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**The Relation between Cell Division and Cell Type
Specification in Floral and Vegetative Meristems
of Tomato and *Arabidopsis***

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The relations between cell division and cell type specification in floral and vegetative meristems of tomato and Arabidopsis.

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ABSTRACT

Meristems were the central issue of our project. Genes that are required for cell division, cell elongation, cell proliferation and cell fate were studied in the tomato system. The analysis of the dUTPase and threonine deaminase genes, along with the dissection of their regulatory regions is completed, while that of the RNR2 and PPO genes is at an advanced stage. All these genes were isolated in our laboratory. In addition, 8 different MADS box genes were studied in transgenic plants and their genetic relevances discovered. We have also shown that a given MADS box gene can modify the polarity of cell division without affecting the fate of the organ. *In vivo* interaction between two MADS box genes was demonstrated and the functional dependency of the tomato *agamous* gene on the TM5 gene product established. We have exploited the *Knotted1* meristematic gene in conjunction with tomato leaf meristematic genes to show that simple and compound leaves and, for that matter, sepals and compound leaves, are formed by two different developmental programs. In this context we have also isolated and characterized the tomato *Knotted1* gene (*TKn1*) and studied its expression pattern. A new program in which eight different meristematic genes in tomato will be studied emerged as a result of these studies. In essence, we have shown that it is possible to study and manipulate plant developmental systems using reverse genetic techniques and have provided a wealth of new molecular tools to interested colleagues working with tomato.

Similarly, genes responsible for cell division, cell proliferation and cell fate were studied in *Arabidopsis* floral meristems. Among these genes are the *TSO1*, *TSO2*, *HANABA TARANU* and *UNUSUAL FLORAL ORGANS* genes, each affecting in its own way the number of pattern of cell divisions, and cell fate, in developing *Arabidopsis* flowers. In addition, new methods have been established for the assessment of the function of regulatory gene action in the different clonal layers of developing floral meristems.

ORIGINAL OBJECTIVES AND DEVIATION

We have shown that it is possible to manipulate floral development in tomato using reverse-genetic techniques. This was particularly important, since mutations that affect specific aspects of development are not available in most crop plants. During the course of this project we have isolated **eight** tomato MADS box genes and studied their developmental role in transgenic plants. Some of these genes, such as TM5, TM3 and TM8, were isolated first in tomato, and the results of their analysis evoked their characterization in other plant species as well. We have shown that, indeed, MADS box genes can modify the rate and orientation of cell division in floral meristems. To further analyse this particular issue, we have isolated the regulatory regions of the threonine deaminase gene and that of the dUTPase gene, and dissected the cis-acting elements *in vivo* in transgenic plants. It is now possible to study the effect of homeotic genes in tomato using legitimate tomato promoters. The analysis of the threonine deaminase promoter and the identification of its jasmonic acid response elements was already published. The discovery that the dUTPase promoter is operating in apical meristems was used to construct transgenes that express the meristem-specific and homeobox-containing gene *Knotted1* in these particular regions, as described in the body of the report. In order to follow cell divisions in meristematic regions, we have also isolated the tomato gene coding for the small subunit of the ribonucleotide reductase and confirmed by *in-situ* hybridization that it is regulated by the cell cycle in floral and vegetative meristems. The recent isolation of the promoter of this gene will permit, in the future, to study tissue-specific regulation of the cell cycle in various organs. Analysis of a variety of transgenes involving RNR2 has already begun.

About two years ago we realized that the understanding of meristems requires the analysis of features common to both floral and vegetative programs. We have, therefore, initiated the study of the *Knotted* genes in tomato, and demonstrated that at least two of the five genes isolated so far are operating in both floral, inflorescence and vegetative meristems. The description of the morphogenetic effect of the *Knotted1* gene on tomato leaves in a variety of meristematic mutants is discussed in more detail in the extended report

Evidently, we have gathered, in a relatively short time, with a very humble budget and limited manpower, a wide range of molecular and genetic tools that promise important results in the future as well. It is important to state that the multifacial research that we have developed also encouraged us to organize a cooperative research program, in the form of the Tomato Genome Project, that was recently granted major support from the Ministry of Science.

