates the presence of one S-like sequence selectively in plant lines containing the S5 allele, independent of their varietal genetic background, whilst a very closely related sequence may be present in lines containing different S-alleles.

Initial studies utilising northern blot hybridisation analysis have demonstrated no SLG-related transcripts in S5

anther tissue at developmental stages during which, on the basis of current hypotheses, male S-gene expression might be expected. Given this apparent lack of nucleotide sequence homology between genes which control self-incompatibility in *Brassica* anthers and stigmas respectively, a strategy has been devised which could allow analysis of an anther-expressed S-gene based on its necessarily close genetic linkage to the SLG-gene. The proposed strategy will be based on the construction of a *Brassica* genomic library in YAC (yeast artificial chromosome) vectors. The YAC library may be screened using an SLG-gene hybridisation probe. Individual YAC clones selected by this procedure should contain 100-200 Kb of DNA encompassing the entire S-locus. Sub-libraries may be constructed in plasmid vectors from the cloned S-locus genomic region and these sub-libraries may then be screened with probes of total cDNA derived from anther mRNA preparations. Sequences selected through this procedure will be anther-expressed, S-locus-specific sequences and hence may control the pollen self-incompatibility reaction.

## The role of major pistil proteins in Self-incompatibility (SI) in *Solanum tuberosum*

Kaufmann, H., Kirch, H.H., Wemmer, T., Salamini, F. and R.D. Thompson
MPI fuer Zuechtungsforschung, KOELN 30 BRD

The gametophytic self-incompatibility system of *Solanum tuberosum* is controlled by a single locus, designated the S-locus. Protein extracts from potato genotyoes of defined S-genotypes were found by analysis on 2D-IEF/SDS PAGE gels to contain a group of basic glycoproteins. Each genetically determined allele S1 to S4 was associated with the presence of one of a number of these polypeptides, differing in isoelectric points (between 8.3 and 9.1) and/or apparent M.W. (23-29kd). Two abundant basic polypeptides were present in all genotypes examined, Sk1, a glycoprotein, and Sk2, which is apparently not glycosylated (Kirch et. al., 1989).

Three S-allele-associated glycoproteins (SLGs), corresponding to S2, S3. and S4, Sk1 and Sk2 have been purified and amino-terminal sequencing carried out. The sequences of S2, S3, S4 and Ski are related to one another and to SLGs from other members of the Solanaceae. Using oligonucleotide probes derived from these sequences, cDNA and genomic sequences have been isolated for the S1 and S2 alleles.

The analysis of these cloned S-alleles underlines the genetic diversity at this locus (Kaufmann et. al., 1991) Genomic clones for S1 and S2- SLG alleles suggest a simple gene structure of two exons separated by a small intron of 113(S1) or 117(S2) base pairs. The predicted protein coding regions of S1 and S2 are only 66% homologous, with the residues which differ between the alleles being scattered throughout the sequence. DNA cross-hybridisation experiments indicate there are at least three classes of S-alleles in potato, as defined by lack of cross-hybridisation at 65deg.C in 3xSSC, corresponding to our characterized alleles S1, S2, and S4. These classes presumably reflect the origin of the S-alleles from a few ancestral S-sequence types, common to different members of the Solanaceae, as the homology between S1 and S2 is similar to that between these alleles and S-alleles from petunia and *N. alata*.

The *S. tuberosum* SLGs show a similar structural organization into conserved and hypervariable regions as the *N. alata* alleles (Anderson et. al., 1989) and those of other members of the

Solanaceae which have been recently analysed. In total, 29% of the residues are invariant when the currently available *S. tuberosum* and *N. alata* alleles are compared, including 8 invariant cysteines and regions surrounding the ribonuclease active site-histidines, suggesting that RNase activity is necessary for function of the SLGs. The potato SLGs S1 and S2 have also been shown to possess RNase activity in vitro, via digestion of 32P-labelled RNA.

When the sequences of two members of one sequence-related SLG-class in potato, S1 and Sr1 were compared, the overall sequence homology was 95% The differences were clustered in one of the hypervariable regions, which is probably located on the surface of the protein, and therefore may play a role in molecular recognition. Ai et al., (1990), have also observed variation in a single hypervariable domain in the comparison of three SLG alleles from *Petunia hybrida*.

The promoter sequences of SLG S1 and SLG S2 alleles have been compared, and reveal little overall homology apart from the first 20bp upstream from the translation start , and around the TATA and CAAT boxes. Two short sequence motifs common to S1 and S2 represent possible cis-acting elements. These sequences are not present in the first 360bp of the Brassica SLR-63 5` untranslated region (Trick, 1990), and it remains to be seen whether they play a role in determining cell-type specific expression.

A crucial experiment in current SI research is to demonstrate that the S-allele-associated polypeptides(SLGs) are responsible for the SI phenotype. We and others have started to address this question by re-introducing the SLG-genes into tobacco by transformation. To date, however, we have not detected expression at the protein or RNA level from our introduced gene, although the Brassica SLG genes are expressed in transgenic tobacco. The simplest explanation is probably that an important cis-acting positive element is missing from our constructs, i.e., is located more than 3kb upstream or 1kb downstream of the coding sequence. Experiments to test this hypothesis are in progress.

One approach to understanding the mode of action of the S-locus is the analysis of mutants. As few induced mutants are available in potato, and the selection of S-locus is technically difficult, we have begun to examine naturally occurring sources of variation which influences the S-locus. An example is a self-compatible(SC) mutation which was identified in dihaploid lines of *S. tuberosum* (Olsder and Hermsen, 1976). The mutation was

previously proposed to have arisen by translocation of an S-allele (S1) to a new chromosomal location. When present in pollen grains of genotype Sx, it overcomes the normal incompatibility reaction seen on styles carrying the Sx allele. However, when present in S1-bearing pollen grains, the normal incompatibility reaction on S1- carrying styles is observed. We have shown using S-allele probes that no DNA fragment or polypeptide derived from the gene encoding the S1 slg can be linked to the presence of the SC mutation(Thompson et. al.,1991). The SC mutation is therefore assumed to have arisen in a sequence other than that encoding the S1 SLG, either in a distinct, pollen-part of the S-locus or at a different locus, giving rise to an S-allele-specific inhibitor.

The most abundant pistil protein in the potato is a 26kd basic polypeptide which was termed Sk2 due to its presence in all genotypes examined, and lack of linkage to the S-locus(Kirch et. al.,1989). This protein has been purified and from protein sequence, oligonucleotides designed to isolate a gene probe using PCR. The sequence of the corresponding PCR product shows 64 % homology to an endochitinase sequence previously published from potato (Gaynor,1988) The purified protein has been found to be a highly active endochitinase. Using an antibody raised to Sk2, we have demonstrated that this endochitinase polypeptide is concentrated in the pistils of potato plants, where it is located in the stylar transmitting tissue. The possible role of this protein in the fertilisation process will be discussed.

References

Ai,Y.,et. al.,(1990) Sex Plant Reprod. 3,130-136
Anderson, M.A.,et. al., (1989) The Plant Cell 1,483-491
Gaynor,J.J.,(1988) Nucl.Acids Res. 16,5210
Kaufmann et.al.,(1991) Molec. Gen. Genet., in press
Kirch et. al.,(1989) Theor.Appl.Genet. 78,581-588
Olsder,D.,and Hermsen,J.G.Th.,(1976) Euphytica 25,597-607
Thompson et. al.,(1991) Molec. Gen. Genet., in press
Trick,M., (1990) Plant Molec. Biol.,15,203-205

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# Anther-specific gene expression and male sterility in plants.

C. Mariani, W. De Greef, M. De Block, R.B. Goldberg* and J. Leemans
Plant Genetic Systems NV, J. Plateaustr. 22, B-9000 Gent, Belgium
* Dept. of Biology, University of California, Los Angeles, California 90024-1606, USA

In flowering plants, male gamete formation is a highly regulated developmental process that occurs in the anthers. In this organ system haploid microspores are produced after meiotic division of the pollen mother cells. The microspores develop further in pollen grains that carry the sperm cells. One of the tissues of the anther, the tapetum, plays an important role in the development and maturation of pollen, functioning as a nutritive tissue for the microspores. Several naturally occurring male sterility mutations, cytoplasmic and nuclear, have been identified amongst various crops. These mutations are often associated with a defective tapetum, suggesting that this cell-type is essential for pollen formation.

Here we report the isolation of a tapetal-specific gene from tobacco and the identification of the region essential for tissue specific expression. We show that in transgenic plants the 5' region of this gene can be used to direct expression of a ribonuclease specifically in the tapetum. The presence of this enzyme impairs the function of these cells and leads to male sterile plants.

We have used the same promoter region to control tapetal expression of a specific ribonuclease inhibitor. Plants carrying this chimaeric gene can be used in crosses to pollinate the engineered male sterile plants. The expression of this gene and the production of the ribonuclease inhibitor restores male fertility in the offspring of these crosses.

The utility of male sterility and restoration of fertility in the production of hybrid seed will be discussed.

MOLECULAR ANALYSIS OF THREE STAMENT-SPECIFIC GENES, **tap 1, tap2,** AND **fil 1,** THAT ARE PUTATIVE GENES OF DEFICIENS IN *Antirrhinum majus*

Beltran, J.P[1].; Nacken, W.K.F.; Huijser, P.; Saedler, H.; Schwarz-Sommer, Zs.; Sommer, H.

Max-Planck-Institut für Züchtungsforschung, 5000 Köln 30, Germany

(1) Instituto de Agroquímica y Tecnología de Alimentos CSIC, 46010 Valencia, Spain

**Deficiens** is a homeotic gene involved in the genetic control of flower morphogenesis in *A. majus*. The loss of its function provokes the transformation of the male organ into an abnormal female organ and causes transformation of petals into sepaloid leaves. Several strategies, such as transposon tagging and chromosome walking, have been proposed to isolated genes with unknown products and functions. To clone **deficiens** we have succesfully used a different strategy based in a combination of differential cDNA cloning and transposon mutagenesis (1). First strand cDNA was synthetized from mRNA isolated from wild type inflorecences of *A. majus* and then substracted with an excess of mRNA from young leaves. The remaining cDNA (enriched for flower specific sequences) was used to prepare a cDNA library by conventional methods. To screen such library, probes enriched for flower specific messages were used, (+) from wild type inflorescences and (-) from mutant inflorescences. As **deficiens** shows homology to the mammalian transciption factor SRF and to the yeast transciption factor MCM1, it is of great interest to search for putative target genes. We have isolated three such genes, **tap 1, tap2,** and **fil 1** by differential screening of the cDNA library mentioned above.

The cDNA of **tap 1** contains an open reading frame that could code for a 107 amino acids long protein (TAP1). The 20 N-terminal amino acids are hydrophobic and display characteristics of a signal peptide. The amino acid sequence of TAP 1 can be divided into three domains, each separated by two prolines. The first domain is the putative signal peptide. Then two cystein-rich domains follow containing 12 cysteines. the third domain displays two putative glycosilation sites. We have not find any significant homology between the deduced amino acid sequence of tap 1 and known proteins in the protein data banks.

The structure of the gene was determined by comparing the DNA sequence of cDNA and genomic clones. The gene consists of two exons separated by a 363 bp intron. Twenty-five bp upstream of the transcriptional start site a putative TATA-box is located. In the promoter of tap 1, which is 66% AT-rich, two perfect repeats of 11 bp and 10 bp respectively were detected. In situ experiments with labelled antisense RNA of tap 1 showed that the gene is expressed transiently in the tapetum.

