Patterning the female side of *Arabidopsis*: the importance of hormones

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Received 26 June 2006; Accepted 6 September 2006

Abstract

The study of floral organ development has been a driving force in plant developmental biology research for the last two decades, and there is now an enormous wealth of information about the genetic networks underlying the specification of floral organ identity and the acquisition of its final morphology and function. These and parallel studies on leaf morphogenesis and development have made evident the common evolutionary origin of all plant lateral organs and the recurrent use of variations in the regulatory circuits involved in the shaping of leaves and flowers. This review summarizes the latest progress on the study of the development of the gynoecium, the female reproductive organ of the flower, stressing the connections with the developmental programme of leaf morphogenesis, and highlighting the common role of hormonal cues in these processes.

Key words: Auxin, carpel, floral organ, gynoecium, lateral organ development, morphogenesis, patterning.

Introduction

We all like flowers: they can be beautiful, attractive and inspiring in many ways, not only for poets and lovers but also for scientists, who have been interested for centuries in unravelling the mystery behind floral organ identity and patterning. But if something defines a flowering plant it is the carpel, the ovule-bearing organ of the flower. In fact, the very term angiosperm comes from the Greek and means ‘seeds enclosed in a vessel’ (*angion*, vessel, and *sperma*, seed). In general, one or more carpels make up the gynoecium, the female reproductive structure and most complex organ of the flower. The gynoecium provides protection for the ovules, helps to discriminate between male gametophytes, and facilitates successful pollination. After ovule fertilization, the gynoecium develops into a fruit, the main functions of this specialized organ being seed protection and dispersal.

Goethe’s early hypothesis that floral organs are ‘metamorphosed’ vegetative leaves has been heavily quoted in the last two decades, as the genetic networks controlling floral organ identity have progressively been revealed. Almost definitive support for it has come from the complete transformation of floral organs into leaves in the quadruple sepallata1 (sep1) sep2 sep3 sep4 mutants, or the ectopic conversion of vegetative leaves into floral organs by the expression of *SEP* and floral homeotic genes (Honma and Goto, 2001; Pelaz et al., 2001). Thus, it is widely accepted that carpels have evolved from modified leaves, probably sporophylls, the gametophyte-bearing leaves, although different theories about the ancestral type of sporophyll involved and how they evolved into a closed gynoecium are still subject to debate (reviewed in Scutt et al., 2006).

Most of our current knowledge on the molecular genetics leading to gynoecium development comes from genetic and molecular research in the model plant *Arabidopsis thaliana*. Comprehensive reviews describing carpel morphogenesis and fruit development in *Arabidopsis* have been published recently (Dinneny and Yanofsky, 2005; Roeder and Yanofsky, 2005). Our aim is to complement those, summarizing the latest progress in this rapidly expanding field, where new genes with a role in carpel development and new roles for known genes have been described recently. An attempt has been made to look into the carpels from the leaf hidden behind, stressing the parallels in leaf and carpel development and highlighting the role of hormonal cues in gynoecium patterning now that molecular genetics are rapidly changing the view on hormone action in relation to development.

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The basic plan in lateral organs

If leaves can be transformed into floral organs by expression of the floral organ identity genes, the distribution of tissues in the floral organ, that is, patterning, must be underlying the basic plan of the leaf, and possibly further refined down-stream in the floral organ identity genes. Accordingly, most of the mutations that affect floral organ patterning also affect the basic development of leaves and all lateral organs.

So, what is known about lateral organ patterning? In the last few years, huge progress has been made in unravelling the genetic networks underlying leaf initiation and development. Several reviews have been published recently that provide an in-depth approach to this field of plant development (Tsukaya, 2003; Byrne, 2005; Fleming, 2005). The aim in this section is to give a schematic picture of the basic plan of lateral organ development in Arabidopsis, that will be used later as the scaffold to build ‘our’ gynoecium.