RELEVANT DATA, RESULTS AND DISCUSSION

Introduction

The major goal of our research is to understand the developmental pathways that shape the morphology of plant organs. Since the fate of plant organs is determined in their meristems we have used several molecular and genetic approaches to study the tomato meristems. In one approach that was discussed in detail in a previous report we have analysed the dUTPase, PPO and RNR2 genes. In a parallel experimental program we have isolated and studied homeotic genes of the MADS box family. Eight different genes of this class were analysed in transgenic plants, their developmental expression patterns determined, and their morphogenetic roles elucidated. Both the MADS box genes and the flowers and meristem-specific gene markers were isolated by virtue of their molecular characteristics, and their developmental role was studied by reverse genetic approaches. During the past two years the study of an additional major pathway in which meristematic homeotic genes affect both floral and vegetative meristems was initiated. The new set of meristematic genes was identified initially by the observed morphogenetic effects of their mutant alleles and the isolation of the genes is done by regular map-based cloning procedures. As will be evident from the description of the first results, the chosen gene mutations alter plant morphology by enhancing the rate and site of cell proliferation in meristematic regions.

The fate of plant organs is determined by developmental events in their respective meristems (see reviews by Walbot 1985; Sussex 1989; Medford 1992; Cutter 1971). All meristems are derivatives of the embryonic shoot or root meristems. They are composed of groups of actively dividing cells that, on the one hand, are conditioned to respond to developmental signals, and on the other, are already differentiated into domains with different potentials. Apical meristems can be classified, however, not only by the fate of the derivative organs but also by their own fate as meristems. Shoot apical meristems may be determinate or indeterminate; the determinate ones will eventually convert to inflorescence meristems, which in turn may be determinate or indeterminate, irrespective of the nature of the progenitor meristem. Determinate and indeterminate meristems may be found in the same plant, as in *Arabidopsis*, where the vegetative meristem is determinate but the inflorescence meristem is indeterminate. Mutations that convert an indeterminate inflorescence meristem into a determinate one, like *tfl* (Shannon & Wagner 1993), or a determinate floral meristem to an indeterminate one, like *agamous* (Coen & Meyerowitz 1991) were found and studied. Genes that are required for the modulation of the timing of determinacy in the form of early or late flowering are also known, of course, in both light-dependent and day-neutral plants.

All types of leaves, regardless of their eventual architecture, arise as dorsiventral appendages from the flanks of the shoot apical meristem. The dorsiventral nature of leaf primordia contrasts with axillary branches or stems which arise as radial primordia (Cutter

1971; Kaplan 1973). It is inferred, therefore, that developmental programs that shape the final forms of leaves are operating on a common background. We assume that different species, or a given plant in different stages or environmental conditions, use the same set of genes in order to shape the morphology of the leaf. Since the genes themselves are identical, we expect that the regulatory patterns in which leaf-form genes are operating will differ in different types of leaves.

In angiosperms, vegetative leaves come mostly in two basic arrangements: simple and compound (see Smith and Hake 1992).

Variations in leaf shape have been analyzed by genetic or anatomical means in maize, pea, cotton and a wide variety of other, and growth parameters of blade units in simple leaves have been studied by clonal analysis (Steeves and Sussex 1989 and Smith and Hake 1992, for review). However, at present no coherent developmental-genetic framework for leaf morphogenesis has been derived, and the pathways that determine a simple vs. compound leaf have not been investigated. Compound leaves have been considered to represent the reiteration of the program which makes simple leaves. **To study the molecular-genetic basis for this dichotomy between simple and compound leaves, we have exploited the maize homeobox containing *Knotted1* gene in conjunction with a range of mutations which alter the tomato compound leaf.**