The deduced **tap 2** protein is 131 amino acids long and has a hydrophobic N-terminus which displays characteristics of a signal peptide. The protein contains 24 basic and 12 acidic residues, and 51 of the total amino acids are glycine, serine, threonine or lysine. No homology to amino

acids sequences of known proteins in the data banks was found. A comparison of the cDNA and genomic sequences revealed that the gene is not interrupted by introns. A putative TATA-box is detected 50 bp upstream of the cDNA start.In situ and Northern blot experiments showed that the tap 2 is transiently expressed in the tapetum.

The fil 1 cDNAs contain an open reading frame that could encode a protein of 93 amino acids with a hydrophobic N-terminus. No homology to any protein sequence in the data banks could be detected with the deduced amino acid sequence of the fil 1 protein. Comparison of the sequences of the genomic cDNA clones reveals the presence of one intron of 266 bp. Analysis of the promoter sequence reveals two partially overlaping repeats of 23 bp and 14 bp respectively. In situ experiments with labelled antisense RNA of fil 1 showed that the strongest signals are detected in the filaments of the stamen and at the basis of the petals. Fil 1 is transiently expressed during flower development.

**The promoters of tap 1, tap 2, and fil 1 contains SRF like motives.** The conserved region of DEF A corresponds to the DNA-binding domain of SRF (serum response factor) that interacts with a DNA sequence motif called SRE (serum response element). Based on a comparison of the different motives bound by SRF or MCM1, a consensus motif CC(A/T) $_6$GG has been postulated. Assuming that not only SRF and MCM1 but also DEF A binds similar motives, the presence of SRE related motives in the promoters of tap 1, tap 2, and fil 1 indicate that they are possible target genes of deficiens.

1.- Sommer, H.; Beltrán, J.P.; Huijser, P. Pape, H.; Löning, W.E.; Saedler, H.; Schwarz-Sommer, Zs. (1991) EMBO Journal 9:605-613

# THIRD SESSION

G. W. HAUGHN
E. M. MEYEROWITZ
H. SOMMER
E. COEN
E. LIFSCHITZ

# Genetic Analyses of FLO10 and LEAFY:

## Two homeotic genes which control floral development in *Arabidopsis thaliana*

**G.W. Haughn** and E. Schultz. Biology Department, University of Saskatchewan, Saskatoon, SK, S7N 0W0, Canada

Recessive mutations in the *FLO10* and *LEAFY* genes of *Arabidopsis thaliana* cause homeotic transformations within the flower and the inflorescence respectively. We have been analyzing the phenotypes of single and double mutants in an attempt to determine the roles of these two genes during plant development and their interaction with several other floral homeotic genes.

Plants homozygous for a recessive mutation (*flo10*) in the *FLO10* gene fail to form a functional gynoecium at the appropriate time and place during flower development. Instead, additional stamens are produced followed by one or more organs which can best be described as intermediate between a stamen and a carpel. Thus the *FLO10* gene appears to be required to stop stamen production and start carpel production at the appropriate time.

Double mutants homozygous for the *flo10* allele and a recessive allele of one of each of the floral homeotic genes *AG* (Bowman et al, 1989; Yanofski et al, 1990), *AP2* (Bowman et al, 1989; Komaki et al, 1988; Kunst et al, 1989), *AP3* (Bowman et al, 1989) and *PI* (Bowman et al, 1989; Hill and Lord, 1989) were constructed. Preliminary analyses

of these double mutants suggest that *pi* and *ap3* are epistatic to *flo10*; *flo10* is epistatic to *ag*, and *ap2* causes partial reversion of *flo10*. One interpretation of these results is that the *AG* gene limits the domain of *FLO10* expression to the carpel whorl. The *FLO10* gene product in turn negatively regulates the expression of *PI* to prevent stamen development.

Wild type inflorescences of *Arabidopsis thaliana* consist of two types of metamers. The first metamers produced by the primary meristem include conflorescences subtended by bracts. Subsequent metamers bear flowers with no subtending bract. Plants homozygous for a recessive mutation (*lfy1*) in the *LEAFY* (*LFY1*) gene are unable to produce flowers. In place of the flower, conflorescence-like shoots, with an indeterminate number of metamers, elongated internodes, bract-like organs, lateral shoots and spiral phyllotaxy, develop. Another difference between wild type and Leafy inflorescences is that an increased number of nodes on the primary shoot have bracts subtending lateral shoots. On the basis of this phenotype, we suggest that the role of the *LFY1* gene is to positively regulate the developmental switch from conflorescence-bearing metamers to flower-bearing metamers.

If the *LFY1* gene indeed positively regulates the development of floral shoots then the *lfy1* should be epistatic to the downstream homeotic genes which control floral organ identity. To test this hypothesis *lfy1/ap2-6* double mutants were constructed and analyzed. As expected, such double mutants could not be distinguished from *lfy1* homozygotes.

References

Bowman, J.L., Smyth, D.R., and Meyerowitz, E.M. (1989). Genes directing flower development in *Arabidopsis*. Plant Cell 1, 37-52.

Haughn, G.W., and Somerville, C.R. (1988). Genetic control of morphogenesis in *Arabidopsis*. Dev. Genet. 9, 73-89.

Hill, J.P., and Lord, E.M. (1989). Floral development in *Arabidopsis thaliana*: A comparison of the wild type and the homeotic pistillata mutant. Can. J. Bot. 67, 2922-2935.

Komaki, M.K., Okada, K., Nishino, E., Shimura, Y. (1988). Isolation and characterization of novel mutants of *Arabidopsis thaliana*. Development 104, 195-203

Kunst, L., Klenz, J.E., Martinez-Zapater, J., Haughn, G.W. (1989). The *AP2* gene determines the identity of perianth organs in flowers of *Arabidopsis thaliana*. Plant Cell 1, 1195-1208.

Yanofsky, M.F., Ma, H., Bowman, J.L., Drews, G.N., Feldmann, K.A., Meyerowitz, E.M. (1990). The protein encoded by the *Arabidopsis* homeotic gene agamous resembles transcription factors. Nature 346, 35-39.

# Genetic Analysis and Molecular Cloning of the Homeotic Genes that Direct *Arabidopsis* Flower Development

E.M. Meyerowitz, Division of Biology 156-29, California Institute of Technology, Pasadena, California 91125, USA

The homeotic genes of plants can be placed in one of two classes: those that affect the identity of plant organs, such as sepals or petals; and those that affect the identity of meristems. In mutants for genes in either class, a normal structure appears in an inappropriate position. The best-studied class is that of the organ homeotic genes, and the best-studied of these genes affect flower development. In *Arabidopsis thaliana* there are three groups of floral homeotic genes. Each alters the identity of organs in two adjacent whorls of the flower. The first group is represented by the *APETALA2* gene, which alters the first two whorls, causing carpels to develop in the places ordinarily reserved for sepals, and stamens to form in the positions normally occupied by petals. The second group includes the *APETALA3* and *PISTILLATA* genes, which change the organs of the second whorl (in wild type, petals) to sepals, and cause carpels to appear in third whorl, where stamens are ordinarily found. The last group is represented by *AGAMOUS*, mutants of which have petals in the places where stamens are found in wild type, and sepals in the fourth whorl, where carpels usually form. The wild-type alleles of these three groups of genes interact in the normal flower, to define each whorl as a separate domain of organ identity, and to specify the organ types that will develop in it. Two of these genes

have been cloned (*AGAMOUS* and *APETALA3*), and they share homology with each other and with known DNA-binding proteins from yeast and humans, as well as with homeotic floral genes from the distantly-related plant *Antirrhinum majus*.

One additional homeotic gene has been cloned from *Arabidopsis*, and it is a member of the meristem homeotic class. Mutants for this gene (*LEAFY*) have inflorescence meristems in the positions where flowers are found in wild type, leading to a plant with many branches and no flowers. This gene also shows homology with a gene of similar phenotype from *Antirrhinum*, indicating that both floral and meristem homeotic genes have been conserved in sequence and in function since the early stages of the evolution of flowering plants.

The availability of cloned probes has allowed the domains and times of expression of some of the genes to be studied. The best studied is *AGAMOUS*, the RNA of which first appears in floral meristems at the stage when the earliest steps of sepal development are occurring. The *AG* RNA is present in a central dome-shaped pattern, which includes the cells that will later give rise to the primordia of the stamens and carpels, but does not include the future petal region. One notable of feature of the pattern of *AG* RNA expression is that it is regulated by the product of another of the homeotic genes, *APETALA2*. In *ap2* mutants, the pattern of accumulation of the RNA expressed by the *AG* locus expands to include much of the floral meristem, including the regions that will alter differentiate into sepals and petals. The provides a partial explanation for the *apetala2* phenotype: in *ap2* mutants, carpels form where sepals would be found in wild type, and stamens where petals

would be found, because of the ectopic expression of *AGAMOUS* activity in these outer floral whorls. This ectopic expression of the substance that directs stamen and carpal development causes these organs to form in abnormal locations.

The molecular cloning of the organ homeotic genes, and the finding that several of them are related by a common DNA-binding region, has led us to examine the *Arabidopsis* genome for additional members of this gene family. There are many, and the sequencing of eight of them, including *AGAMOUS* and *APETALA3*, shows that they share both a common DNA-binding region at their amino-terminal ends, and an additional region with structural conservation (the K box) near their middles. The K box region of the proteins can form a pair of amphipathic alpha helices, and it may be that this region is involved in protein-protein interactions between members of this gene family and each other, or between these proteins and other regulatory proteins, to allow the sort of combinatorial specification of organ identity that we predict from our genetic results. One indication that the K box performs an important role in these proteins is that a single amino acid change at one conserved point in the K box of the *APETALA3* protein gives a mutation with a phenotype indicating a nearly-complete loss of *AP3* function.

Selected References:

1. Pruitt, R.E., Chang, C., Pang, P. P.-Y., and E.M. Meyerowitz (1987) Molecular genetics and development of *Arabidopsis*. In: Genetic Regulation of Development: 45th Symposium of the Society for Developmental Biology, ed. W.R. Loomis, A.R. Liss, New York, pp.327-338.
2. Meyerowitz, E.M. (1987) *Arabidopsis thaliana*. Ann. Rev. Genet. **21**, 93-111.

3. Bowman, J.L., Yanofsky, M.F. and E.M. Meyerowitz (1988) *Arabidopsis thaliana*: a review. In: Oxford Surveys of Plant Molecular and Cell Biology, vol. 5, ed. B.J. Miflin, Oxford, pp. 57-87.

4. Meyerowitz, E.M. (1989) *Arabidopsis*, a useful weed. Cell **56**, 263-269.

5. Bowman, J.L., Smyth, D.R., and E.M. Meyerowitz (1989) Genes directing flower development in *Arabidopsis*. Plant Cell **1**, 37-52.

6. Meyerowitz, E.M., Smyth, D.R. and Bowman, J.L. (1989) Abnormal flowers and pattern formation in floral development. Development **106**, 209-217.

7. Ma, H., Yanofsky, M.F., and Meyerowitz, E.M. (1990) Molecular cloning and characterization of *GPA1*, a Gα gene from *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA **87**, 3821-3825.

8. Yanofsky, M.F., Ma, H., Bowman, J.L., Drews, G.N., Feldmann, K.A., and Meyerowitz, E.M. (1990) The protein encoded by the *Arabidopsis* homeotic gene *agamous* resembles transcription factors. Nature **346**, 35-39.

9. Smyth, D.R., Bowman, J.L., and Meyerowitz, E.M. (1990) Early flower development in *Arabidopsis*. Plant Cell **2**, 755-767.

10. Bowman, J.L., Smyth, D.R. and E.M. Meyerowitz (1991) Genetic interactions among floral homeotic genes of *Arabidopsis*. Development, in press.

11. Ma, H., Yanofsky, M.F. and E.M. Meyerowitz (1991) *AGL1-6*, an Arabidopsis gene family with similarity to floral homeotic and transcription factor genes. Genes and Development, in press.

