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Constructing a Petal

Pattern formation in multicellular organisms is a sequential process: successive patterning events cause the formation or delineation of distinct regions or primordia that then undergo further patterning. Floral patterning, for instance, involves not only the specification of the identities of the floral organ primordia but also the elaboration of these primordia into mature, fully formed sepals, petals, stamens, and carpels. Although much has been learned about the processes that occur within the floral meristem and lead to the initiation of floral organs, many questions remain about later steps in organ development. That is, after organ primordia form, how are different regions and cell types then specified during organogenesis?

One organ in which to examine pattern formation during organogenesis is the petal. The petal is a relatively simple structure, but in some plants, including tobacco, Antirrhinum, and petunia, the corolla is divided into two distinct regions, a fused tube region and an unfused limb (or lobe, in Antirrhinum) region. This obvious morphological difference is presumably a reflection of patterning events that occur early in petal development. In fact, the bases of the individual petals fuse to form the tube at a very early stage in petal development, while the tips, which will form the limb regions, remain separate. By this stage, therefore, the cells of the petal must "know" whether they are destined to contribute to tube or limb. Another regional difference within petals can be found in the pattern of anthocyanin pigmentation, which is often coupled to the tube/limb division. The limb of tobacco, for instance, is pigmented, whereas the tube is unpigmented. The entire Antirrhinum flower is normally pigmented, but the lobe becomes pigmented slightly earlier than the upper part of the tube. Mutants of both Antirrhinum and petunia have been found that restrict pigmentation to certain regions of the petal (Wiering and de Vlaming, 1984; Martin et al., 1987).

Two major approaches can be taken to identifying the regulatory molecules that lead to region-specific gene expression in the petal (or in any organ that shows clear regional differentiation). One approach is genetic: mutations that change petal morphology in a defined way or that alter the expression patterns of genes normally expressed in limited regions of the petal may define molecules that are involved in petal pattern formation. For instance, mutants in which the global pattern of anthocyanin pigmentation is disrupted may result from lesions in genes that control petal region–specific gene expression. Mutants have also been identified with obvious changes in petal structure: in the petunia mutant *blind*, the limb region is eliminated and replaced with antheroid structures that crown the tube (Gerats, 1991). In addition to pinpointing potential regulatory genes, mutant phenotypes can give a sense of the range of patterns or fates that are possible for cells in an organ to adopt as well as revealing regional domains that may be under common—or distinct—genetic control. For instance, the *blind* mutant shows that the organ identities of the tube and limb regions are not necessarily linked.

Although the genetic approach may yield important and interesting regulatory genes, it has significant limitations. The principal problem is that if a spatial regulator of petal gene expression also has an essential function much earlier in development or plays a role in the initiation of petal primordia, it will rarely if ever mutate to change the pattern of petal gene expression. Moreover, there is no guarantee that genes for which mutant alleles alter petal morphology or the pattern of anthocyanin biosynthesis are those that actually establish the basic coordinates of the petal. Such genes may function far downstream of the actual pattern formation process.

A complementary approach to finding the molecules that specify the petal pattern is to isolate genes that are expressed in specific regions of the petal and use biochemical techniques to learn how the differential expression of these genes is achieved. From comparing the promoters of genes expressed in the same regions of the petal, a common, potentially regulatory, motif may emerge; it should then be possible to isolate a protein that binds to this motif. The distribution and regulation of this protein may, in turn, point the way to even earlier events in petal patterning. The spatial and temporal expression patterns of differentially expressed genes may also yield clues about the general features of pattern formation in the petal and may reveal subdivisions of the petal that are not apparent from morphology or pigmentation alone.

The biochemical approach avoids the major limitation of the genetic approach—any regulatory molecule, even one that has a function at another time in development, can, in theory, be identified. But this approach poses its own problems, among them determining whether a protein that binds to the promoter of a regionally expressed gene actually dictates the expression pattern in vivo. And, as with molecules identified genetically, those identified biochemically may well respond to, but not establish, the petal pattern.