Lateral organ primordia are produced in the periphery of the shoot apical meristem (SAM). SAM maintenance is mainly controlled by several related class-I KNOX homeobox transcription factors (Hake et al., 2004). Down-regulation of KNOX genes occurs at positions for lateral organ primordia initiation, and auxins have been proposed as a major factor influencing this process. In the SAM, auxin efflux facilitators such as PINFORMED (PIN) direct auxin flux to sink positions, where they reach high concentrations, pinpointing the location of primordium emergence (Reinhart et al., 2000). In these auxin peak positions, the KNOX genes are excluded, and ‘boundary’ genes such as the CUP-SHAPED COTYLEDON (CUC) genes delimit the primordium borders. Concomitantly, auxin transporters appear to change their intracellular polar localization, redirecting auxin flux to a new position in the meristem (Heisler et al., 2005). KNOX gene repression in the emerging primordium is maintained by the heterodimer formed by the transcription factors ASSYMMETRIC LEAVES1 (AS1), of the Myb family, and ASSYMMETRIC LEAVES2 (AS2), which encodes a LOB domain protein. Conversely, AS1 and AS2 are excluded from the SAM by the KNOX proteins (reviewed by Byrne, 2005). In addition to auxin, other plant hormones are important for inducing primordia differentiation. Thus, low levels of cytokinins (CK) and high levels of gibberellins (GAs) are needed to induce a differentiated state (Jasinski et al., 2005). KNOX proteins appear to control hormonal balance in the SAM to maintain the undifferentiated state, inducing CK biosynthesis and reducing the level of active GAs (Frugis et al., 2001; Hay et al., 2002; Yanai et al., 2005). In addition to the determination of primordia initiation, auxin distribution in the developing leaf appears to be an important factor controlling final shape and size, as well as venation patterning. It has been proposed that directed transport of auxin established the developing leaf primordial tip as an auxin sink (Reinhart et al., 2003), thus generating a proximodistal auxin gradient with its maximum at the tip of the developing primordium (Benkova et al., 2003), which could provide positional information for midvein development (Zgurski et al., 2005). As they develop, primordia would become auxin sources, auxin biosynthesis taking place first at the leaf tip and then at the margins (hydathodes). This proposed sink–source transition coincides with the lateral growth of primordia and the formation of the secondary veins (Avsian-Kretchmer et al., 2002; Aloni et al., 2003). The symmetric pattern of auxin foci at the leaf margin has been postulated to direct symmetric blade expansion and vascular development, and, interestingly, auxin sources in the leaf primordia are asymmetrically positioned in as mutants (Aloni et al., 2003; Zgurski et al., 2005).

Dorsoventrality, that is, development along the abaxial–adaxial axis, is immediately specified in the emerging primordium. It has been proposed that this polarity requires a signal provided by the SAM, of yet unknown nature, as primordia microsurgically separated from the SAM develop as radial abaxialized structures (Reinhart, 2005). The genes involved in establishing this polarity are expressed in their corresponding adaxial or abaxial domains from primordia inception, although it is still not clear how this polar expression is first established. Adaxial identity is primarily conferred by members of the class III homeodomain-leucine zipper (HD-ZipIII) transcription factors PHABULOSA, PHAVOLUTA, and REVOLUTA, which also have a role in SAM initiation and maintenance (Emery et al., 2003; Green et al., 2005; Prigge et al., 2005), with the partially redundant help of AS1 and AS2. Abaxial fate specification is mainly carried out by two groups of genes expressed in abaxial positions: the KANADI genes, encoding putative transcription factors of the GARP family (Eshed et al., 2001; Kerstetter et al., 2001) and several YABBY genes such as FILAMENTOUS FLOWER (FIL), YABBY2 (YAB2), and YABBY3 (YAB3) (Eshed et al., 1999, 2004; Sawa et al., 1999; Siegfried et al., 1999). Recently, ANTIGUAMENTA (ANT), a gene encoding a transcription factor of the AP2/ERF family and one of the earliest genes expressed in lateral primordia founder cells has been postulated as a positive regulator of both abaxial and adaxial factors because of the mixed abaxial and adaxial characters of both leaf surfaces caused by the loss of ANT function in a yabby mutant background (Nole-Wilson and Krizek, 2006). Later, in the developing primordium, the antagonistic action of abaxial and adaxial genes and a prominent role for microRNA-dependent transcriptional regulation define both domains and strengthen the maintenance of polarity in the developing leaf. Thus, KAN genes negatively regulate the adaxial HD-ZipIII genes, which, in addition, are targeted by microRNAs (miRNAs) (Engstrom et al., 2004; Williams and Fletcher, 2005; Grigg et al., 2006). The auxin response
factor encoding genes ETTIN (ETT) and ARF4 have been shown to promote abaxial development in parallel with the KAN genes (Pekker et al., 2005) and to be regulated by trans-acting siRNAs (ta-siRNAs) (Fahlgren et al., 2006). The role of Auxin Response Factors in abaxial specification suggests a possible role for auxin in the adaxial/abaxial patterning process. This hypothesis is strengthened by the higher presence of AUX1, an auxin influx facilitator, in the abaxial epidermis of the leaf (Reinhardt et al., 2005), which could result in the formation of an abaxial–adaxial gradient of auxin concentration in the leaf.