12. Meyerowitz, E.M., Bowman, J.L., Brockman, L.L., Drews, G.N., Jack, T., Sieburth, L.E. and D. Weigel (1991) A genetic and molecular model for flower development in *Arabidopsis thaliana*. Development, in press.

# ANALYSIS OF HOMEOTIC GENES CONTROLLING FLORAL ORGANOGENESIS IN *Antirrhinum majus*

Hans Sommer, Peter Huijser, Jose-Pio Beltran*, Wolfgang Nacken, Wolf-Ekkehard Lönnig, Heike Pape, Peter Flor, Rolf Hansen, Heinz Saedler, Zsuzsanna Schwarz-Sommer

Max-Planck-Institut für Züchtungsforschung, 5000 Köln 30, FRG

*Instituto de Agroquimica y Tecnologia de Alimentos, CSIC, 46010 Valencia, Spain

Floral morphogenesis in *Antirrhinum majus* seems to be controlled by a set of homeotic genes (1). Three of these genes [*deficiens* (2), *globosa*, *squamosa*] have been isolated and their structure and expression pattern partially characterized by us. Mutations in *deficiens* and *globosa* lead to homeotic transformation of petals to sepals and stamens to carpels, while mutational inactivation of *squamosa* causes the production of "shoots" instead of flowers in the axils of the bracts, thus affecting an early step in flower morphogenesis.

Analysis of the temporal and spatial expression patterns of the three genes by *in situ* hybridization revealed that *deficiens* and *globosa* are turned on after the sepal primordia have appeared, at positions of the floral meristem where later the petal and stamen primordia arise. In contrast, *squamosa* is expressed much earlier, at the time when the flower primordium appears in the axils of bracts. Thus it is possible that *squamosa* is controlling early expression of *deficiens* and *globosa*.

The proteins encoded by the three genes are putative transcription factors: they have a conserved domain at the N-terminus in common that displays extensive homology to two known transcription factors, SRF of mammals and MCM1 of yeast. The conserved putative DNA-binding domain [the so-called MADS-box (1)] was found to be present in twelve aditional genes, the majority of which is expressd only in floral organs, in a specific manner. We speculate that specific combinations of these MADS-box genes, expressed in the respective organs, specify organ identity in the process of organogenesis.

1) Schwarz-Sommer,Zs., Huijser,P., Nacken,W., Saedler,H. and Sommer,H. (1990) Genetic control of flower development by homeotic genes in *Antirrhinum majus*. Science 250, 931-936

2) Sommer,H., Beltran,J.P., Huijser,P., Pape,H., Lönnig, W.-E., Saedler,H. and Schwarz-Sommer,Zs. (1990) *Deficiens*, a homeotic gene involved in the control of flower morphogenesis in *Antirrhinum majus*: the protein shows homolology to transcription factors. EMBO J. 9, 605-613

## HOMEOTIC GENES CONTROLLING FLOWER DEVELOPMENT IN *ANTIRRHINUM*

Enrico Coen, Rosemary Carpenter, Sandra Doyle, Ruth Magrath, José Romero, Robert Elliott, Justin Goodrich and Desmond Bradley, John Innes Institute, John Innes Centre for Plant Science Research, Colney Lane, Norwich NR4 7UH, UK

In order to isolate and study genes controlling floral development, we have carried out a large-scale transposon-mutagenesis experiment in *Antirrhinum majus*. Ten independent floral homeotic mutations were obtained and these can be divided into three classes depending on whether they affect (1) identity of organs within the same whorl, (2) identity and sometimes also the number of whorls and (3) the fate of the axillary meristem that normally gives rise to the flower. The classes of floral phenotypes suggest a combinatorial model for the genetic control of primordium fate.

Using transposons as probes, we have isolated and analysed a class (3) gene, *floricaula* (*flo*). Plants carrying the *flo* mutation cannot make the transition from inflorescence to floral meristems and have indeterminate shoots in place of flowers. *In situ* hybridization shows that the *flo* gene is expressed from a very early stage in wild-type inflorescences in a specific temporal and spatial sequence. The earliest expression seen is in bract primordia and is followed by expression in sepal, petal and carpel primordia but no expression is seen in stamen primordia. Expression in each is transient and is not observed in later stages of floral development. This pattern of expression has implications for how *flo* affects phyllotaxis, organ identity and determinacy. In particular, we propose that *flo* interacts in a sequential manner with other homeotic genes (class 2) that affect the identity of whorls in the flower.

**References**

Carpenter, R. and Coen, E.S. (1990) Floral homeotic mutations produced by transposon-

mutagenesis in *Antirrhinum majus*. Genes & Development, 4, 1483-1493.

Coen, E.S., Romero, J.M., Doyle, S., Elliott, R., Murphy, G. and Carpenter, R. (1990) *floricaula*: a homeotic gene required for flower development in *Antirrhinum majus*. Cell 63, 1311-1322.

# FLOWER DEVELOPMENT IN TOMATO: IDENTIFICATION AND CHARACTERIZATION OF MOLECULAR MARKERS AND REGULATORY GENES

Eliezer Lifschitz
Department of Biology
Technion-Israel Institute of Technology
Haifa 32000 Israel
Tel: 04-293959
FAX: 04-225153

It was proposed that upon induction, meristematic cells fated to form an inflorescence, acquire a basic cell-autonomous floral program that distinguishes a floral cell from a vegetative cell. This distinction persists throughout development and is a prerequisite for the proper execution of the homeotic transformations, i.e. organogenesis, as well as the ensuing organ-specific gene activity.

In an attempt to dissect the developmental processes in tomato flowers we undertook the isolation of gene markers common to all floral organs and others that are particularly upregulated during meristematic stages. It is hoped that "common denominator" gene markers will allow the identification of regulatory elements that may characterize the presumptive floral program. Furthermore, gene markers shared by all organs, but not necessarily by all tissues of all organs, will be very useful in tracing unknown developmental shifts in seemingly identical cells or in detecting homeology among tissues of different floral organs. Examples of these possibilities will be illustrated.

We are also trying to isolate and characterize genes with direct regulatory role. Several such genes which comprise the MADS box gene family of tomato were isolated by virtue of their homology with the MADS box sequence of the *deficiens* gene from *Antirrhinum*. Another class of regulatory genes is represented by the *anantha* mutation. It is an unmapped recessive mutation whose development of flower primordia is blocked before organogenesis. The arrested floral meristems multiply and branch indefinitely to give rise to cauliflower-like inflorescences. To prepare for the cloning of *anantha*, the chromosomal localization was determined and closely linked RFLP markers assigned. The mapping procedure, which is efficient and useful for other morphogenetic mutations, will be briefly described.

We also took advantage of the homogeneous floral primordia tissue provided by the *anantha* inflorescence in order to distinguish early and late expression of marker genesor regulatory genes, and to identify genes specific to meristems. More detailed description of our efforts in each department are summarized below.

(A) MARKER GENES: The repertoire of proteins from leaves, mature flowers and *anantha* floral meristems was compared and 16 polypeptides, specific to mature flowers or floral primordia were identified. Antibodies for proteins P3, P5, P2, P18 and P17 were raised and

corresponding cDNA clones from *anantha* and floral expression libraries (λgt11) isolated. P3 and P5 are expressed at low level in leaves and floral meristems but are upregulated dramatically in developing floral organs. Their stepwise differential upregulation and detailed tissue and organ compartmentalization will be described.

The P3 gene encodes the plant biosynthetic threonine deaminase. It is unique in the genome, mapped to chromosome IX and is trnslated to a 595 residue long polypeptide. It is expressed more than 50-fold higher in sepals and more than 500-fold higher in the rest of the flower than in leaves or roots. Its overexpression, like that of P5 is strictly confined to the parenchyma cells and possible explanations of this phenomenon will be discussed.

The genes for P2 and P18 were also studied. P2 is a plastid-bound, insoluble protein. The gene (587 residues) is tandemly duplicated. Both copies are expressed and localized to a site on chromosome VIII. It is upregulated in floral meristems as well as in the very early leaf primordium, but its expression subsides in growing leaves while being persistently high in floral organs. We will show that in the very early floral primordia, P2 is expressed in ground and pith tissues but not in provascular strands or apical cells. This pattern is contrasted with that of the meristem-specific gene P18. The later involves in DNA metabolism and is over-expressed in apical cells and provascular strands but not in ground tissue or pith cells.

(B) REGULATORY GENES: Six genes with homology to the floral homeotic genes *deficiens* of *Antirrhinum* and *agamous* of *Arabidopsis* were isolated from tomato. Each of the six genes is unique in the genome and could be localized to a different chromosome by RFLP mapping. Five of the tomato genes (hereafter TM) are flower-specific with distinguishable temporal expression. TM4 and TM8 are upregulated in *anantha* floral meristems and downregulated in mature flowers. They are considered, therefore, "early" genes. By the same token, TM5 and TM6 are "late" genes, while TM16 is upregulated in *anantha* meristem, but is even higher in mature flowers. TM4 is homologous to *squamosa* and TM6 is similar to *deficiens* which are, respectively, "early" and "late" bona fide homeotic genes in *Antirrhinum*. The proteins encoded by the tomato genes, like known homeotic genes from other plants, contain within their N-terminus a highly conserved DNA binding domain, the MADS box. All known plant MADS box genes share, however, other properties as well. They all contain a central, moderately conserved, and rather basic domain, and a highly divergent or even missing C-terminal domain. Furthermore, molecular modeling predicts the presence of an amphipatic alpha helix, composed of 17 residues, at a constant distance from the MADS box in each of these proteins.The common properties of eight MADS box proteins from three plant families indicate that all their domains were coded for by the same ancestor gene. The sequence homology between pairs of MADS genes from different species indicates that the MADS ancestor gene multiplied and diverged in an ancestor plant common to several dicotyledon families. Possible roles for the MADS genes in flower development in tomato and some preliminary observations on transgenic plant will be considered.

# FOURTH SESSION

R. JORGENSEN
J. PAZ-ARES
C. MARTIN
H. SAEDLER

Homology-based trans interactions and developmental control of petal pigmentation

Richard Jorgensen[1], Tim Robbins[2], and Carolyn Napoli[1]
[1]University of California, Davis, and [2]DNA Plant Technology Corp.

A recently discovered trans interaction phenomenon known as "co-suppression" appears to be a fundamental genetic mechanism involving efficient interactions between homologous genes in trans, resulting in the suppression of the expression of both homologues. For instance, the introduction of a chalcone synthase (CHS) transgene to an ectopic position in the petunia genome can result in the coordinated and reversible suppression of the transgene and both alleles of the homologous, endogenous CHS gene. In addition, co-suppression of CHS elicits highly regular, non-clonal pigmentation patterns in flower petals. These patterns are subject to stochastic reprogramming events which appear to occur in vegetative meristems. Furthermore, it appears that all the cells of a meristem are reprogrammed cooperatively such that all subsequently produced flowers produce a new pattern. In addition, reprogramming events are heritable, and heritable reprogramming occurs cooperatively in adjacent cells. Lastly, transgene alleles carrying different pattern programs interact in the same nucleus such that one alters the other in a manner that persists even after the segregation of the alleles away from each other, as in paramutation in maize and snapdragon.

This system has many parallels with the phenomenon of phase change, except that phase change is not imposed on the germ line, nor is it usually so easily reversed. If somatic reversibility and imposition of somatic changes on the germ line are considered to be abnormal manifestations of a fundamental developmental programming mechanism, this petal pigmentation system may be seen as a potentially "simple" model for flexible developmental programming mechanisms requiring the use of positional information.

# MOLECULAR ANALYSIS OF THE PETUNIA GENE FAMILY RELATED TO THE MAIZE REGULATORY C1 GENE AND TO ANIMAL myb PROTO-ONCOGENES.