The genetic approach has been especially fruitful for studying the spatial regulation of anthocyanin biosynthesis in Antirrhinum, in which a large collection of transposon-induced pigmentation pattern mutants has been identified (Martin et al., 1987). The best characterized of the genes that are required for the normal pigmentation pattern of the Antirrhinum corolla is *Delila*, loss-of-function mutations of which abolish tube but not lobe pigmen-

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tation. The *Del* gene has recently been sequenced, and it appears to encode a helix-loop-helix protein of the *myc* type (Goodrich et al., 1992). DEL's closest relatives are the members of the maize R gene family, which also regulate anthocyanin biosynthesis. The *del* phenotype arises from a dramatic reduction in the expression of four structural genes for anthocyanin biosynthesis in the tube, but not the lobe (Almeida et al., 1989; Martin et al., 1991), and an obvious possibility is that DEL binding to these genes boosts their transcription.

All of the genes that require *Del* for tube expression encode enzymes that catalyze late steps in anthocyanin biosynthesis. By contrast, the genes for three enzymes that act earlier in the biosynthetic pathway, including chalcone synthase (CHS), are expressed at normal levels in both the tube and lobe of *del* mutants (Martin et al., 1991). Detailed studies of the cell type specificity and regional expression patterns of both the early and late genes reveal that the four late genes are expressed in identical spatial and temporal patterns (Jackson et al., 1992). Their RNA levels peak just before the buds open, and their transcripts accumulate in the epidermal layers, reaching their highest concentrations in the inner epidermis of the lobe and the outer epidermis of the base of the tube. Thus, these four late genes are probably coordinately regulated. Although CHS gene expression in the tube is independent of *Del* and its transcript accumulates earlier than those of the four late genes, it shows an identical spatial expression pattern to those genes.

The phenotype of *del* mutants suggests that DEL plays a role in establishing spatial patterns of gene expression in the petal. However, DEL is clearly not the only regulator of pigment pattern in the Antirrhinum corolla. *Del* is not required for activation of the anthocyanin biosynthesis genes in the lobe, which implies that there exists a lobe-specific regulator of these genes. Moreover, the spatially heterogeneous expression of CHS in the tube arises not through the action of *Del*, but from that of some other regulator. These observations suggest that *Del* does not itself establish the basic coordinates of the petal pattern; instead, it responds to or interprets an underlying prepattern that is established by the action of other genes. By studying the regulation of expression of *Del* and other, as-yet-unidentified, region-specific regulators, it should be possible to learn more details of the mechanisms that establish spatially restricted gene expression in the petal.

In this issue, Drews and coworkers (pages 1383–1404) describe the use of the alternative, molecular approach to examine gene regulation during petal organogenesis. They isolated putative limb- and tube-specific genes from tobacco by constructing cDNA libraries for each of these two regions from fully developed petals. They then screened these libraries with limb and tube cDNA, respectively, and, to eliminate genes whose expression is not specific to the corolla, counter-screened with cDNA representing pooled mRNA from other organs. Of the petal-specific cDNAs, half correspond to RNAs that accumulate to about equal levels in the limb and tube and half to RNAs that accumulate at higher levels in the limb. None represents RNAs expressed preferentially in the tube region. This may indicate that tube identity is a basic or default state and that additional genetic information is required to specify the limb. The authors also used a parsley CHS cDNA probe to isolate a CHS genomic clone from tobacco; the CHS gene also turns out to be expressed at higher levels in the limb than the tube, which is not surprising, given that CHS catalyzes an early step in anthocyanin biosynthesis.

To further investigate the expression patterns of these genes, the authors hybridized several of the limb-specific probes to corolla sections in situ. Most of the genes are expressed in both the inner and outer layers of the petal tip epidermis; however, one of the genes is expressed only in the parenchyma cells of the mesophyll layer. This observation is particularly interesting because there is no morphological evidence for any difference between tube mesophyll and limb mesophyll.