The identification of *Knotted1* as a meristematic homeobox gene permitted, for the first time, molecular genetic analyses of leaf morphogenesis. Dominant mutations in the *Knotted* locus (hereafter *Kn1*) of maize result in formations of pocketed outgrowths (knots) on lateral veins, overall growth retardation, wider leaves, distorted patterns of lateral veins, disappearance of the ligule appendage and ectopic formation of sheath tissues on blades characterize the KNOTTED syndrome (Sinha and Hake 1994 and references therein). When the *Kn1* gene was misexpressed in dicot plant species with simple leaves, like tobacco and *Arabidopsis*, severe morphogenetic alterations were induced (Sinha et al. 1993; Lincoln et al. 1994). The leaves of these species turn lobed and rumpled, as well as shorter and wider and, in extreme cases, ectopic shoots appear on the main veins. Growth retardation and loss of apical dominance have also been observed. Significantly, and despite the excessive meristematic activities causing all the growth malformations, leaves of *Arabidopsis* and tobacco plants overexpressing *Kn1*, like those of maize *Kn1* mutant plants, remain simple.

The Tomato *Knotted* System

The prototype compound leaf is composed of a midvein (rachis), a terminal (distal) leaflet, 3-4 pairs of lateral petiolated and dented leaflets and some additional folioles that emerge between leaflets. Up to 2,000 leaflets, organized in compound reiterated units, are formed in tomato plants expressing *Kn1* under the control of the ubiquitous 35S promoter. Following this amazing observation we came to realize that the **simple vs. compound** problem

provides a new rationale for the experimental dissection of leaf development. Nine seemingly unrelated mutations were judged to alter the basic prototype of the compound leaf (the term “phyllomer” is suggested for convenience) and selected for further studies. We found that, as in wild-type, expression of *Kn1* in *Petroselinum* mutant plants, which are already subdivided to the third order, resulted in further subdivision. However, ectopic expression of *Kn1* in the simple leaves of *Lanceolate* mutant plants results in the same phenotypic manifestation as in tobacco or *Arabidopsis*. Furthermore, misexpression of *Kn1* in mutant plants where only two pairs (i.e., *potato-leaf*, *c/c*) or one pair (i.e., *trifoliate*) of lateral leaflets are allowed, indicated that *Kn1* can ramify the wild-type or a given mutant pattern but will not restore the wild-type arrangement in mutant plants and will not increase the complexity of the wild-type pattern (Hareven et al., in press).

We have also isolated the tomato *Kn1* ortholog (*Tkn1*) and showed that it is not identified by either *Pts* or *La* and that, unlike the *Arabidopsis* and maize genes, it is expressed in leaf and floral primordia (Hareven et al. 1996). Four more tomato *Knotted* genes were also isolated and are now being studied.

Fortuitously, leaves of all species in which misexpression of *Kn1* was previously examined (i.e., maize, tobacco, rice and *Arabidopsis*), were simple. The above experiments demonstrate that misexpression of *Kn1* affects compound leaves of tomato in a very different way than it affects simple leaves, and we argued that this seminal observation reflects inherent fundamental differences in the development of simple and compound leaves. This hypothesis implies that simple and compound leaves are determined by two different developmental programs, and that the gene systems that condition them are conserved among species with simple and compound leaves, respectively.

Working hypothesis

An attempt to develop an experimental framework for the genetic dissection of leaf arrangement requires the formulation of a likely developmental scenario, a judicious classification of the many genes involved, and their isolation and characterization. Formally, the formation of the prototype compound unit of tomato entails the establishment, in a regular basipetal pattern, of pairs of lateral meristems along the peg-like structure of the primary dorsiventral primordium, and the concomitant inhibition of lamina expansion. Additional orders of subdivision require the reiterated formation of lateral meristems on the secondary primordia before lamina expansion ensues. We speculate that the interplay between apical growth, formation of lateral meristems and activation of the diffused leaf meristems that condition the expansion of the lamina, determine whether a leaf will be compound or simple. As long as the primordium apex continues to “grow”, emanating signals that induce the formation of lateral meristems, and a coupled system delays the activation of the diffused meristem, the leaf will be compound. Maintaining this balance between the developmental programs allows for more

subdivisions to ensue. Presumably, an additional, species-specific developmental plan dictates the developmental window in which "active" cell types, i.e., apical, provascular, lateral lamina, are allowed to proliferate.