In addition to the adaxial/abaxial axis, leaves also show a proximal/distal axis of asymmetry. In the Arabidopsis simple leaf, proximal positions are occupied by a narrow petiole, while the leaf blade develops at the distal end (Fig. 1B). Growth of the leaf primordium into a flat expanding structure depends initially on the activity of a short-lived marginal meristem (Donnelly et al., 1999), and it has been proposed that the juxtaposition of the abaxial and adaxial domains could direct the specification of this marginal meristem (Waites and Hudson, 1995). Subsequently, leaf growth depends on a diffuse cell proliferation, and the final shape is acquired by the combination of cell division and differences in polar cell elongation. In contrast to the increasing knowledge on the specification of the adaxial/abaxial domains, a clear picture of the genetic network controlling development in the proximal–distal axis has yet to emerge, although significant advances have been made recently when mutations that specifically affect the blade or the petiole domains have been described. Thus, the JAGGED (JAG) gene encodes a C2H2 zinc-finger transcription factor involved in the maintenance of cell-division activity in the growing leaf (Dinneny et al., 2004; Ohno et al., 2004). In jag loss-of-function mutants, distal growth of all lateral organs is suppressed, resulting in short and serrated organs. Constitutive expression of JAG leads to ectopic blade formation in the proximal end of the leaf, a similar phenotype to that caused by the loss of function of the BLADE ON PETIOLE (BOP1 and BOP2) genes.

In the leaf primordium, JAG and the BOP genes are expressed in non-overlapping domains: JAG in distal positions and BOP at the proximal end. From genetic analyses, it appears that the BOP proteins restrict JAG expression to the distal part of the developing leaf, limiting cell division to this domain. BOP proteins also appear to act co-operatively with AS1 and AS2 in repressing KNOX gene expression in the emerging leaf primordia (Ha et al., 2003; Hepworth et al., 2005; Norberg et al., 2005).

In summary, complex robust genetic networks ensure the maintenance of the meristematic-differentiated, adaxial–abaxial or proximal–distal dichotomies (Fig. 1). Cross-talk and common regulators are multiple in these pathways, as well as the general co-ordination provided by the interplay of hormonal balances, with auxin as a major morphogen.

The Arabidopsis gynoecium

The Arabidopsis gynoecium is derived from two congenitally fused carpels that appear as a single primordium in the centre of the flower, around stage 5 of flower development (according to Smyth et al., 1990). Subsequently, a central invagination forms and the primordium elongates as an open hollow cylinder (stages 6 to 8). At later stages, two opposing internal meristematic outgrowths form at medial positions, which, in turn, produce the placenta and ovules laterally and fuse in the centre to form the septum (Fig. 2A, B). Shortly before anthesis, the developing gynoecium closes at its apical end and the first signs of tissue differentiation are visible. At anthesis, all the tissues required for fertilization have fully developed and those required for fruit maturation and dehiscence are already present, but will develop further after fruit set (Bowman et al., 1999; Roeder and Yanofsky, 2005).

Figure 2C depicts the different regions of a mature gynoecium along the different axis of development. In the apical–basal axis, from top to base: a single cell layer of papillar cells or stigma; a short, solid style; the ovary, papillar cells or stigma; a short, solid style; the ovary,
which contains the ovules and spans most of the length of the gynoecium; and, basally, a short stalk-like structure, the gynophore, which attaches the ovary to the flower base. A transverse section of the ovary shows the arrangement of tissues in the medial–lateral axis: the valves correspond to the two carpel walls and are placed in lateral positions; they end in a column of smaller cells, forming a longitudinal crease, called the valve margin. In medial positions the fused margins of the carpels are found, as well as the abaxial side of the septum or replum, the post-genitally fused septum and derived ovules. The transmitting tract, a specialized tissue that facilitates pollen tube growth, runs along the entire length of the gynoecium from the stigma and through the centre of the style and the septum. All these medial tissues, together with the apical style and stigma, are collectively termed marginal tissues as they arise from the margins of the fused carpels. These marginal tissues clearly show adaxial–abaxial polarity. Abaxial positions are occupied by the replum, which forms a narrow stripe between the valve margins, whereas the septum, the transmitting tract, placentae, and ovules, are adaxial (Fig. 2B, C).

In mature fruits, different tissues have developed to ensure dehiscence, that is, the shattering process that opens the pod and frees the seeds to facilitate dispersal. The region that develops at the valve margin, sandwiched between the valves and the replum, is called the dehiscence zone (DZ). The DZ comprises a separation layer of small cells, which defines a longitudinal plane of rupture at both sides of the replum, and a patch of adjacent lignified cells. The valve internal subepidermal cell layer (or endocarp) is also lignified, and when the mature fruit dries, it provides, together with the lignified patch at the DZ, mechanical tensions that facilitate pod opening (Ferrándiz, 2002).

Four longitudinal major veins run through the gynoecium, two lateral and two medial. The lateral veins are placed at the centre of the valves and terminate at their apical end, being ontogenetically related to the middle vein in the leaf. The medial veins run through the replum and bifurcate extensively where valve and style meet, forming vascular fans in the lateral plane of the style. The medial veins are highly lignified and it is possible that they could also contribute to the dehiscence process.

Genetic factors controlling gynoecium development

During the last few years, several mutations affecting gynoecium development in Arabidopsis have been identified. Some of them primarily caused the loss of carpel identity, while others affected the differentiation of specific tissues within the gynoecium. The cloning of the...
corresponding genes and the determination of their expression patterns showed that, in general, loss of function mainly affected those domains where the gene was expressed. From these studies and analyses of genetic interactions it was suggested that most of the different tissues developed quite independently (Bowman et al., 1999; Ferrándiz et al., 1999). However, as will be seen later, it is becoming increasingly clear that the functional connections among them are stronger than anticipated and that development of the different compartments along the main axes of development shares many common regulators and is largely co-ordinated.