Avila, J., Nieto, C., Cañas, L., and Paz-Ares, J.

Centro de Investigaciones Biológicas-CSIC. Velázquez, 144. 28007 Madrid.

One characteristic of regulatory genes encoding transcriptional factors in higher eukaryotes is that they form part of gene families with several members in each species. In plants, one of these families is that represented by the C1 gene of Zea mays.

The C1 gene regulates the anthocyanin biosynthetic pathway of the aleurone and scutellum tissues of the maize kernel. It encodes a protein with the characteristics of transcriptional activators: it contains, at its N-terminus, a region of 110 amino acids rich in basic residues with homology to the DNA binding domain of myb proto-oncoproteins and a region rich in acidic amino acids, putatively representing its activator domain.

Recently, several other genes from maize and barley have been described that, like the C1 gne, contain the myb and the acidic (activator) domains. The functions of these additional genes remains unknown.

We are interested in the elucidation of the physiological role of the different myb-related genes in plants. Towards this propose, we have chosen Petunia hybrida as a plant system, since this plant can be easily transformed and regenerated and therefore, should allow full explotation of recombination DNA technology.

Three myb-related Petunia genes have been cloned. Their proteins contain myb-related domains with no additional sequence homology to each other or to any other known MYB protein. Each myb gene has been shown to be part of a small subfamily of highly related genes (2-4 members) and to have a specific expression pattern. For instance, Myb.Ph3 is transcribed only in the flower (except sepals).

DNA binding properties of MYB.Ph3 have been studied, and a consensus sequence of its DNA binding site could be stablished: CAG(T/G)T(A/G). This consensus sequence is partially overlapping with that of animal c-MYB protein (C(A/C)GTT(A/G)).

Since the similarities among each other of the plant MYB-proteins is higher than that between any plant MYB-protein an animal c-MYB protein, it appears likely that the DNA-binding sites of the majority of the plant MYB proteins so far known will be very similar, if not identical, and therefore they might regulate partially overlapping groups of genes in each plant species.

Experiments to obtain transgenic plants with constructs aimed to interfere with Myb.Ph3 function have been initiated. Preliminary data indicating that some of the transgenic plants have alterated flower coloration are consistent with the above mentioned hypothesis on the role of plant myb genes.

## THE CONTROL OF GENE EXPRESSION IN *ANTIRRHINUM* FLOWERS

**Cathie Martin, Francisco Culianez-Macia, Andy Prescott, David Jackson**

The production of anthocyanin in flowers is an important determinant of their ability to attract pollinators. Anthocyanin biosynthesis involves a complex metabolic pathway including the activity of between eight or nine committed enzymes. The activity of these enzymes appears to be controlled primarily through their biosynthesis. Patterns of anthocyanin production within flowers reflect patterns of expression of the biosynthetic genes.

In *Antirrhinum majus* the expression of the biosynthetic genes is not uniform within or across the tissues of the flower. Some genetic components that regulate the expression of the biosynthetic genes have been identified. *Delila* appears to be an activator of four later steps in the pathway within the tube of the flower. *Delila* does not appear to influence chalcone synthase expression within the flower tube, although it appears to act as a repressor within the mesophyll tissues of the lobes. *Eluta* modifies the expression of the four late steps in specific regions of both lobes and tubes but has the opposite influence on chalcone isomerase expression in the flower lobes. The control of the pathway by regulatory genes appears to operate independently on early and late steps. However, genes that act as activators in one area of the flower may serve a different function in the expression of the other biosynthetic genes in other parts of the flower.

We have searched for regulatory genes at the molecular level by homology to the *R* and *C1* regulators of anthocyanin biosynthesis in maize. *Delila* appears to be homologous to *R*. *C*1 is related to the *myb*-protooncogenes in animals and the BAS1 transcriptional activator in yeast. We have identified 6 genes from *Antirrhinum* flowers with homology to the DNA-binding domain of *C*1 and c-*myb*. Evidence from expression patterns suggest that these *myb* genes may not be functionally homologous to *C*1 but may be involved in regulation of other developmental or metabolic functions in flowers. The control of anthocyanin biosynthesis may therefore provide a useful model system for understanding the control of these other metabolic pathways in flowers.

We have shown differences in DNA binding specificities between the *myb* genes from *Antirrhinum* flowers suggesting that a component in the specificity of the processes they control is vested in differences in their binding sites. To identify the functional roles that these genes play in plant development we have initiated a programme of analysis using antisense in tobacco which indicates that *myb*-type genes may play a broad role in regulating aspects of growth and flower function and longevity.

Methylation and Development

Heinz Saedler, Max-Planck-Institut für Züchtungsforschung, Carl-von-Linne-Weg 10 5000 Köln 30, FRG
Peter Meyer, Max-Delbrück-Laboratorium, Carl-von-Linne-Weg 10, 5000 Köln 30, FRG

Tissue-specific gene expression and the maintenance of developmental phases are believed to involve methylation/demethylation of genes. Switching of one developmental phase, say indeterminant vegetative growth, to another phase, i.e. flower evocation could possibly be accomplished by methylation/demethylation system.

What do we now about methylation in developmental processes. There are 3 lines of suggestive evidence.

a) During maize development transposable element have a tendency to become progressively methylated.

b) Azacytidine treatment of rice leads to dwarfism due to undermethylation. This is a reversible process.

c) In a transgenic petunia line - containing the maize A1 colour gene - colourformation is switched off in subsequent flowers due to methylation of the 35 S promoter driving A1 gene expression.

This letter system will be described in more detail, since it reveals exogenous as well as endogenous factors influencing the degree of gene expression.

References

Meyer, P.; Heidmann, I.; Büssmann, K.; Saedler, H.: A new petunia flower colour generated by transformation of a mutant with a maize gene. Nature, 330, 677-678, 1987

Linn, F.; Heidmann, I.; Saedler, H. and Meyer P.: Epigenetic changes in the expression of the maize A1 gene in Petunia hybrida: role of numbers of integrated gene copies and state of methylation; Mol Gen Genet, 222, 329-336, 1990

# POSTER SESSIONS

# CALCIUM-EFFECTED PEROXIDASE ACTIVITY IN LEAVES OF SOYBEAN UNDER INDUCTIVE AND NON-INDUCTIVE FLOWERING CONDITIONS.

Silvia V. Caffaro and C. Vicente

Department of Plant Physiology, Faculty of Biology, Complutense University, 28040 Madrid, Spain.

SD timing produces flowering induction in soybean plants cultured in laboratory conditions, whereas LD timing behaves as non-inductive. Perception of inductive SD promotes transient decrease of peroxidase activity obtained after isolation of microsomal fractions of leaves, changes that are not observed for peroxidase isolated from leaves receiving non-inductive LD timing. In order to study the causes of this lower activity, the possible implication of $Ca^{2+}$ as a second messenger for phytochrome-regulated responses on *in vivo* and *in vitro* peroxidase activities was examined. Soluble peroxidase shows to be inhibited *in vitro* by $Ca^{2+}$, with a maximum per cent inhibition of 28.14% for 1 mM $Ca^{2+}$ in LD treatments and of 42.3% for 2.5 mM $Ca^{2+}$ in SD treatments. Inhibition is impeded by both 0.5 mM EDTA and 0.5 mM EGTA only when 1.0 mM $Ca^{2+}$ is included in the reaction mixtures. Chelating agents inhibit peroxidase activity when $Ca^{2+}$ is not added to the incubation mixtures. This is in agreement with that previously described for peroxidase from other plant sources about the occurrence of calcium into protein molecule.

To investigate the effect of calcium ions on peroxidase activity *in vivo*, $Ca^{2+}$ was directly applied on leaves of both LD and SD plants. When indicated, calcium ionophore or verapamil were also applied. Pelletable 30 Kp peroxidase activity from control ( without additions ) LD plants was higher than that obtained from SD plants only after the first inductive day. This behaviour was clearly reversed for the second inductive night or day. Addition of $Ca^{2+}$ clearly inhibits pelletable peroxidase obtained from LD leaves only after the first inductive day, but it does not significantly affect peroxidase activity from this time as well as that isolated from SD leaves. Ionophore also inhibits peroxidase activity but only in LD-treated plants during the first inductive day. Verapamil, a $Ca^{2+}$ channel blocker, partially reverses inhibition produced by $Ca^{2+}$ or the ionophore. However, this effect was changed by an inhibitory effect during the 2$^{nd}$ inductive day. This may be due to a residual perturbation of membrane structure rather than to a direct effect on $Ca^{2+}$ uptake.

Soluble peroxidase activity was also inhibited by $Ca^{2+}$, being this inhibition coincident to that obtained by $Ca^{2+}$ applications *in vitro*. However, neither ionophore nor verapamil exert a clear effect. These results give evidences about a transient $Ca^{2+}$ uptake during the first hours of SD flower induction that produces the observed decrease in pelletable peroxidase.

# SELF-INCOMPATIBILITY IN *Papaver rhoeas:* MOLECULAR BASIS OF THE POLLEN STIGMA INTERACTION

V.E. Franklin-Tong, G.J. Thorlby, K.K. Atwal,
M.J. Lawrence and F.C.H. Franklin.
School of Biological Sciences, University of Birmingham,
BIRMINGHAM B15 2TT, U.K.

We have developed a bioassay which permits the self-incompatibility (SI) responses to be included in pollen of Papaver rhoeas grown in vitro(1). This has allowed us to identify and partially purify biologically active stigmatic S-gene products. The bioassay has also enabled characterization of these proteins, which are tissue specific and developmentally expressed. They are glycoprotins of similar molecular weight, whith S-alletes varying in pI(2). Ribonuclease assays have revealed that, in contrast to the S-glycoproteins from Nicotiana alata, there is no detectable ribonuclease activity that correlates with the presence of the functional stigmatic S-gene product in P. rhoeas(3). The S-glycoproteins in this species are, therefore, not ribonucleases. This suggests a different mechanism for the operation of SI in these two species, although they have genetically the same SI system.

We are currently exploiting the bioassay to investigate the responses induced in pollen as a result of the SI response. Experiments with metabolic inhibitors and stigmatic extracts indicate that both glycosylation and de Novo transcription of pollen genes which are specific to an incompatible SI response play an important role in the inhibition of pollen tube growth in this species. While dry, germinated and compatible pollen all have the same protein profile on 2D gels of mRNA translations, we have detected the appearance of novel proteins appearing in pollen as a response to an incompatible reaction(4). A cDNA library has been constructed from this incompatible-challenged pollen, with the intention of cloning the pollen SI "response" genes.

1. Franklin-Tong, Lawrence & Franklin(1988), New Phytologist 110, 109-118.
2. Franklin-Tong, Lawrence, Ruuth & Franklin(1989) New Phytologist 112, 305-307.
3. Franklin-Tong, Lawrence & Franklin(1990) New Phytologist 116, 319-324.
4. Franklin-Tong, Atwal, Howell, Lawrence & Franklin: In press.

## Development of the gynoecium and silique of *Arabidopsis*

**M. Harris, P. Gates and J. Spence**

University of Durham, Department of Biological Sciences, Durham DH1 3LE, UK

Using the silique of *Arabidopsis* as a model system, we are studying ovary and fruit development by a combination of cytological, cytochemical, genetical and molecular techniques.

From the floral bud the early gynoecium develops with the differentiation of two meristematic regions. These give rise to the ovules and combine to form the septum which separates the two loculi of the maturing fruit. Development of the ovary wall results from differentiation of specific endocarp, mesocarp and exocarp layers. An abscission zones forms between these and the major vascular supplies to the developing ovules. The patterns of differentiation result in the dehiscence mechanism which disperses seeds from the shattering fruit. Such fruit shatter and seed dispersal mechanisms represent significant problems in a number of agricultural crops.

