Transcriptional Networks Controlling Plant Development

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The rise of developmental biology to the fore of modern biology can be traced back to the efforts of three people: Ed Lewis, Christiane Nüsslein-Volhard, and Eric Wieschaus. Lewis had been studying the homeotic Bithorax gene complex in fruitfly (*Drosophila melanogaster*) for several decades and came to the conclusion that the genes in this complex control body pattern along the anterior-posterior axis (7). This seminal insight, published in 1978, was followed 2 years later by another landmark publication in which Nüsslein-Volhard and Wieschaus described the results of a mutant screen from which they had isolated several classes of mutations that affected specific elements of the larval body pattern (14). Previous to this work it was generally believed that most developmental regulators would control a wide spectrum of activities, and that disrupting their function would lead to pleiotropic phenotypes that would be very difficult to interpret. With the development of recombinant DNA techniques around the same time, it took only a few years until many of the genes identified by Lewis, Nüsslein-Volhard, and Wieschaus could be studied at the molecular level. One of the first lessons was that many of the early acting genes encode transcription factors that are part of a network of regulatory interactions. For example, the maternal morphogen BICOID, a homeodomain protein, activates the zygotic gap gene *HUNCHBACK*, which encodes a zinc finger factor. Gap proteins, which are expressed in broad domains, in turn regulate pair rule genes, which are expressed in periodic stripes. Gap and pair rule proteins are required for region-specific expression of homeotic genes such as *ULTRABITHORAX* and *ANTENNAPEDIA*, founding members of the homeobox family of transcriptional regulators. In addition to hierarchical interactions, there are also many cross-regulatory and autoregulatory interactions among gap, pair-rule, and homeotic genes. It is intriguing that although much has been learned about the regulation of the regulators, the question of how homeotic proteins subsequently specify position-dependent elaboration of different organs has largely remained a mystery.

Inspired by *Drosophila* sp., several scientists began in the mid-1980s detailed studies of plant mutations that caused specific developmental defects, with much of the initial focus on mutations that changed floral organ pattern. It was initially unclear whether any of these were strictly homeotic mutations, as most of them caused more complex phenotypes than only organ transformations. For example, *agamous (ag)* mutants in Arabidopsis show a conversion of stamens into petals, but also produce a new flower where the carpels normally form in the center of the flower. Furthermore, whereas homeotic mutations in the fly show a consistent directionality with anterior larval segments transformed into more posterior fates, a similar regularity was not apparent in the floral homeotic mutants, since there were changes from central to more peripheral fates, as in *ag*, and from peripheral to more central fates, as in the stamen-to-carpel conversions seen in *apetala3* mutants. Thus it was a major breakthrough when Elliot Meyerowitz and Enrico Coen proposed the ABC model, according to which the combinatorial activity of three classes of homeotic genes specifies the four major types of floral organs (3) (Fig. 1).

At the time the ABC model was proposed, it was not known how the floral homeotic genes controlled floral organ identity, but when the first two were cloned in the laboratories of Meyerowitz and Heinz Saedler (18, 20), it turned out that they encode transcription factors and that they are expressed in specific domains of the flower. As with animal homeotic genes, most floral homeotic genes belong to a single gene family, but instead of homeodomain proteins they encode MADS domain proteins. Moreover, as is the case for the animal homeotic genes, plant homeotic genes encode developmental switches, and ectopic expression experiments showed that they are both necessary and sufficient to confer specific organ fates (11, 13). Another similarity with the animal homeotic genes is the multitude of cross- and autoregulatory interactions among floral homeotic genes.

Although the floral homeotic genes were cloned about a decade ago, we still know little about the molecular basis for the region-specific expression of floral homeotic genes. For example, although the early acting transcription factor LEAFY (LFY) has recently been demonstrated to be a direct regulator of several homeotic genes including *AG* (2), we still do not understand why *AG* is normally activated only in a subset of LFY-expressing cells. Several transcrip-

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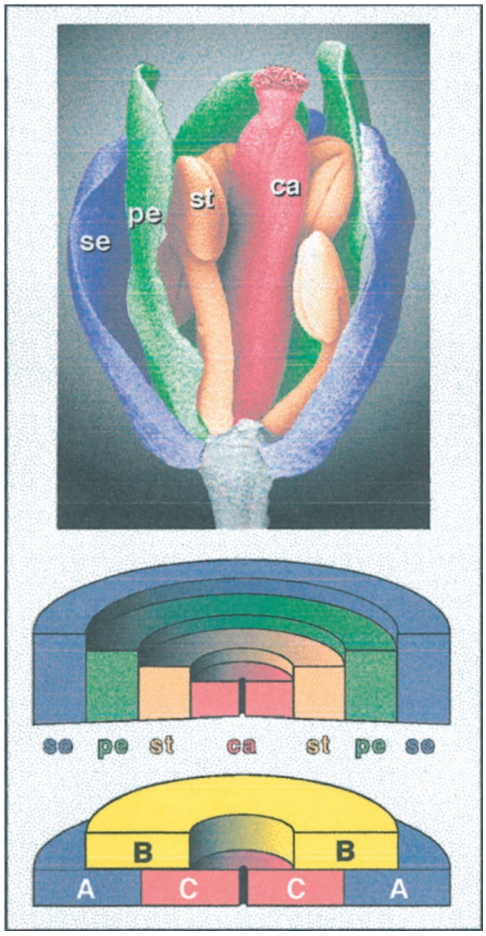