Most of the genes affecting gynoecium development also have a role in leaf patterning. Thus, the genetic networks that have previously been summarized for lateral organ development are essentially working in the same way in the two carpels that form the Arabidopsis gynoecium. Interestingly, many of these genes with a role in both gynoecium and leaf pattern were first identified by their phenotypes in gynoecium development, suggesting that redundancy must be more robust in leaves and, possibly, reflecting the shorter evolutionary trajectory for the acquired roles of these genes in carpel development.

From the leaf to the carpel: identity genes

As discussed above, and confirming the prediction of the widely accepted quartet model for floral organ identity (Jack, 2001; Krizek and Fletcher, 2005), a leaf can be transformed into a carpel by expressing the corresponding organ identity genes, specifically AGAMOUS (AG) and one or more of the SEPALLATA (SEP) genes (Honma and Goto, 2001). Conversely, simultaneous loss-of-function of the redundant SEP genes leads to a complete absence of carpel development, which in place develop as leaf-like organs (Ditta et al., 2004; Pelaz et al., 2000). ag mutants also lack carpels completely, although due to the homeotic transformations caused by the expansion of A-function genes when AG activity is lost, carpels are transformed into sepals (Bowman et al., 1989). Both AG and SEP are members of the MADS box family of transcription factors, and they are already expressed at the inception of the carpel primordia, before any morphological sign of differentiation (Yanofsky et al., 1990; Savidge et al., 1995; Mandel and Yanofsky, 1997). However, in spite of the pivotal role of AG in carpel identity specification, most carpel-associated tissues, with the exception of valve-like tissue, can still develop in the absence of AG activity, such as, for example, in the ectopic first whorl organs of the double apetala2 (ap2) ag mutant (Bowman et al., 1991). This fact led to the proposal that additional factors could specify, at least partially, carpel identity in an AG-independent pathway. Such factors have turned out to be a pair of completely redundant and highly similar MADS box genes, SHATTERPROOF1 and 2 (SHP), closely related to AG and primarily involved in specifying valve margin identity (see below; Liljegren et al., 2000). Thus, all carpelloid features disappear in the ap2 ag shp1 shp2 quadruple mutant. Complementation studies have shown that SHP and AG proteins are largely equivalent at the functional level, and that their distinct roles mostly derive from their different expression patterns (Pinyopich et al., 2003).

Two additional factors involved in the AG-independent carpel identity pathway are SPATULA (SPT) and CRABS CLAW (CRC). SPT encodes a bHLH transcription factor and is widely expressed in different specific tissues throughout vegetative and reproductive development (Heisler et al., 2001), while CRC belongs to the YABBY family but is exclusively expressed in nectaries and carpels (Bowman and Smyth, 1999). spt mutants show defects in the development of most carpel specific tissues (see below), whereas crc gynoecia are shorter and wider than wild type and partially unfused at the top. The crc spt gynoecium develops as two unfused organs with very reduced amount of ovules and of stigmatic and stylar tissue and, furthermore, loss of SPT and CRC function in the ap2 ag background mimics the phenotype of ap2 ag shp1 shp2 mutants (Alvarez and Smyth, 1999).

The precise hierarchy leading to carpel identity is not completely understood, although some hints have been revealed in the last few years. AG is activated in the floral meristem by the joint action of the products of the floral meristem identity gene LEAFY and the meristem maintenance homeobox gene WUSCHEL (Lenhard et al., 2001; Lohmann et al., 2001). Different studies placed the SHP genes downstream of AG, but this appears to be only partially true since, as seen in ap2 ag mutants, they can be activated independently of AG activity. Recently, FIL, YAB3, and JAG, present both in leaves and carpels, have been shown jointly to activate SHP (Dimmeny et al., 2005; see below). Thus, SHP might be placed at the top of the carpel identity AG-independent pathway, and both AG and SHP could then, directly or indirectly, activate SPT and CRC independently (Fig. 3A).