We have described the cytological events associated with the differentiation of the major tissue types associated with carpel and septal development, and identified a number of probes which act [by immunoctyochemistry, *in situ* hybridisation or enzyme cytochemistry] as appropriate markerts for specific phases of differentiation. We have compared the development of meristic and homeotic mutants with that of the wild type, and are screening EMS-mutated stock for other appropriate lines

Having obtained parthenocarpic fruit set, we are currently constructing cDNA libraries which will be screened for tissue-specific or tissue-enhanced sequences.

**α,1-4-D-OLIGOGALACTURONIDES STIMULATE THE FORMATION OF FLOWERS AND INHIBIT THE FORMATION OF ROOTS IN TOBACCO EXPLANTS.** Victòria Marfà, S. Eberhard, D. Mohnen, A. Darvill and P. Albersheim, Complex Carbohydrate Research Center, 220 Riverbend Road, The University of Georgia, Athens GA 30602; F. Cervone and G. de Lorenzo, Dipartimento di Biologia Vegetale, U. di Roma "La Sapienza", Roma, Italy 00100.

We modified a tobacco thin cell layer morphogenesis bioassay for testing morphogenesis-regulating activity of plant cell wall fragments. Pectic fragments were released from suspension cultured sycamore (*Acer pseudoplatanus*) cell walls by treatment with a purified endopolygalacturonase (EPG) from *Aspergillus niger*. Addition of pectic fragments to a culture medium containing 1.5 μM IBA and 0.9 μM kinetin induced the formation of flowers on the explants. The EPG-released pectic fragments are known to be primarily composed of the polysaccharides rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II), and a-1,4-linked-oligogalacturonides (OGs). Pectic components were purified and tested for flower-inducing activity. The larger OGs induced flowers to form on the explants. RG-I, RG-II and small OGs did not induce flowers to form. Highly purified OGs obtained by partial acid hydrolysis of citrus pectin were also able to induce flowers to form. OGs with a degree of polymerization (DP) of 12-14 were the most active at inducing flowers exhibiting half maximum activity at ~ 0.4 μM. OGs with DPs<10 showed little or no activity at 4 μM. OGs with DPs>10 inhibit the formation of roots on the explants incubated in a medium containing 15 μM IBA and 0.5 μM kinetin. OGs also inhibit the formation of roots on tobacco leaf disc explants. The ability of oligogalacturonides to regulate morphogenesis is another biological activity of this pleiotropic oligosaccharin. Acknowledgements: This work is supported in part by U.S. Department of Energy grant DE-FG09-85ER13425, and by U.S. Department of Energy grant DE-FG09-87ER13810 as part of the USDA/DOE/NSF Plant Science Centers program.

*floricaula*: A HOMEOTIC GENE CONTROLLING FLOWER DEVELOPMENT IN *Antirrhinum majus*.

J.M. Romero, D. Bradley, R. Carpenter, E.S. Coen, S. Doyle, R. Elliott and S. Hantke.
John Innes Institute, John Innes Centre for Plant Science Research, Colney Lane, Norwich NR4 7UH, U.K.

The *floricaula* (*flo*) mutant was obtained in a large-scale transposon mutagenesis experiment in *Antirrhinum majus*. The *flo* mutant initiates vegetative growth and the transition to inflorescence meristem in a similar manner to wild-type plants. However, *flo* plants cannot make the transition from inflorescence to floral meristem and have indeterminate shoots in place of flowers.

The *flo*-613 allele carries a Tam3 transposon insertion, thus allowing isolation of the *flo* locus. The *floricaula* gene produces a 1.6 kb transcript with the potential to encode a protein, (FLO), of 396 amino acids. The FLO protein contains a proline-rich N-terminus (including a strech of 7 consecutive prolines) and a highly acidic region containing 15 glutamic or aspartic acid residues out of 18 amino acids. This acidic region was preceeded by a basic region.

*In situ* hybridization shows that the *flo* gene is expressed from a very early stage in wild type inflorescences in a specific temporal and spatial pattern. The earliest expression seen is in bract primordia and is followed by expression in sepal, petal and carpel primordia, but no expression is detected in stamen primordia. This pattern of expression suggests that *flo* interacts in a sequential manner with other homeotic genes that affect the identity of organs in the flower.

In order to study the type of interactions that FLO may establish with other floral homeotic genes, we are at present carrying out experiments to express the FLO protein using both *in vivo* and *in vitro* translation systems.

## Cloning of apex specific genes from tomato using the PCR technique

Claus Roßbach, Rüdiger Simon and Klaus Theres

Institut für Genetik, Universität zu Köln, D-5000 Köln 41, F.R.G.

The aim of our studies is the isolation and characterisation of apex specific genes from Lycopersicon esculentum. Since the shoot apex consists of a small number of cells apex cDNA was amplified using the PCR technique. We have enriched for apex specific sequences by substracting apex cDNA with biotinylated leaf, stem and flower cDNA. The substracted library was differentially screened with cDNA probes from various plant tissues. So far two clones ( apex1, apex2 ), which hybridized to the apex probe but not to the other probes ( leaf, stem, flower ), were further tested in Northern and Southern experiments.

Both genes are highly expressed in the shoot apex but the transcripts were also found in young flower buds. Even in long exposures of autoradiographs the 600 nucleotide transcript apex2 was undetectable in samples of total mRNA from young leaf, stem, root and callus tissue. However apex1 ( 600 bp ) is also weakly expressed in root and callus tissue. The sequence analysis revealed that apex1 has a significant similarity to the tomato proteinase inhibitor II. Surprisingly apex2 is similar to a potato gene which has a similarity to the soybean proteinase inhibitor CII ( Bowman-Birk). Furtheron a Southern blot experiment indicated that apex2 belongs to a gene family of about seven genes.

Presently the isolated clones are tested in in-situ hybridisation experiments to localize the transcripts in tomato tissue sections. In addition we would like to express the antisense as well as the sense transcript in transgenic tomato plants trying to generate plants with an altered phenotype either by inhibition or by overexpression.

TITLE: Maize HRGP RNA as an early marker for vascular differentiation and axis development during maize embryogenesis

Luis Ruiz-Avila, Shirley Burgess, Maria Dolors Ludevid and Pere Puigdomènech. Departamento de Genética Molecular, CID-CSIC. Jordi Girona 18-24, Barcelona 08034, Spain.

In situ hybridization studies with a maize HRGP RNA probe have revealed a precise pattern of label distribution in intermediate stages (13 to 20 days after fertilization) of embryo development. The maize HRGP has been shown to be a protein extractable from the cell walls of many tissues by alcoholic-acid or saline procedures. Its mRNA is accumulated in association with the divisional activity of a given tissue. The maize HRGP accumulates in the early stages of root and leaf vascular differentiation, as it has been demonstrated by in situ hybridization of young plants.

In whole embryos the HRGP mRNA is detectable at lower levels than in others parts, stages or organs of the plant. A strong diluting effect of the scutellar tissues has been proposed. We found that maize HRGP is neither detectable nor extractable from scutellar cell walls or tissue extracts. The mRNA accumulation is at least 10 times higher in embryo axis than in scutellum. The in situ hybridization studies we present here reveal that the HRGP mRNA accumulates in a similar fashion between 13 and 20 days after fertilization. The hybridization is restricted to well defined cellular types, associated mainly with procambium and pre-vascular elements. The maize cell wall HRGP mRNA accumulation is prior to cellulose organization in cell walls of the embryo tissues, as revealed by studies of polarized light microscopy. Hybridization of *decussate*, a symmetry mutant of maize, at equivalent stages of embryo development, has revealed that the pattern changes as the morphology does. We propose that the HRGP mRNA could be used as a marker for the early commitment of vascular cells, as well as for the distinction of axis from scutellar structures.

**Characterization of some alleles of *deficiens*, a homeotic gene controlling flower morphogenesis in *Antirrhinum majus*.**

H. Sommer, F. Tetens, R.Hansen, P.J. Flor, J.-P. Beltran[1], H.Pape, H. Saedler and Zs. Schwarz-Sommer
Max-Planck-Institut für Züchtungsforschung
5000 Köln 30, FRG
[1]Instituto de Agroquimica y Tecnologia de Alimentos, CSIC,
46010 Valencia, Spain

The homeotic gene *deficiens* is involved in the control of flower development in *Antirrhinum majus*. The *deficiens* gene product belongs to a group of transcription factors, including SRF and MCM1, which share a common DNA binding domain. We therefore suppose, that *deficiens* also acts at the transcriptional level.

To learn about the regulation of the gene and its role in flower development, several morphoalleles (alleles of *deficiens* with altered flower morphology) were characterized.

In *deficiens*$^{globifera}$ a 7kb transposable element (Tam7) is integrated at an intron exon boundary. No *deficiens* mRNA can be detected in northern-blot analysis. In homozygous *globifera* plants the petals are transformed to sepal-like organs and stamens are transformed to carpels.

Homozygous *deficiens*$^{chlorantha}$ plants show a phenotype very similar to the wildtype, only the petals are slightly sepaloid. The amount of mRNA is decreased to one tenth compared to wildtype. The *chlorantha* genomic sequence differs from the wildtype sequence by a four base pair alteration 1.2kb upstream of the transcriptional start.

*Deficiens*$^{nicotianoides}$ plants display an altered phenotype (petals are sepaloid, stamens are sterile), but the amount of mRNA is similar to wildtype. Within the DNA-binding domain we find a single amino acid exchange (Gly to Asp).

Recently, several additional morphoalleles were obtained by transposon mutagenesis; two of them are analyzed so far:

*Deficiens 186b* (heterozygous *186b/def*$^{wt}$) plants display slightly sepaloid petals and stamens, which are petaloid and curled. A 3kb Tam1-derived element is integrated at an intron exon boundary. The amount of *deficiens*-mRNA is similar to the amount of mRNA of the *chlorantha* allele (1/10 of wildtype).

In the other, *deficiens 23* (heterozygous *23/def*$^{wt}$), stamens are transformed to carpels and petals to sepaloid organs. The amount of *deficiens*-mRNA is strongly reduced and hardly detectable. A 3kb transposable element (Tam8) is integrated in the last exon, 36 base pairs upstream of the stop codon.

We discuss the molecular data with respect to the altered phenotypes.

TITLE: Maize HRGP RNA as an early marker for vascular differentiation and axis development during maize embryogenesis

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In whole embryos the HRGP mRNA is detectable at lower levels than in others parts, stages or organs of the plant. A strong diluting effect of the scutellar tissues has been proposed. We found that maize HRGP is neither detectable nor extractable from scutellar cell walls or tissue extracts. The mRNA accumulation is at least 10 times higher in embryo axis than in scutellum. The in situ hybridization studies we present here reveal that the HRGP mRNA accumulates in a similar fashion between 13 and 20 days after fertilization. The hybridization is restricted to well defined cellular types, associated mainly with procambium and pre-vascular elements. The maize cell wall HRGP mRNA accumulation is prior to cellulose organization in cell walls of the embryo tissues, as revealed by studies of polarized light microscopy. Hybridization of *decussate*, a symmetry mutant of maize, at equivalent stages of embryo development, has revealed that the pattern changes as the morphology does. We propose that the HRGP mRNA could be used as a marker for the early commitment of vascular cells, as well as for the distinction of axis from scutellar structures.

**Characterization of some alleles of *deficiens*, a homeotic gene controlling flower morphogenesis in *Antirrhinum majus*.**

H. Sommer, F. Tetens, R.Hansen, P.J. Flor, J.-P. Beltran[1], H.Pape, H. Saedler and Zs. Schwarz-Sommer
Max-Planck-Institut für Züchtungsforschung
5000 Köln 30, FRG
[1]Instituto de Agroquimica y Tecnologia de Alimentos, CSIC,
46010 Valencia, Spain

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To learn about the regulation of the gene and its role in flower development, several morphoalleles (alleles of *deficiens* with altered flower morphology) were characterized.