The inherent inability of simple leaves to respond "properly" to *Kn1* overexpression is reflected by ectopic formation of sheath tissues and "knots" in blade territories in maize (Sinha and Hake 1994) and irregular expansion of the lamina and the ectopic formation of shoots on tobacco and *Arabidopsis* leaves (Sinha et al. 1993; Lincoln et al. 1994). The developmental requirements for compoundness cannot be satisfied under the restrictions imposed by the "simple" program, and the *Kn1* gene product which enhances meristematic activity in leaves, affects only secondary growth parameters resulting in a variety of malformations. The reiterated ramification of the compound prototype unit of the tomato leaf is, according to this view, due to developmental programs that distinguish compound from simple leaves.

In accord with the proposed developmental scenario, we suggest that the recessive *potato leaf* and *trifoliate* mutations, as well as the dominant *Pts* and *Kn1* alleles, modify preferentially the balance between lamina expansion and lateral leaflet meristems. The *Lanceolate* dominant mutation subdues, preferentially, meristematic activities without which the potential for compoundness, and thus ramification, cannot be materialized (Hareven et al., in press).

Homeotic genes of the floral system

Two most important observations associated with the tomato *Agamous/TM5* gene system and with a newly discovered inflorescence gene were recently made. Initially, the TAG1 (tomato *agamous*) gene was chosen as a model system to assess the validity of the reverse genetic approach in tomato. This is because phenotypic consequences of inhibition or ectopic expression of *agamous* were studied thoroughly in *arabidopsis* and *antirrhinum* and its pivotal role in the organ identity programs established. Indeed, it was shown that the TAG1 gene is expressed in cells fated to differentiate into stamens and carpels and its expression becomes later restricted to specific cell types within these organs in analogy to the expression pattern in *arabidopsis*. The homeotic alterations conferred by the expression of TAG1 antisense RNA in all floral organs were, in general, in agreement with those caused by the legitimate *agamous* and *plena* mutations in their respective species. Third whorl stamens were converted into petaloid structures and fourth whorl carpels were replaced by pseudocarpels bearing indeterminate floral meristems with nested perianth flowers. A complementary phenotype was observed in transgenic plants expressing the TAG1 sense RNA in all floral organs under the transcriptional control of the ubiquitous 35S promoter. Here the first whorl sepals were converted into pericarpic leaves and sterile stamens replaced the second whorl petals. Although the overall pattern of phenotypic alterations is indeed what one expects of legitimate *agamous* mutations there are several important deviations from the analogous

phenotypes of TM5 antisense plants suggest that TM5 mediates two unrelated secondary regulatory systems. One system is the early function of the floral meristem identity genes, and the other system is the function of the major floral organ identity genes (Pnueli et al. 1994b).

Since the late effect of TM5 involves the tendency of organs in all whorls to acquire at least some sepal markers, we have tested the possibility that the TM5 product is required for the proper function of *agamous*. This was done by studying the interaction of the two genes in the two-hybrid system, by analysis of chimeric MADS box genes and genetic interactions between the two genes in transgenic plants (in preparation).

The results of these experiments clearly support the idea that TM5 is required for the proper function of *agamous*, but neither of the two genes directly control the transcription of the other. We have shown that it is possible, for example, to "disrupt" the interaction between the two gene products by introducing into the plant by adding a chimeric MADS box gene in which the *agamous* MADS box was replaced by a "neutral" MADS box shown before not to interfere in any way with the processes under study. Thus, misexpression of the *TM16:TAG1* gene fusion elicits no recognizable phenotypic alterations. Other combinations were reported in another context in Hareven et al. (1994), where the contribution of BARD was acknowledged.

Flower-specific markers and inflorescence genes

Since the last progress report we have completed three major tasks, all of them associated with the regulatory regions of the threonine deaminase (TD) dUTPase and RNR2 genes. Detailed analysis of the TD gene revealed an intricate developmental regulation pattern and we have decided to dissect its promoter region in order to obtain a parenchyma-specific promoter for the analysis of meristematic genes. In a recent publication (Samach et al. 1995) we have shown that the upregulation of TD in floral organs, the response to MeJa and the parenchyma-specific expression are all mediated by the cis-elements within the proximal 192bp. Similarly, we have shown that the proximal 240bp of the dUTPase promoter are sufficient for the meristem and provascular-specific expression and that the 380bp 5' fragment, as well as the 5' upstream 1200bp contain no enhancers or silencers of this activity. The RNR2 putative promoter region of 1300bp was identified, sequenced and subdivided into 4 fragments and constructs introduced into tomato plants. This analysis is not completed as yet.