Setting up territories

Once organ identity is specified, the gynoecium primordium becomes partitioned in different domains. Adaxial–abaxial patterning is established at the early stages of development. As for leaves, the KAN genes become initially expressed in abaxial domains together with the Auxin Response Factors ETT and ARF4 (Sessions et al., 1997; Kerstetter et al., 2001; Pekker et al., 2005). Leaf adaxial YABBY genes such as FIL or YAB3 are also restricted to abaxial domains in the carpel primordia, together with CRC, another member of the family that is not expressed in leaves (Bowman and Smyth, 1999; Siegfried et al., 1999). Single mutants in any of these loci show weak phenotypes related to adaxialization, but mutant combinations lead to strong polarity defects,
indicating that all these functions act together to specify abaxial fate. Conversely, the HD-ZIP genes are expressed in their expected adaxial positions. Although the effect of both gain-of-function and loss-of-function mutations in gynoecium development has not been described in detail, it appears to be milder than in leaves, perhaps indicating that additional factors could have a more prominent role in directing adaxial fate (McConnell and Barton, 1998; Alvarez et al., 2006). A strong candidate for such activity has been identified recently. NUBBIN (NUB) is a C2H2 zinc-finger transcription factor closely related to JAG. While JAG is expressed in all lateral organs in a non-polar manner, NUB is restricted to the adaxial side of leaves and, in the flower, of stamens and carpels. nub single mutants do not show obvious phenotypic defects, but jag nub double mutants exhibit severe defects in organ growth and the abaxialization of stamens and carpels. Based on careful characterization of the expression patterns of polarity genes, it has been proposed that NUB/JAG activity could work downstream or in parallel to the KAN/YAB/HD-ZIP network, although it is still unclear whether the role of NUB/JAG in promoting adaxial fate is direct or reflects a requirement of correct organ growth to maintain organ polarity (Dinneny et al., 2006).

A significant particularity of carpel morphogenesis is the early onset of mechanisms directing polarity in the gynoecial medial–lateral axis and subsequent partition of the gynoecium primordia into lateral and medial regions. The lateral domains later develop into the valves, while the medial domains give rise to the marginal tissues. The specification of the medial–lateral axis is clearly reflected by the early specific expression of different genes in lateral or medial regions. The medial domain retains certain meristematic identity, possibly needed to differentiate the full complement of medial tissues in later stages. Accordingly, several of the KNOX genes involved in SAM maintenance are expressed in this domain, as well as the ‘boundary’ genes CUC1 and CUC2 (Long et al., 1996; Aida et al., 1997; Ori et al., 2000; Pautot et al., 2001). Because of the severe developmental defects in mutants for most meristem maintenance genes, usually unable to form flowers, the precise role of these genes in gynoecium development is largely obscure. However, the importance of meristematic activity in medial regions is suggested by the phenotype of flowers produced out of calli regeneration of cuc1 cuc2 mutants, where marginal tissues fail to develop fully (Ishida et al., 2000).

Interestingly, many of the genes directing adaxial–abaxial polarity also appear to be at work in the establishment of medial–lateral domains. FIL, YAB3, and CRC, the abaxial YABBY genes, are excluded from the medial domains in early stages of gynoecium development, although CRC later becomes expressed also in the abaxial medial regions (Bowman and Smyth, 1999; Siegfried et al., 1999). Specific expression in lateral domains is also observed for JAG and NUB (Dinneny et al., 2004, 2006). CRC, JAG, and NUB activities in the lateral domains are important to maintain growth in these regions, that later will develop into the valves. In contrast to the YAB genes, the expression pattern of KAN1 initiates in the abaxial side of gynoecium primordia but, intriguingly, this expression domain switches to the medial zones at later stages.

**Fig. 3.** (A) Model for genetic interactions directing the specification of valves (green), valve margins (blue), and replum (orange), according to Dinneny et al. (2005). (B) Model for patterning along the apical–basal axis, based on the auxin gradient hypothesis (Nemhauser et al., 2002). Auxin levels (represented in red) would be highest in apical regions, directing stigma and style formation; intermediate levels would promote ovary development; and low levels towards the base would direct gynophore formation. Synthesis of auxin at source positions could be under the control of STY/SHI genes, while translation of auxin levels input into tissue differentiation could be mediated by the action of SPT and ETT in their respective domains.
(Kerstetter et al., 2001). kan1 kan2 mutants show an extreme phenotype, with adaxial marginal tissues such as ovules and transmitting tract developing all over the abaxial side of the gynoecium, indicating that, in addition to the adaxialized phenotype, the medial–lateral boundaries are lost, and therefore suggesting that KAN activity could be also be required to maintain lateral domain specification (Eshed et al., 2001). However, even with these emerging clues, we are still far from understanding how the medial and lateral domains are specified. An appealing hypothesis is that genetic networks that maintain the SAM and lateral organ boundaries are translated to the gynoecium context, and both antagonistic and co-operative activities fix the position of medial and lateral territories. Some recent work supports this view. Thus, the homeobox gene REPLUMLESS (RPL, aka BELLRINGER, PENNYWISE, and VAAMANA) interacts with class-I KNOX factors in the SAM to maintain the undifferentiated meristematic fate (Byrne et al., 2003; Smith and Hake, 2003; Bhatt et al., 2004). RPL is also expressed in the gynoecium medial domains from the early stages of development, where it has been shown to repress FIL, YAB3, and JAG activities (see below; Roeder et al., 2003; Dinneny et al., 2005). Interestingly, AS genes are also expressed in carpels, as well as some class I KNOX genes (Byrne et al., 2000). Our own unpublished results indicate that AS activity could have a role in restricting class I KNOX gene expression in the lateral domains of the gynoecium, thus maintaining the antagonistic relationship observed in SAM-lateral organ primordia (H Alonso, JJ Ripoll, I Ochando, A Vera, C Ferrándiz, A Martinez-Laborda, unpublished data).