Despite the similar tissue specificity of most of the limb-specific genes, they show distinct temporal expression profiles. The mRNA levels of all the limb-specific genes examined increase throughout most of corolla development, but with different kinetics. Therefore, these genes are subject to diverse, as well as common, regulatory influences. In this respect, they are unlike the late anthocyanin biosynthesis genes of Antirrhinum, which do appear to be regulated coordinately in the corolla.

Although all of the limb-specific genes were identified in a cDNA library representing transcripts present in the petals of mature, open flowers, most of their transcripts begin to accumulate at low levels early in petal development. In at least one case, that of CHS, gene expression is regionally specific early in petal development. After petal fusion has taken place but when there are few apparent morphological differences between the incipient petal regions, CHS is expressed at higher levels in the upper region of the corolla than the lower. This indicates that very early in petal development, when the limb-specific genes (at least CHS) are activated, regulatory processes have already subdivided the corolla into distinct regions.

Whatever the mechanism by which limb-specific gene expression is achieved, it apparently operates, at least in part, at the transcriptional level. The authors show that this is the case by comparing the transcription rates of the limb-specific genes with the steady state levels of RNA: the relative RNA accumulation roughly parallels the transcription rate in the two regions of the corolla and in different organs. Promoter–GUS fusions indicate that cell type specificity within the limb is subject to transcriptional regulation as well. Therefore, although post-transcriptional processes may influence RNA accumulation to some extent, the organ-, region-, and cell-specific expression patterns of many of these genes arise mainly through transcriptional regulation. This result suggests

that differentially expressed or active DNA binding proteins probably dictate the regional specificity of gene expression in the petal and further implies that these regulators can be identified as proteins that bind to the promoters of the limb-specific genes.

Although each approach has its limitations, genetic and molecular analysis are both likely to yield molecules that confer spatial asymmetry to gene expression in the petal. However, it is in the very early events of petal development that the initial asymmetries that dictate the pattern of the petal are formed. As Drews and coworkers show, the major cell differentiation events occur shortly after the petal primordia emerge from the floral meristem. The events that occur much later—the cell type– and region-specific expression of the genes identified by Drews and coworkers and the anthocyanin biosynthesis genes—are ultimately under the control of these early events. An exciting challenge for the future will be learning how early petal asymmetries are established and how these processes are integrated into the pattern elaboration that occurs later in petal development.

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Petunia Flowering Revisited

This letter is in response to the PLANT CELL issue of August 1992 in which a report on the Amsterdam meeting on Floral Development was presented (Plant Cell **4,** 867–870). We would like to point out some unfortunate inaccuracies that were stated in the section on Flower Architecture.

First, the suggestion was raised that the petunia MADS box gene *green petal* (*gp*, cloned by Anil Kush at Rockefeller University, New York) and *fbp1* (cloned by Angenent et al. in Wageningen, Holland) are the same gene. This is not true; we did report the cloning of another MADS box gene, *pGlo-like*, which is very similar to *fbp1*. Both *pGlo-like* and *fbp1* share homology to the Antirrhinum MADS box gene *globosa*. The *gp* gene shares homology with the Antirrhinum MADS box gene *deficiens-A* (within and outside the MADS box region).

Both the meeting report and the cover title of the August issue suggest that *fbp1* and *fbp2* have a homeotic function during floral development. These genes are expressed in specific floral organs and are likely to play important roles during petunia floral development. However, a true homeotic function, e.g., that mutations in these genes cause a homeotic transformation of floral organ identity, has not been demonstrated. At this point it is premature to assign a homeotic function based solely on (partial) homology to a homeotic gene that has been identified in another plant species.

The meeting report also mentioned that the *green petal* mutant phenotype has been obtained by antisense-mediated suppression of the petunia *gp* gene. This was not reported at the meeting. We did present data that show that the *green petal* phenotype can be obtained by cosuppression of the *gp* gene.

We also have one comment that is related not to the meeting report but to Figure 1 of the paper by Angenent et al. (Plant Cell **4,** 983–993). There is a discrepancy between the DNA and amino acid sequence from nucleotides 582 to 663 of the *fbp2* gene.

We hope your readers will benefit from these corrections.

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