Figure 1. Top, Scanning electron micrograph of a nearly mature Arabidopsis flower, with sepals (se), petals (pe), stamens (st), and carpels (ca) color-coded. Middle, Schematic half flower. Bottom, The ABC model, showing the domains of A, B, and C homeotic activities in wild type. A function alone specifies sepals; A plus B petals; B plus C stamens; and C alone carpels. The MADS box gene *APETALA1* is expressed in the A domain, the MADS box gene *APETALA3* and *PISTILLATA* in the B domain, and the MADS box gene *AG* in the C domain.

tion factors that prevent precocious and ectopic expression of *AG* have been identified, but it is not known how their effects are integrated, and how repression by these factors is overcome in the center of the stage 3 flower.

As with animal homeotic genes, it has been difficult to identify downstream effectors of homeotic genes by genetic screens, although molecular approaches show some promise (15). Most progress has been made in understanding the later events during carpel development. One of the most intriguing observations has been that *AG* controls some aspects of carpel development by activating two redundant genes, *SHATTERPROOF1 (SHP1)* and *SHP2*, whose sequences are very closely related to that of *AG*, indicating that they evolved through gene duplication of *AG*. The *SHP* genes, in turn, are negatively regulated by another MADS domain protein, FRUITFULL (8). However, not all floral patterning is due to a single hierarchy of transcription factors. For example, expression of *ETTIN*, which encodes a member of the auxin response factor (ARF) family of plant-specific transcription factors and which plays a key role in apical-basal patterning within the gynoecium, is independent not only of *AG*, but also of the floral identity gene *LFY* (17).

Whereas the largest body of work to date is on floral development, a growing network of interactions between transcription factors controlling root development is becoming apparent as well. In the late 1980s and early 1990s several groups began to apply genetic analyses to formation of the Arabidopsis root, which, because of its simple organization, was particularly attractive for studying tissue patterning. Nevertheless, in contrast to floral development for which horticultural varieties and phylogenetic comparisons provided some idea of what sorts of mutations one might obtain from a screen, there was no basis for predicting what kind of phenotypes were possible for the root.

One of the first screens, conducted by John Schiefelbein in Chris Somerville's laboratory, was for alterations in root hair formation (16). Subsequent screens have uncovered mutations that dramatically affect the radial pattern of the root, as well as root meristem function. The root epidermis consists of adjacent files of hair cells and non-hair cells, and several mutations that cause the ectopic formation of root hairs on non-hair cells or the loss of root hairs from hair cells have been found. Molecular analysis of genes that control the number or spacing of root hairs has identified several transcription factors, which appear to form a regulatory network. *GLABROUS2* (*GL2*) encodes a homeodomain protein that is expressed in non-hair cells where it is required to maintain the non-hair fate (12). *GL2* in turn is activated in non-hair cells by the MYB type transcription factor WEREWOLF (WER; 6) and is repressed in hair cells by another MYB factor, CAPRICE (CPC) (19). It is surprising that not only *WER*, but also *CPC* RNA is expressed in non-hair cells, suggesting a non-cell autonomous mode of action for CPC. MYB factors often function as heterodimers with bHLH transcription factors, and evidence for the role of a bHLH transcription factor in root hair patterning comes from the observation that overexpression of the maize bHLH factor R in Arabidopsis roots results in a lack of root hairs (9).

Underlying the epidermis are several tissues that make up the radial organization of the root, including the endodermis and cortex, both of which derive from the same set of stem cells. Two genes are required for the generation of these two tissue types from common precursors, *SHORT-ROOT (SHR)* and *SCARECROW (SCR)*, and mutations in these cause endodermis and cortex to be replaced by a single tissue layer. A recurring theme is that these two

genes, which affect a common developmental process, are also related in sequence, both encoding members of the GRAS family of putative transcription factors (4, 5). *SHR* is required for the transcriptional activation of *SCR*, but *SHR* RNA is expressed in the tissues internal to the endodermis in which *SCR* is expressed, again indicating a non-cell autonomous mode of regulation (5).

The analysis of these factors has already revealed some new insights. Although it was generally thought that the shoot and root meristems share only superficial similarities, recent findings indicate that the transcription factor networks that control epidermal and radial patterning in the root perform a similar function in the shoot (1). This argues for evolutionary homology, suggesting that one meristem was derived from another or that ancient patterning processes predated the origin of either meristem and that root and shoot meristems incorporated these primitive regulatory systems.

What we have described above provides a glimpse of the importance of transcription factor networks in development. To understand how these networks work in detail we ultimately need to know the temporal and spatial expression patterns of all transcription factors. Such knowledge, in conjunction with information on loss- and gain-of-function phenotypes, in turn, will allow prediction of new interactions, as has been demonstrated already in yeast. However, knowing simply the RNA expression patterns of transcription factors may not be enough. Although plants have animal-like systems for cell-to-cell signaling via secreted molecules, plants can go further and make use of transcription factors in direct cell-to-cell signaling, as first proposed 5 years ago by Bill Lucas and Sarah Hake (10). Thus plant transcription factor networks may after all turn out to be quite different from those in animals.

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