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Homozygous *deficiens*$^{chlorantha}$ plants show a phenotype very similar to the wildtype, only the petals are slightly sepaloid. The amount of mRNA is decreased to one tenth compared to wildtype. The *chlorantha* genomic sequence differs from the wildtype sequence by a four base pair alteration 1.2kb upstream of the transcriptional start.

*Deficiens*$^{alcotianoides}$ plants display an altered phenotype (petals are sepaloid, stamens are sterile), but the amount of mRNA is similar to wildtype. Within the DNA-binding domain we find a single amino acid exchange (Gly to Asp).

Recently, several additional morphoalleles were obtained by transposon mutagenesis; two of them are analyzed so far:

*Deficiens 136b* (heterozygous *136b/def*$^{wt}$) plants display slightly sepaloid petals and stamens, which are petaloid and curled. A 3kb Tam1-derived element is integrated at an intron exon boundary. The amount of *deficiens*-mRNA is similar to the amount of mRNA of the *chlorantha* allele (1/10 of wildtype).

In the other, *deficiens 23* (heterozygous *23/def*$^{wt}$), stamens are transformed to carpels and petals to sepaloid organs. The amount of *deficiens*-mRNA is strongly reduced and hardly detectable. A 3kb transposable element (Tam8) is integrated in the last exon, 36 base pairs upstream of the stop codon.

We discuss the molecular data with respect to the altered phenotypes.

## Functional Analysis of Pollen specific-Proteins

Guy Vancanneyt, Judy Yamaguchi and Sheila McCormick,
Plant Gene Expression Center
800 Buchanan Street
Albany CA 94710 USA

Several tomato genes (LAT52, LAT56, LAT59) have been isolated which are predominantly expressed in the male gametophyte . Whether these proteins are important for pollen formation and/or fertilisation remains largely unknown. Although the LAT56 and LAT59 cDNAs do not cross-hybridise, their deduced protein sequences share 54% amino acid identity. Both LAT56 and LAT59 show significant sequence similarity to the major Short Ragweed pollen allergen (antigen E). On the other hand a pistil specific cDNA of tomato reveals a striking sequence sequence similarity to both cDNAs. Hence these proteins seem to cover a multi protein family, encoded by non cross-hybridising genes.
In addition these DNA sequences show a sequence similarity to both procaryotic (*Erwinia*) pectate lyases and a eucaryotic (*Aspergillus*) pectin lyase. Whether or not the these LAT clones express allergen properties and/or pectin degrading activities remains to be elucidated. The LAT52 gene encodes a potential 18 Kd cysteine - rich protein which shows significant homology to a maize pollen-specific cDNA clone ( Zmc13). Moreover it shows partial similarity to several Kunitz trypsin inhibitors.
In order to characterize the three pollen specific proteins, the respective cDNAs (or parts of the cDNA) were overexpressed in *E.coli* using the *trp*E expression system.These overexpressed proteins were used as antigens for raising polyclonal antisera in both mice and rabbits. The antisera are currently being tested for their specificity. A biochemical characterisation of the LAT52, LAT56, and LAT59 proteins using these antisera will be presented.

**Genetic and Developmental Analyses of Maize Inflorescences**
Bruce Veit and Sarah Hake, Plant Gene Expression Center, 800 Buchanan St., Albany, CA 94710 USA

Maize is a monecious species that bears two inflorescence types, the staminate tassel and the pistillate ear. To better understand the role of specfic genes during inflorescence development and how their expression is coordinated, we have assessed the manner in which specific mutations perturb normal inflorescence development and performed tests of epistasis with various double mutant combinations.

Normal inflorescence development can be divided into two broad phases. During the first phase, the pattern of ear and tassel development is essentially identical with the acropetal formation of perfect florets on the main inflorescence axes. This phase can be further subdivided to describe the sequential formation of distinct floral structures. Branch primordia borne on the main inflorescence axis divide to form pairs of spikelet initials, with each spikelet giving rise to a pair of perfect florets. During the second phase, ear and tassel development diverge with the respective abortion of stamen and pistil primordia to give female and male inflorescences.

Through developmental studies, we have defined several mutants that perturb normal development in a stage specific manner. Two mutants, *ramosa1* (*ra1*) and *ramosa2* (*ra2*) interfere with spikelet determination to give indeterminate branches. By contrast, *branched silkless* (*bd*) blocks a later step, the determination floret primordia. Several tassleseed mutants (*ts1* and *ts2*) apppear to act even later, reversing the normal pattern of organ abortion in the tassle to give a pistillate structure.

Most double mutant combinations of inflorescence mutants give additive phenotypes, suggesting that the corresponding genes act relatively independently of each other. For example, a *ts2; ra1* double mutant gives a highly branched (*ramosa* like), pistillate tassel. However, some double mutants give epistatic or synergistic phenotypes, suggesting the expression of the corresponding genes is in some way coordinated. For example, the branching conditioned by *bd* is greatly potentiated by *ts1* or *ts2* to produce cauliflower like inflorescences. These data provide an essential basis for models that can be tested and further refined through molecular studies.

PROGRESSION OF CELL CYCLE AND CHANGES IN GENE EXPRESSION DURING INDUCTION OF EMBRYOGENESIS IN ISOLATED POLLEN CULTURES.

O.Vicente[1], V.Zarsky[2], D.Garrido[1], M.Pfosser[3], J.Tupy[2] and E.Heberle-Bors[1].

1) Institute of Microbiology and Genetics, University of Vienna.
2) Institute of Experimental Botany. Czechoslovak Academy of Sciences. Prague.
3) Institute of Botany. University of Agriculture and Forestry Vienna.

An in vitro starvation treatment, followed by culture in a sugar-containing medium, induces immature pollen grains of tobacco to deviate from their normal gametophytic development towards cell division and embryogenesis. We are using cytological, biochemical and molecular techniques to study the events associated with induction of pollen embryogenesis, with the final aim of understanding the molecular mechanisms controlling this developmental switch. Experiments involving cytophotometry of Feulgen stained nuclei and autoradiography of nuclei labelled with ($^3$H)Thymidin show that the vegetative nucleus of the polen grain (which will give rise to the embryo) is arrested in the G-1 phase of the cell cycle during normal pollen development, but it is committed to S-phase (e.g. it goes through the START point of the cell cycle) under starvation conditions. Actual DNA replication can take place already during starvation, or in the embryogenic medium. Changes in gene expression associated with this transition are under study. Starvation causes an overall decrease in RNA and protein synthesis in the pollen grain, but de novo transcription of specific genes seems to be required for embryogenesis: inhibitors of transcription added to the starvation medium effectively block the formation of embryos after transfer to the sugar-containing medium. Accordingly, at least two major species of mRNA, that are not present in the young pollen before starvation, have been detected in embryogenic pollen by 2-d gel electrophoresis of their in vitro translated products. A similar comparative analysis of in vivo synthesised proteins, after in situ labelling with pulses of ($^{35}$S)Met, did not detect new protein spots, suggesting that those mRNAs accumulate in the embryogenic pollen in a translationally inactive form. Isolation and characterisation of the starvation-induced messenger RNAs, via cDNA cloning, is in progress. Post-translational covalent modification, and specifically phosphorylation, of proteins could also be involved in the induction of pollen embryogenesis, mediating the effect of starvation as the trigger of this process, as it has been demonstrated in other systems in which a hunger signal induces similar developmental switches (e.g. in yeast). In agreement with this idea, assays of protein kinase activities in pollen extracts, before and after starvation, revealed a change in the phosphorylation patterns of endogenous polypeptides.

**Study on sex determination in the dioecious Melandrium album: Mutagenesis in sex expression**

D. Ye, P. Installé, M. Jacobs, I. Negrutiu, M. Oliveira2.

1. Inst. Molekulaire Biologie, Vrije Univ. Brussel, 1640 St.-Genesius-Rode, Belgium.
2 Dep. Biologia Vegetal, Fac. Ciências Lisboa, Campo Grande, 1700 Lisboa, Portugal.

Melandrium album (2n=24), also often known as Silene alba, is a dioecious species with heteromorphic sex chromosomes (XY, male; XX, female) which, for a long time, has been considered to be a model system for sex determination in plants. Its genetic control is by far the most strict of all such plant species. The only known way to modify sex expression in this species is by the infection with the fungus Ustilago violacea. It was postulated that there are male determiners on the Y-chromosome. The absence of the X-chromosome seems to be lethal at least at definite stage of embryo (haploid) regeneration (Ye et al, Sex Plant Prod. 3: 179-186). Mutants are an useful tool for the study on genetic mechanisms of development. Recently, the mutant approach has been successfully used in sutides on genetic control of flower development and the molecular cloning of corresponding genes involved in flower development. The role Y-chromosome plays in controlling sex determination as a dominat factor (XY = male) and in a haploid condition provides an ideal experimental system for identifying mutation in sex expression.

Here we report our first result on isolation of mutants in sex expression by pollen irradiation. The mature pollen grains were collected from male plants in greenhouse and irradiated by y-rays (5-25 Krads), then used to pollinate to the female flowers. Mutans in flower development were screened from the population of the resulting progeny by checking flower patterns. So far, three types of such mutants have been isolated: asexual, agamons and pseudohermaphrodite mutants. The asexual mutants: the flower contains normal sepals and petals, but no sexual organs. In the agamous mutant, the anthers are modified into petal-like structures or "normal" petals. This plant contains nerrow leaves. The pseudohermaphrodite mutants can be divided into two types. One is male fertile, the flower containing normal anther producing fertile pollens and an abnormal carpel. The other is female fertile, in its flower, the carpel developing completely, but the development of the anthers stops at a relatively early stage. The genetic and cytological analysis of these mutants will be presented.

# CONCLUSIONS AND PERSPECTIVES

H. SAEDLER
J. P. BELTRAN
J. PAZ-ARES

The topics that were presented during the two and a half-day meeting cover four broad areas of research : (1) Signals affecting floral induction and flower development ; (2) Self-incompatibility and male sterility ; (3) Flower homeotic mutants as a tool to study flower development and (4) Control of gene expression in flowers.