The availability of two new characterized promoters permitted the analysis of the *Kn1* gene in two alternative developmental domains. For example, O. Coen showed that expression of the *Kn1* gene under the control of the dUTPase promoter is sufficient to elicit ramification of the tomato leaf and malformation of the tobacco leaf (in preparation).

Finally, we wish to summarize the work done thus far with respect to the structure and development of inflorescence meristems. Tomato inflorescence meristems are determinate, while those of *Arabidopsis* are indeterminate. A single gene mutation in tomato, *anantha*,

conditions the formation of cauliflower-like inflorescences, the result of uncontrolled ramification of floral meristems. In *Arabidopsis*, mutations in two genes, AP1 and CAL, were shown to condition exactly the same phenotype (Bowman et al. 1993). The *Arabidopsis* genes were isolated and shown to belong to a sub-class of MADS box genes, represented in tomato by TM4 (Pnueli et al. 1991). Since TM4 is not localized to the same chromosome as *anantha*, we have tried to isolate additional MADS box genes of this sub-family and to examine their role in the formation of the cauliflower-like inflorescence in tomato. Note that TM4, in either sense or antisense orientations has no effect on inflorescence structure (unpublished). Extensive screen resulted in the isolation by S. Yosef of two members of this sub-family, designated TM17 and TM18. The MADS box of TM18 is identical, residue by residue, to that of TM4, but the rest of the gene diverges slightly. TM17 is 88% similar to TM4 or APICAL along the gene. None of the two genes is located to the site of *anantha*, and the study of their effect in transgenic plants along with efforts to rescue *anantha* with the *Arabidopsis* AP1 and CAL genes continues.

In the meantime, we found two mutations which, in the double mutant plant, confer the formation of *anantha*-like inflorescence, i.e., endless ramification by bifurcation, but instead of floral meristems, completely normal leaves are formed. The analyses of inflorescence meristems and leaf meristems in tomato are now converged.

Meristematic Programs in *Arabidopsis*

Arabidopsis apical meristems consist of three layers: L1, L2 and L3. Cells in the L1 and L2 layers divide anticlinally while cells in the L3 layer divide both anticlinally and periclinally. During plant development, cells in different layers develop into different tissues and the developmental fate of a cell is determined by its position within the context of meristematic structures. Thus, mutations in genes required for establishing and maintaining meristematic architecture can also disrupt development.

Two recessive mutations that cause callus-like green flowers were isolated in a genetic screen. These two mutants were named tso. Complementation tests indicated that these two mutations identify two genes, *TSO1* and *TSO2*. While *tso2* exhibits defects in both flowers and leaves, *tso1* primarily affects flowers.

Scanning Electron Microscopy (SEM) analysis shows that *tso1* flowers produce 4 to 6 morphologically abnormal sepals and then produce large and abnormal cells in the center. These callus-like cells do not divide further, resulting in complete sterility. Thus, unlike floral homeotic mutations that specifically change floral organ identity but not cellular morphology, the *tso1* mutation causes abnormal cellular morphology, apparently by blocking cell division, and therefore blocks proper floral organ initiation and differentiation.

Using the Confocal Laser Scanning Microscopy (CLSM) we have observed that the *tsol* floral meristem sometimes lacks the properly organized L1, L2 and L3 layers. In addition, the nuclei of *tsol* flowers are irregular in size and shape. Furthermore, there are fewer cells in a young *tsol* flower than in a wild type flower at similar stages. These observations suggest that the primary defect of *tsol* is likely at cell division. Further evidence for this comes from microspectrophotometry of DAPI-stained *tsol* nuclei. There is much more than the 2C or 4C amount of DNA found in wild-type nuclei, indicating that the defect in the *tsol* mutant is failure of nuclear division and cytokinesis, but not failure of DNA replication.