Development of the lateral domains

The lateral domains differentiate into the valves, including the valve margins that eventually will develop into the dehiscence zones. Major factors directing valve margin formation have been studied for some time (reviewed in Dinneny and Yanofsky, 2005; Ferrándiz, 2002). shp1 shp2 double mutants do not differentiate the dehiscence zones and therefore shp1 shp2 mutant fruits do not shatter at maturity (Liljegren et al., 2000). Similar indehiscent phenotypes are found in mutants in INDEHISCENT (IND) or ALCATRAZ (ALC), two additional transcription factors of the bHLH family (Rajani and Sundaresan, 2001; Liljegren et al., 2004). Somehow opposite phenotypes are observed in fruitfull (ful) mutants, where the small lignified cells typical of dehiscence zones are ectopically found in place of valve cells, and, as a consequence, fruits do not elongate properly and prematurely break out from the internal pressure of the growing seeds (Gu et al., 1998). Accordingly, SHP, IND, and ALC, which in wild-type fruits are expressed in the valve margin, are ectopically expressed in ful mutant valves (Ferrándiz et al., 2000; Liljegren et al., 2004). Similarly, in rpl mutants, the replum (the abaxial medial region of the ovary) disappears, taking valve margin identity and expressing the corresponding valve margin genes (Roeder et al., 2003).

A beautiful solid model has been put forward recently that explains the precise development of valves and valve margins in the ovary and accounts for the phenotypes and genetic interactions observed (Fig. 3A; Dinneny et al., 2005). According to the model, the co-operating activities of FIL, YAB3, and JAG positively regulate the transcription of the MADS-box gene FUL and the SHP genes in the valve and the presumptive valve margin, respectively, in such a way that high levels of FIL/JAG activity in the valves would activate FUL expression while the transcription of SHP genes only would require the weaker FIL/JAG activity present in the valve margins. The FUL product, in turn, represses SHP gene expression, reinforcing the division of FUL-expressing valve and SHP-expressing valve margin territories (Ferrándiz et al., 2000). In the replum, RPL blocks FIL/JAG activity, therefore preventing SHP activation (Roeder et al., 2003; Dinneny et al., 2005). Thus, by the action of FUL and RPL, SHP activity is restricted to a narrow strip of cells sandwiched between the valve and the replum, where it promotes the expression of IND and ALC. These two activities, IND and ALC, are, ultimately, the main factors responsible for the correct differentiation of tissues in the dehiscence zone (Rajani and Sundaresan, 2001; Liljegren et al., 2000, 2004).

Marginal tissue development

Marginal tissues, such as replum, septum, transmitting tract, placenta, style, and stigma, are derived from the medial regions of the gynoecial primordium. Several genes are involved in the specification of these different tissues, as deduced from the phenotypes associated with mutations in these genes. As discussed previously, meristem-associated genes are expressed in these regions, although whether they are required for marginal tissue development or not is largely unknown due to the non-flowering phenotype of most of the corresponding mutants.

No single mutation has been identified so far that completely blocks marginal tissue formation. One of the most severe disruptions corresponds to mutations in the transcriptional co-repressor LEUNIG (LUG). lug mutant gynoecia are partially unfused at the apical end, forming horn-like projections at the top of the valves, and exhibit defects in septum fusion and ovule development. Again, LUG activity is not restricted to gynoecium development, but is also involved in leaf blade expansion and the negative regulation of AG in the first and second whorls of the flower (Liu and Meyerowitz, 1995; Conner and Liu, 2000; Cnops et al., 2004). Mutations in ANT, a further gene involved in leaf blade growth through maintenance of cell proliferation in lateral primordia, cause similar though weaker defects in gynoecium development, and have been also regarded as an AG repressor in the outer whorls of
the flower (Krizek et al., 2000). Marginal tissue development is fully dependent on the co-operative activities of ANT and LUG as ant lug double mutants completely lack replum, style, septum, and placental tissues (Liu et al., 2000). A third factor appears to contribute also to this pathway. Like LUG, SEUSS (SEU) encodes a transcriptional co-repressor, and seu mutants show similar though weaker defects in gynoecium development as lug mutants. LUG and SEU proteins are able to interact physically and they have been proposed to act co-operatively to regulate their targets throughout plant development (Franks et al., 2002; Sridhar et al., 2004). In fact, the double mutant shows severe developmental phenotypes, such as a strong reduction of flower size and plant height, a significant decrease in floral organ numbers and floral organ homeotic transformations. Usually, the fourth whorl organs of the flower fail to develop, but first whorl organs are carpelloid, although aberrant, developing as filamentous organs with no trace of marginal tissues. In addition to ANT/LUG/SEU, several lines of evidence point to YABBY genes as additional players in this pathway. First, fil ant double mutants carpels resemble those of both ant lug and lug seu mutant combinations (Nole-Wilson and Krizek, 2006). Second, FIL also appears to be a negative regulator of AG in the outer whorls of the flower (Chen et al., 1999). Third, the Antirrhinum LUG orthologue STYLISH1 (STY) has been shown to interact physically with YABBY proteins (Navarro et al., 2004). Finally, a further functional connection has been recently reported: LUG, SEU, and ANT appear to be involved in maintaining abaxial–adaxial polarity by positively regulating FIL expression and/or activity (Franks et al., 2006; Nole-Wilson and Krizek, 2006). However, the big question remains as to whether the phenotypes described above could reflect a role for ANT/LUG/SEU/YAB in specifying medial tissue identity, or, alternatively, could be the consequence of an arrest of the proliferative capacity of the medial region, unable then to grow and differentiate.