Floral induction and flower development are very complex processes controlled by genetic and environmental factors. Thus, the induction of flower formation is the result of the interaction of integrated events whose basis are changes of the signalling system in the whole plant. As it was shown by **G. Bernier** (Liège, Belgium), all plant parts seem to participate in the exchange of signals, so that the different tissues and organs are rapidly instructed about the changes of the environmental conditions. Despite the past and present research effort, the physiological dissection of such processes appears to be insufficient to untangle these fundamental regulatory processes. A systematic mutant analysis of floral induction has only been possible in Arabidopsis , which small size and short life cycle has allowed the identification and characterization of a large number of mutant plants with an altered floral phenotype, although no mutant totally unable to initiate flowers has been obtained. Mutagenesis of early flowering ecotypes has resulted in the isolation of mutants that are delayed in flowering time under floral inductive conditions. The genetic and physiological analysis of these late flowering mutants is permitting the understanding of the function of the loci involved ( **J.M. Martinez-Zapater**, Madrid, Spain). He also proposed the mutagenesis of late flowering ecotypes as a way to identify possible inhibitors of flower induction.
**V.K. Sawhney** (Saskatoon, Canada) described physiological studies carried out with the flower development mutant of tomato *stamenless-2*. This mutant produce flowers in which stamens have microspores in the distal part of the anther and ovules near the base. Mutant plants treated with gibberellic acid produce phenocopies of normal flowers. In contrast, plants treated with indole-3-acetic acid produce carpel-like structures in place of stamens, suggesting that stamen identity might be determined by concentrations or gradients of two morphogens. The analysis of endogenous plant growth regulators in this system combined with a molecular approach would provide a connection between physiology and molecular genetics of flower development. **P. Albersheim** (Athens, GA, USA) presented evidence that oligosaccharins, structurally defined fragments of plant cell wall polysaccharides, can affect flower formation *in vitro* in the Thin Cell Layer system. Oligosaccharins must be the product of enzymatic action upon plant cell wall (i.e. endopolygalacturonase). Whether this process happens or not in response to environmental conditions we still do not know. If true, a new class of signals should be taken into consideration to explain flowering. **K.Tran Thanh Van** (Gif sur Yvette, France) reported the identification of thaumatin like proteins as markers of floral meristems both in *in vitro* (Thin Cell Layer) and in *in vivo* systems.
Outbreeding is favored in many angiosperm species by a mechanisms that prevents self-fertilization denominated sel-incompatibility (SI). Plants bearing a SI system reject self pollen by the female somatic tissues. Self-incompatibility is controlled by a single S-locus with approximately fifty allelic forms. Several cDNAs corresponding to

glycoproteins of the female tissues that segregate with particular variants encoded by the S-locus have been cloned. Progress made in experimental systems corresponding to two major types of SI, gametophytic (*Solanum tuberosum*) and sporophytic (*Brassica oleracea*) was presented. **C.P. Scutt** (Durham, UK) showed evidence of a new class of expressed S-locus related sequence present in plant lines of *B.oleracea* containing the S5 allele. Northern blot hybridization analysis demonstrated no S-locus related transcripts in S5 anther tissues. A major unanswered question of this area of research is that of the nature of the pollen product of the S-locus. The products of the S-locus in pollen and style must be identical or closely linked as the two functions have never be separated by conventional breeding. **C.P. Scutt** proposed a strategy to isolate anther-expressed, S-locus specific sequences, based on the screening of a *B.oleracea* genomic library in YAC vectors using an S-locus sequence as hybridization probe.Then , sublibraries constructed in plasmid vectors from the previously cloned S-locus genomic region will be screened using as probe total cDNA derived from anther mRNA. **R.D. Thompson** (Köln, Germany) reported the cloning of cDNA and genomic sequences corresponding to S1 and S2 alleles in S.tuberosum. These S-locus sequences show a similar structural organization into conserved and hypervariable regions as the *Nicotiana allata* alleles and those of members of the *Solanaceae* recently analyzed. Similar to the *N.allata* glycoproteins, the potato glycoproteins corresponding to S1 and S2 alleles posses RNase activity in vitro.

The offspring of crosses between different plant varieties are more productive than their parents, an effect known as heterosis. A crucial step in hybrid breeding is the cross between two different inbred lines. Pollen from one line is transferred to the pistils of a second line (mother line). To make sure that hybrid seeds are produced, it is important to avoid that pistils of the mother line plants receive pollen from themselves. Usually, male sterile mutants of the mother lines are used in hybrid production. If hybrid plants have the mutant phenotype (male sterility), they are therefore unable to produce fertile pollen. To solve this problem, a certain number of restorer plants, capable of pollinating the male sterile hybrid plants are grown together with them. **J.Leemans** (Gent, Belgium) reported results showing the high potential of gene technology in hybrid production. He described the isolation of a tapetal specific gene from tobacco and the identification of the region essential for tissue specific expression. The 5´region of this gene can be used to direct expression of a ribonuclease specifically in the tapetum in transgenic tobacco plants leading to male sterile plants. The same promoter region was used to drive tapetal expression of a specific ribonuclease inhibitor. Plants carrying this chimaeric gene can be used in crosses to pollinate the engineered male sterile plants. The expression of this gene and the production of the ribonuclease inhibitor restored male fertility in the offspring of these crosses.

The molecular and genetic analysis of homeotic mutants in animal systems has led to deep insight into the underlying regulatory principles of development. Similarly, the understanding of plant development has taken a big step forward with the cloning of flower homeotic genes. **H. Sommer** (Köln, Germany) reported the molecular cloning of three homeotic genes from *Antirrhinum majus*: *deficiens*, *globosa* and *squamosa*. Mutations in *deficiens* and *globosa* lead to homeotic transformation of petals to sepals

and stamens to carpels, while mutational inactivation of *squamosa* causes the production of shoots instead flowers. The strategy used to clone *deficiens* was a combination of transposon mutagenesis and differential screening of a cDNA library enriched by subtraction for flower specific messages.The *globosa* and *squamosa* genes were cloned by virtue of their homology with the deficiens gene. The proteins encoded by the three genes are putative transcription factors : they have a conserved domain at the N-terminus in common that displays extensive homology to two known transcription factors, SRF of mammals and MCM1 of yeast. The conserved putative DNA-binding domain (the so-called **MADS-box**) is present in twelve additional genes, the majority of which is expressed only in floral organs. MADS-box genes might be essential to specify organ identity in the process of organogenesis. An additional output of the differential screening of subtracted libraries was the isolation of several putative target genes ( *tap1*, *tap2* and *fil1*) of deficiens and /or other MAD-box genes in *A. majus* as reported by **J.P.Beltrán** (Valencia, Spain). The promoters of these genes contain motives similar to the targets of MAD-box containing proteins (i.e. serum response elements, SRE). **E. Lifschitz** (Haifa, Israel) reported the isolation, using as a probe the deficiens gene of *Antirrhinum*, of six genes from tomato that constitute the MADS-box gene family of tomato. He reported also the isolation of tomato gene markers common to all floral organs (*common denominator* gene markers). The identification of regulatory elements in these genes could help to characterize the presumptive floral program. **E.S. Coen** (Norwich, UK) reported the molecular cloning of the *floricaula* gene from *Antirrhinum*. Plants carrying the *floricaula* mutation cannot make the transition from inflorescence to floral meristems and have indeterminate shoots in place of flowers. The strategy used in this case was transposon tagging. This homeotic gene, required for flower development, encodes a putative protein containing a proline-rich N-terminus and a highly acidic region. *In situ* hybridization shows that the *floricaula* gene is transiently expressed in the very early stages of flower development. It was proposed that *floricaula* interacts in a sequential manner with other homeotic genes affecting floral organ identity.

Homeotic genes affecting the identity of meristems as well as floral organ homeotic genes have also been described in *Arabidopsis thaliana*. **G. Haughn** (Saskatoon, Canada) presented the characterization of two homeotic loci that control identity of floral meristems ( *leafy*) and carpels ( *stud*) respectively. Phenotypic analysis of *leafy* mutants and double mutants ( *leafy*, *apetala 2* ) indicated that the product of the *leafy* gene is required for the transition from floral meristem to inflorescence, in an analogous way as the function of *floricaula* gene in *Antirrhinum* . Similar analysis of mutants under stud locus indicated that the *std 1* product is required for correct formation of carpels and that it could act by inhibiting expression of *pistillata* and / or *apetala 3* in carpel primordium. Three groups of floral homeotic genes were presented by **E.M. Meyerowitz** (Pasadena, CA, USA). The first group is represented by the *apetala 2* gene, which mutation causes development of carpels in place of sepals and formation of stamen in the positions occupied by petals. The second group includes the *apetala 3* and *pistillata* genes, which mutation changes petals to sepals and stamens to carpels. The last group is represented by *agamous*, mutants of which have petals in the places where stamens are normally formed and sepals instead carpels. Meyerowitz

reported the molecular cloning of three homeotic mutants from Arabidopsis : *agamous* , *apetala 3*, and *leafy*. The cloning of *agamous* was facilitated by a T-DNA insertion mutation. Both, *agamous* and *apetala 3* ( highly homologous to the deficiens gene of *Antirrhinum*) belong to the MADS-box kind of regulatory genes. The *leafy* gene was isolated using as heterologous probe the *floricaula* gene from *Antirrhinum majus*. These similarities indicate that both floral and meristem homeotic genes have been conserved in sequence and function since the early stages of evolution of flowering plants.

Flowers produce anthocyanin pigments and alterations on the expression of either structural or regulatory genes of each pathway will result in modifications of flower coloration, an easily scorable trait. Therefore, flowers can be used as a tool to study the control of gene expression in plants. J.Paz-Ares (Madrid, Spain) reported the cloning and characterization of *Petunia* genes expressed in the flower related to the animal myb protooncogenes and to the C1 gene of *Zea mays* which regulates the anthocyanin biosynthetic pathway. He has shown that the product of one of these genes can bind DNA in a sequence specific manner and that the sequence which it binds is partially identical with that of animal c MYB protein. Since the similarities among each others of the plant MYB proteins is higher than between any plant MYB protein and animal MYB proteins, he suggested that the DNA binding sites of most of the plant MYB proteins will be very similar. C.Martin (Norwich, UK) however has shown that there are at least differences in the binding affinities of different *Antirrhinum* MYB proteins with the high affinity binding sequence of cMYB. Se also showed that regulatory genes of anthocyanin biosynthesis can exert different effects on different structural genes of the pathway. Thus, *Delia* appears to act as an activator of the four later steps in the pathway within the epidermal cells of the tube on the flower and to be a repressor of chalcone synthase gene within the mesophyll cells of the flower lobe.

R. Jorgensen (Davis, CA, USA) reported that transgenic Petunia plants transformed with a gene homologous to a *Petunia* endogenous one can result in the reversible suppression of both the transgene as well as the two alleles of the endogenous gene using the chalcone synthase gene as a model system. This fact termed co-suppression can result in flowers with highly regular non clonal coloration patterns which are subject to stochastic reprogramming events apparently occurring in the vegetative meristems.

Finally, H.Saedler (Köln, Germany) reported on the first field experiment performed in Germany with transgenic plants. Using a *Petunia* line transformed with a color gene of maize (the A 1 gene) under the control of 35 S promoter it was shown that the expression of the transgene was affected by both endogenous (flower position) and exogenous (temperature) factors.These changes in expression were caused by methylation. He suggested that methylation might be important in the switching of one developmental phase to another one.

In conclusion, considerable breakthroughs have been achieved in all of the four areas of research covered by the workshop. Some of the progress, as it is the case of the flower homeotic mutants cloned and characterized in the last two years, promises a very exciting near future to the field of flower development.

# LIST OF INVITED SPEAKERS

Workshop on

*FLOWER DEVELOPMENT*

List of Invited Speakers


Peter Albersheim - Complex Carbohydrate Research Center and the Department of Biochemistry. The University of Georgia. 220 Riverbend Road. Athens, GA. 30602 (USA). Tel.: 404 542 4401 Fax : 404 542 4412

José Pío Beltrán - Instituto de Agroquímica y Tecnología de Alimentos. C.S.I.C. 46010 Valencia (Spain). Tel.: 34 6 3690800 Fax : 34 6 3930001

Enrico Coen - John Innes Institute. John Innes Centre for Plant Science Research. Colney Lane. Norwich NR4, 7UH (U.K.).

George Haughn - Department of Biology. University of Saskatchewan. Saskatoon, Saskatchewan S7N 0W0 (Canada). Telf.: 306 966 4457 Fax : 306 966 4461

Jan Leemans - Plant Genetic Systems N.V. J. Plateaustraat 22. B-9000 Gent (Belgium). Tel.: 32 91 358 448 Fax : 32 91 240 694

Eliezer Lifschitz - Department of Biology. Technion-Israel Institute of Technology. Haifa 32000 (Israel). Tel.: 04 293959 Fax : 04 225153

Cathie Martin - John Innes Institute. John Innes Centre for Plant Science Research. Colney Lane. Norwich NR4 7UH (U.K.).