Additional, careful studies used transmission electron microscopy. With thin sections of glutaraldehyde-fixed flowering apices, we observed that cell division in floral meristems becomes abnormal shortly after the time of floral specification. Characteristic of the cells in early (Stage 3) *Arabidopsis* floral meristems are incomplete cross walls, large nuclei with irregular boundaries (as contrasted to the spherical nuclei of wild-type cells), mitotic abnormalities that include disorganization of the metaphase plate of condensed chromosomes, and in some cells apparent failure of formation of a phragmoplast, and failure of fusion of the vesicles that contain cell wall material in the region where, in wild-type flowers, new cell walls would be forming.

The striking aspect of the mutant is thus that there is a cell and nuclear division deficit that is highly specific to floral meristems, starting just at the time when the floral organ identity are expressed (Stage 3).

By *in situ* hybridization, we found that the expression level of floral organ identity genes *AP3* and *AG* was decreased in *tsol* flowers. This may indicate a requirement for continued cell division in the maintenance of organ identity gene expression, or some other level of interaction between cell division and the specification of cell fate.

The *UFO* gene

Study of *Arabidopsis* flower development has provided insights into how genetic pathways function to establish complex patterns during plant development. More and more, we are seeing the interaction of pathways of differentiation with pathways of cell division control, and the *UFO* (*Unusual Floral Organs*) locus appears to be one point of connection between these two processes. We have used genetic and molecular approaches to characterize the *Arabidopsis* *UFO* gene. *UFO* is likely to function in (1) the determination of floral meristem identity, (2) the maintenance of floral determinacy, (3) the maintenance of boundaries between floral organs, and (4) the regulation of floral organ identity genes.

We have proposed these roles for the *UFO* gene based on the following evidence. First, *ufo* mutations may cause a partial conversion of floral meristems to inflorescence meristems

because there is an increase in the number of secondary inflorescences formed on the primary inflorescence, and a leaf subtends the first flower on about two-thirds of inflorescences. (In wild-type plants, a leaf subtends an inflorescence, but not a flower). Similar effects on floral meristem identity have been observed previously for mutants in the *LEAFY* gene. It appears that *UFO* functions downstream of, or at the same step as *LEAFY* in floral development, because the pattern of *LEAFY* expression is not altered in a *ufo* mutant and a strong leafy mutation is epistatic to a *ufo* mutation. Second, an agamous;*ufo* double mutant forms fasciated flowers indicating that the *UFO* gene has a role in controlling the proliferation of the floral meristem. Third, it seems that the *UFO* gene is involved in maintaining the borders between floral organs based on our observation that the second and third whorls of *ufo* mutant flowers often contain mosaic organs and/or fusions of two adjacent organs. Finally, *in situ* hybridization and antibody studies suggest that the expression of the *AP3* gene is reduced in *ufo* mutant flowers and this is consistent with the similarity seen in the identity of floral organs in *ap3* and *ufo* mutants.

In summary, the experiments indicate that *UFO* plays a role in regulating both organ identity and cell division in developing flowers.

HANABA TARANU

A final gene we have identified and are studying is *HANABA TARANU*, which causes there to be too few cells in developing shoot and floral meristems. In flowers the result of this is too few floral organs. The studies of this gene are in an early phase, but we can already say that double mutant studies show a series of interactions of *han* mutants with other mutations that affect cell division in growing meristems, including the *ufo* mutants.

New methods for mosaic studies

Finally, we have started to establish a new system for the formation of genetic mosaics in *Arabidopsis* meristems, to assess the relative contributions of activity of genes from the L1, L2 and L3 cell layers. We used the site-specific recombinase systems Cre/Lox (of phage P1) and Flp/Frt (of yeast) to generate genetic chimeras. These systems each contain two components: the first component is a site-specific recombinase (Cre and Flp) and the second component is the target site for the recombinase (Lox and Frt). Constructs that place the recombinase genes, Cre and Flp, under the control of the *Arabidopsis* heat shock promoter HSP18.2, have been made and introduced into plants. We have also made constructs that place *35S-GUS* between direct repeats of the target sequences, Lox and Frt. These have also been introduced into plants. Plants carrying these constructs have been analyzed for copy number and were crossed to generate F1 plants that contain both components of a system (i.e., Heat