Putative downstream effectors of the ANT/LUG/SEU/YAB pathway are the members of the SHI gene family, encoding zinc-finger transcription factors that exhibit an unusual degree of functional redundancy. STYLISH1 (STY1) is the only one of them that shows subtle defects in style development when knocked out. Mutations in the other nine members of the family do not show phenotypic defects in carpel development or elsewhere, but gradually enhance styl phenotypes when two or more mutations are combined with styl loss of function. Multiple mutants in styl and other SHI-related genes have strongly reduced style and stigmatic tissues, unfused gynoecia in the apical end and incomplete sepal. In addition, they exhibit defects in leaf development, such as serrated margins and partial loss of abaxial–adaxial polarity, resembling lug mutants and also very similar to those observed in multiple mutants in the ANT/LUG/SEU/YAB loci (Fridborg et al., 1999; Kuusk et al., 2002, 2006). lug mutations are epistatic over sty phenotypes and, moreover, overexpression of STY1 is able to rescue lug mutant phenotypes, placing STY1/Shi genes genetically downstream of LUG. 35S::STY1 also causes ectopic development of style-like epidermal cells and the proliferation of style-like vascular fans along the valves, may be favouring the idea that STY1/Shi genes are involved in the specification of style identity instead of merely controlling cell proliferation. However, these possibilities are not mutually exclusive, since 35S::STY1 plants also have an additional leaf phenotype, with ectopic blades developing at the petioles. STY/Shi proteins have been proposed to mediate auxin signalling (see below), hence providing an additional functional link between STY and the ANT/LUG/SEU/YAB complex, as both LUG and SEU have been also related to auxin responses (Navarro et al., 2004; Pfluger and Zambryski, 2004).

SPT, which is expressed in medial domains throughout gynoecium development, is another major factor involved in the differentiation of a subset of medial tissues. In spt mutants, transmitting tract tissue does not form, and other marginal tissues such as the style, the stigma, and the septum, are reduced (Alvarez and Smyth, 1999). SPT has also been proposed to mediate auxin signalling, since chemical inhibition of polar auxin transport restores normal gynoecium development in spt mutants (Heisler et al., 2001). In addition, a strong synergistic interaction of spt and sty/shi mutations has been observed, although in some genetic backgrounds spt mutations are epistatic over some sty/shi phenotypes, suggesting that they could be involved in the same genetic pathway (Kuusk et al., 2002, 2006). It is important to note that most of the functional characterization of the SPT role in carpel development has been derived from the characterization of the spt-2 mutant allele, which carries a missense mutation in the putative DNA binding domain and had been regarded as the strongest allele. However, in an independent study on the role of SPT in the control of cold responses in seed germination it was observed that null alleles for spt show subtler gynoecium phenotypes, and that, probably, spt-2 behaves as a dominant negative allele in carpel development. Interestingly, in this work, the authors also find that SPT can work as a repressor of GA3ox expression, which encodes a key enzyme in the GA biosynthetic pathway, although in a very different developmental context (Penfield et al., 2005). While unrelated in scope, this work raises important questions that will have to be addressed in the future, such as the molecular basis of the dominant negative phenotype found in spt-2 mutants, or a possible role of SPT in mediating hormone responses, by regulation of both auxin and gibberellin signalling pathways. Interestingly, SHI, one of the members of the STY1/Shi family, has been proposed to work as a repressor of GA responses (Fridborg et al., 2001). In contrast to auxin, a role for GA in gynoecium patterning has not
been described, although it is tempting to translate the antagonistic relationship between auxins and GA in the SAM to the ‘meristematic’ medial domain of the gynoecium, and it could be interesting to explore whether SPT and STY/SHI genes could work together in promoting auxin responses, while reducing GA signalling, and if this balance could have a role in directing gynoecium medial tissue development.