José Manuel Martínez-Zapater - Departamento de Protección Vegetal CIT-INIA. Ctra. de la Coruña Km. 7. 28040 Madrid (Spain). Tel.: 34 1 357 22 86 Fax : 34 1 357 31 07

Elliot Meyerowitz - Division of Biology 156-29. California Institute of Technology. Pasadena, CA. 91125 (USA). Fax : 818 4490756

Javier Paz-Ares - Centro de Investigaciones Biológicas.
C.S.I.C. Velázquez, 144.
28007 Madrid (Spain).
Tel.: 34 1 261 1800
Fax : 34 1 262 7518

Heinz Saedler - Max-Planck Institut für Züchtungsforschung. D-5000 Köln 30 (Germany).
Tel.: 49 221 5062100
Fax : 49 221 5062113

C.P. Scutt - Department of Biological Sciences.
University of Durham.
Durham DH1 3LE (U.K.).
Tel.: 091 374 2000
Fax : 091 374 3741

Hans Sommer - Max-Plank-Institut für Züchtungsforschung. D-5000 Köln 30 (Germany).
Tel.: 49 221 5062110
Fax : 49 221 5062113

Richard Thompson - Max-Planck Institut für Züchtungsforschung. D-5000 Köln 30 (Germany).
Tel.: 49 221 5062110
Fax : 49 221 5062113

K. Tran Thanh Van - Laboratoire Physiologie Végétale.
Centre National de la Recherche Scientifique.
91190 Gif-Sur-Yvette (France).
Tel.: 33 1 69 82 36 12
Fax : 33 1 69 28 86 59

# LIST OF PARTICIPANTS

Workshop on

*FLOWER DEVELOPMENT*

List of Participants

| | |
|---|---|
| Eduardo R. Bejarano | - Imperial College of Science. Centre for Biotechnology. London SW7 2AZ (U.K.)<br>Tel.: 071 589 5111 (Ext: 7094)<br>Fax : 071 225 8942 |
| Georges Bernier | - Laboratory of Plant Physiology. Université de Liège. B22 Sart Tilman. B-4000 Liège (Belgium).<br>Tel.: 3241 563830<br>Fax : 3241 563840 |
| Silvia Veronica Caffaro | - Department of Plant Physiology. Faculty of Biology. Complutense University. 28040 Madrid (Spain). |
| Luis Antonio Cañas Clemente | - Instituto de Agroquímica y Tecnología de Alimentos. C.S.I.C. Valencia (Spain).<br>Tel.: 34 6 3690800<br>Fax : 34 6 3930001 |
| Bart Den Boer | - Laboratorium Genetika. Ruksuniversiteit Gent. Gent (Belgium)<br>Tel.: 32 91 200195 Ext. 344/352<br>Fax : 32 91 220581 |
| Veronica Elsa Franklin-Tong | - School of Biological Sciences The University of Birmingham. Birmingham B15 2TT (U.K.).<br>Tel.: 021 414 5924<br>Fax : 021 414 5925 |
| José Antonio Godoy López | - Instituto de Recursos Naturales y Agrobiología de Sevilla. C.S.I.C. Sevilla (Spain).<br>Tel.: 95 462 4711<br>Fax : 95 462 4002 |
| Nick Harris | - Department of Biological Sciences. University of Durham. Durham DH1 3LE (U.K.)<br>Tel.: 091 374 2000<br>Fax : 091 374 3741 |
| Alicia Hidalgo Downing | - Madrid (Spain)<br>Tel.: 91 307 91 70 |

Richard Jorgensen - University of California. Davis, CA. (USA). Tel.: 916 758 0310 Fax : 916 758 5672

Helgard Kaufmann - Department of Plant Breeding and Yield Physiology. Max-Planck-Institut für Züchtungsforschung. Köln (Germany) Tel.: 0221 5062 440 Fax : 0221 5062 413

Victoria Marfà - Complex Carbohydrate Research Center. The University of Georgia. 220 Riverbend Road. Athens, GA. 30602 (USA). Tel.: 404 542 4401 Fax : 404 542 4412

C. Mariani - Plant Genetic Systems N.V. Gent. (Belgium). Tel.: 32 91 35 84 11 Fax : 32 91 24 06 94

Siegbert Melzer - ETH - Institut für Pflanzenwissenschaften. Zürich (Switzerland). Tel.: 01 256 22 11 Fax : 01 252 08 29

A.J.M. Peeters - Department of Exp. Botany. University of Nijmegen. Nijmegen (The Netherlands). Tel.: 080 61 Fax : 080 553450

José Luis Riechmann - Centro de Biología Molecular. C.S.I.C. Universidad Autónoma de Madrid. (Spain). Tel.: 34 1 397 50 70 - Fax : 34 1 397 47 99

Tim Robbins - DNAP - DNA Plant Technology Corporation. Oakland, CA. (USA). Tel.: 415 547 2395 Fax : 415 987 7445

José M. Romero - Instituto de Bioquímica Vegetal y Fotosíntesis. Universidad de Sevilla. C.S.I.C. Facultad de Biología. Sevilla (Spain) Tel.: 34 5 461 70 11 Fax : 34 5 462 01 54

Claus Josef Rossbach
- Institut für Genetik.
Universität zu Köln.
D-5000 Köln 41.
Köln (Germany).
Fax : 49 221 5062513

Joaquín Royo Carcamo
- Departamento de Bioquímica.
E.T.S.I. Agrónomos. Madrid
(Spain).

Luis Ruiz Avila
- Departamento de Genética Molecular. CID-C.S.I.C. Jordi Girona Salgado, 18-24. 08034 Barcelona (Spain).
Tel.: 34 3 204 06 00
Fax : 34 3 204 59 04

V.K. Sawhney
- Department of Biology. University of Saskatchewan.
Saskatoon, SK, S7N 0W0 (Canada).
- Tel.: 306 966 4400
- Fax : 306 966 4461

Frank Tetens
- Max-Planck-Institut für
Züchtungsforschung. D-5000
Köln 30 (Germany).
Tel.: 49 221 5062110
Fax : 49 221 5062113

Guy Vancanneyt
- Plant Gene Expression Center.
800 Buchanan Street.
Albany, CA. 94710 (USA).
Tel.: 415 559 5918
Fax : 415 559 5678

Miguel Angel Vega Palas
- Instituto de Bioquímica Vegetal y Fotosíntesis. C.S.I.C.
Sevilla (Spain).
Tel.: 34 5 461 70 11
Fax : 34 5 462 40 02

Bruce Veit
- Plant Gene Expression Center.
800 Buchanan Street.
Albany, CA. 94710 (USA).
Tel.: 415 559 5918
Fax : 415 559 5678

Oscar Vicente Meana
- Institute of Microbiology and Genetics. University of Vienna.
Althanstrasse 14
A-1090 Vienna (Austria).
Tel.: 1 31336
Fax : 1 31336-700

De Ye
- Inst. Molekulaire Biologie.
Vrije Univ. Brussel.
1640 St.-Genesius-Rode (Belgium).
Fax : 32 2 3590399

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**Caracterización molecular de las estructuras de colágeno.**

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240 **Genetic Strategies in Development.**
Symposium in honour of Antonio García Bellido. Lectures by S. Ochoa, S. Brenner, G. S. Stent, E. B. Lewis, D. S. Hogness, E. H. Davidson, J. B. Gurdon and F. Jacob.

244 **Course on Genome Evolution.**
Organized by E. Viñuelas. Lectures by R. F. Doolittle, A. M. Weiner/N. Maizels, G. A. Dover, J. A. Lake, J. E. Walker, J. J. Beintema, A. J. Gibbs, W. M. Fitch, P. Palese, G. Bernardi and J. M. Lowenstein.

246 **Workshop on Tolerance: Mechanisms and implications.**
Organized by P. Marrack and C. Martínez-A. Lectures by H. von Boehmer, J. W. Kappler, C. Martínez-A., H. Waldmann, N. Le Douarin, J. Sprent, P. Matzinger, R. H. Schwartz, M. Weigert, A. Coutinho, C. C. Goodnow, A. L. DeFranco and P. Marrack.

247 **Workshop on Pathogenesis-related Proteins in Plants.**
Organized by V. Conejero and L. C. Van Loon. Lectures by L. C. Van Loon, R. Fraser, J. F. Antoniw, M. Legrand, Y. Ohashi, F. Meins, T. Boller, V. Conejero, C. A. Ryan, D. F. Klessig, J. F. Bol, A. Leyva and F. García-Olmedo.

248 Beato, M.:
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249 **Workshop on Molecular Diagnosis of Cancer.**
Organized by M. Perucho and P. García Barreno. Lectures by F. McCormick, A. Pellicer, J. L. Bos, M. Perucho, R. A. Weinberg, E. Harlow, E. R. Fearon, M. Schwab, F. W. Alt, R. Dalla Favera, P. E. Reddy, E. M. de Villiers, D. Slamon, I. B. Roninson, J. Groffen and M. Barbacid.

251 **Lecture Course on Approaches to Plant Development.**
Organized by P. Puigdoménech and T. Nelson. Lectures by I. Sussex, R. S. Poethig, M. Delseny, M. Freeling, S. C. de Vries, J. H. Rothman, J. Modolell, F. Salamini, M. A. Estelle, J. M. Martínez Zapater, A. Spena, P. J. J. Hooykaas, T. Nelson, P. Puigdoménech and M. Pagès.

252 **Curso Experimental de Electroforesis Bidimensional de Alta Resolución.**
Organizado por Juan F. Santarén. Seminarios por Julio E. Celis, James I. Garrels, Joël Vandekerckhove, Juan F. Santarén y Rosa Assiego.

253 **Workshop on Genome Expression and Pathogenesis of Plant RNA Viruses.**
Organized by F. García-Arenal and P. Palukaitis. Lectures by D. Baulcome, R. N. Beachy, G. Boccardo, J. Bol, G. Bruening,

J. Burgyan, J. R. Díaz Ruiz, W. G. Dougherty, F. García-Arenal, W. L. Gerlach, A. L. Haenni, E. M. J. Jaspars, D. L. Nuss, P. Palukaitis, Y. Watanabe and M. Zaitlin.

254 **Advanced Course on Biochemistry and Genetics of Yeast.**
Organized by C. Gancedo, J. M. Gancedo, M. A. Delgado and I. L Calderón.

255 **Workshop on The Reference Points in Evolution.**
Organized by P. Alberch and G. A. Dover. Lectures by P. Alberch, P. Bateson, R. J. Britten, B. C. Clarke, S. Conway Morris, G. A. Dover, G. M. Edelman, R. Flavell, A. Fontdevila, A. García-Bellido, G. L. G. Miklos, C. Milstein, A. Moya, G. B. Müller, G. Oster, M. De Renzi, A. Seilacher, S. Stearns, E. S. Vrba, G. P. Wagner, D. B. Wake and A. Wilson.

256 **Workshop on Chromatin Structure and Gene Expression.**
Organized by F. Azorín, M. Beato and A. A. Travers. Lectures by F. Azorín, M. Beato, H. Cedar, R. Chalkley, M. E. A. Churchill, D. Clark, C. Crane-Robinson, J. A. Dabán, S. C. R. Elgin, M. Grunstein, G. L. Hager, W. Hörz, T. Koller, U. K. Laemmli, E. Di Mauro, D. Rhodes, T. J. Richmond, A. Ruiz-Carrillo, R. T. Simpson, A. E. Sippel, J. M. Sogo, F. Thoma, A. A. Travers, J. Workman, O. Wrange and C. Wu.

257 **Lecture Course on Polyamines as modulators of Plant Development.**
Organized by A. W. Galston and A. F. Tiburcio. Lectures by N. Bagni, J. A. Creus, E. B. Dumbroff, H. E. Flores, A. W. Galston, J. Martin-Tanguy, D. Serafini-Fracassini, R. D. Slocum, T. A. Smith and A. F. Tiburcio.

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