shock-Cre with Lox constructs and Heat shock-Flp with Frt constructs). Exposure of these F1 plants to heat-shock conditions induce expression of the recombinase, which then acts on the two target sites to delete the intervening sequences. By controlling the level of induction, we have found conditions that result in excision in only a subset of cells, thus resulting in plants that are chimeric for *35S-GUS*. Excision is assessed by staining for *GUS* activity and monitoring white (non-staining) sectors. Analysis of these plants should allow us to understand the clonal relationship between cells of the inflorescence and flower meristem and the cells that are incorporated into the mature flowers. Through these studies, we hope to determine whether the *Arabidopsis* inflorescence meristem is organized into L1, L2 and L3 layers as has been described for other plants. We have also made a series of constructs that contain both *35S-GUS* and the *AGAMOUS (AG)* gene between target site direct repeats. Plants containing these constructs, and plants containing the heat shock-recombinase constructs, are being crossed into an agamous mutant background. Eventually, we will obtain an F1 plant in which the only functional *Ag* allele is located between the recombinase target sites. Heat-induction of the recombinase in these plants should result in excision of both the *35S-GUS* and the functional *AG* gene. Through analysis of flower development in these plants, we hope to assess cell autonomy of *AG* and identify the subset of cells in which *AG* expression is essential for normal differentiation, and therefore find the role of regulatory gene expression in each of the L1, L2 and L3 layers of developing floral meristems.

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DESCRIPTION OF THE COOPERATION

The cooperative results include the exchange of appropriate probes and gene promoters between the U.S. and Israeli labs, and visits between the labs for the exchange of conformation, materials, and for discussion of new results. The most recent U.S. to Israel visit was from Dr. Joshua Zvi Levin of the Caltech lab to the Technion in January 1994, and a visit by Prof. Eliezer Lifschitz, director of the Technion lab, to Caltech in January of 1995.

EVALUATION AND CONCLUSIONS

In tomato we have demonstrated that it is possible to use the reverse genetic approach to dissect complex developmental processes. By now we have isolated 17 genes and analysed their molecular and developmental properties. Eight MADS box genes, four regular genes (PPO, TD, dUTPase, RNR2) and thus far five homeotic genes of the *Knotted* family. All these genes were sequenced, genomic clones isolated and 5 promoters identified and tested in transgenic plants. Moreover, the detailed expression patterns of more than half of these genes determined by *in-situ* hybridization and other means, and the overexpression or inhibition of most of the genes were examined in tomato transgenic plants. All this material, as well as the expertise needed to execute the experiments were made available to many colleagues in Israel and abroad. The analysis of all these systems revealed several features unique to tomato, both with respect to flower development and meristematic programs in general. We have established that ortholog regulatory genes may play different roles in different species and thus lead to the phenotypic variations observed in the plant world. Of particular importance in this respect is our recent paper published in *Cell* on the genes that control the development of compound and simple leaves. This work presents a completely new approach to the analysis of leaf morphogenesis and may have many practical ramifications.

The results we have described dictate two future experimental lines. In the first one we will study mutations that suppress meristematic activity, such as *Lanceolate*, and mutations that modify the morphology of sepals. Sepals of tomato are simple leaves and, similar to foliage leaves, their venation pattern and areole size is altered in *Kn1*-expressing flowers. Unveiling the developmental pathways that convert the compound foliar leaf to the simple leaf of the calyx is complementary to the study of the simple vs. compound phenomenon in the *Lanceolate* system. In a second future line we will try to clone and study non-*Knotted* tomato genes which enhance meristematic activity in sepals and leaves, leading to the appearance of shoot initials on sepals to the ramification of the compound leaf, and to ectopic formation of shoots under a variety of conditions. Likewise, the recent discovery reported here for the first time of a gene system that converts every single floral meristem in a regular inflorescence to a single normal leaf will, we believe, be a basis for exciting experiments on flower development and the genes that regulate the conversion of putative leaf meristems in the inflorescence to floral meristems.

Publications in which the support of BARD was acknowledged

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