Apical–basal patterning

As described above, several mutations disrupt normal patterning of the apical tissues of the gynoecium. Since they mainly affect carpel fusion and style/stigma development, the role of the corresponding genes in marginal tissue development has been discussed, but SPT and STY/SHI genes could also be considered as major factors directing the development of the apical regions of the gynoecium. In addition to spt or sty/shi, other mutants display an altered distribution of tissues along the apical–basal axis. Mutations in ETT, which encodes an Auxin Response Factor, cause severe defects in apical–basal patterning, with increased basal (gynophore) and apical (style/stigma) regions, and strongly reduced ovaries (Sessions et al., 1997; Sessions and Zambryski, 1995). The role of ETT in promoting the formation of the ovary is largely mediated through the repression of SPT expression in this domain. Thus, spt mutations are epistatic to ett, suppressing the ectopic development of apical and adaxial tissues observed in ett mutants (Heisler et al., 2001). Other mutants affecting auxin transport or signalling such as pinoid or pinformed also show enlarged apical and basal regions and reduced ovaries (Okada et al., 1991; Bennett et al., 1995). Moreover, chemical inhibition of auxin transport leads to very similar defects and differentially modifies the phenotype of apical–basal mutants, for instance, enhancing ett weak mutations or suppressing spt phenotypes (Nemhauser et al., 2000). Taking all this evidence together, Nemhauser et al. (2000) proposed that the apical–basal patterning is dependent on an auxin gradient spanning the gynoecial primordium. Auxin would be synthesized at the apical end of the gynoecium and transported basally, forming a decreasing gradient. According to this hypothesis, high levels of auxin would induce style and stigma differentiation, intermediate levels would form the ovary, and low levels the basal gynophore. Inhibition of auxin transport in the gynoecium would lead to auxin accumulation in source tissues, hypothesized to be the apical parts, and depletion in the basal regions (Fig. 3B; Nemhauser et al., 2000).

The auxin gradient model provides a nice framework to explain apical–basal patterning in the gynoecium, but we still need to understand the mechanisms of action of the genes involved. A key contribution towards this goal could be the recent finding of Sohlberg et al. (2006) which involve STY1/SHI genes in the regulation of auxin synthesis. In this work, the authors identified YUCCA4, a gene encoding a key enzyme in the auxin biosynthetic pathway, as a primary target for STY1 activation. In addition, STY1 and STY2 are expressed in the apical region of the developing gynoecium, the proposed auxin source according to the auxin gradient model, and levels of active auxin and auxin metabolites are reduced in sty/shi mutant combinations. Additional support for this hypothesis has been found on the functional characterization of YUCCA4 and related genes throughout plant development. Several YUCCA genes are expressed in the apical regions of the developing gynoecium and, when mutated, severe phenotype defects in carpel morphogenesis are found (Cheng et al., 2006).

Taking all these findings together, we can then speculate on a model that would place YUCCA-dependent auxin synthesis directly under the control of STY/SHI genes at the apical end of the developing gynoecium. SPT could mediate the response to high levels of auxin to direct stigma, style, and transmitting tract differentiation and/or participate in the formation of the auxin gradient by regulation of its polar transport, both alternatives in accordance with the observed suppression of spt phenotypes by pooling of auxin in the apical regions caused by chemical inhibition of its transport. ETT, in addition to the spatial regulation of SPT expression, may respond to intermediate levels of auxin to establish the size of the medial region in the gynoecium where the ovary will develop. ETT, a member of the Auxin Response Factor family, is a likely candidate to directly interpret auxin gradients during gynoecium development and translate them into target gene activation/repression. The importance of ETT as a possible fine-tuning sensor of auxin morphogenetic inputs is suggested by the tight regulation of ETT mRNA and protein levels found throughout floral development: ETT is target for ta-siRNA directed degradation, and, in addition, upstream open reading frames are found in ETT 5’-transcript leader sequences that have been shown to reduce both mRNA stability and the rate of translation of the downstream major ETT ORF. However, little is known about the nature of ETT downstream targets, and this should be an important question to address in the near future.

Auxin at the top (or in the middle)

One clear conclusion jumps out from this overview on gynoecium patterning: auxin is related to all the major pathways directing polarity in the gynoecium. However, how auxin signalling is translated into gene regulation and the specific nature of its targets, ultimately directing specific tissue differentiation, is still far from understood. Even the precise pattern of auxin accumulation is not fully resolved, although in the last few years, diverse attempts to visualize the localization and concentration of auxin during gynoecium development have been carried out (Avsian-Kretchmer et al., 2002; Aloni et al., 2003, 2006; Benkova
et al., 2003; Heisler et al., 2005). The use of auxin responsive promoters to drive the expression of reporter genes or auxin tissue immunolocalization with specific monoclonal antibodies are providing useful clues on auxin dynamics throughout development, that will probably be refined in the future with further studies and the aid of new technical innovations. While no other plant hormone has such a prominent role in gynoecium morphogenesis, increasing evidence points to gibberellins and cytokinins as secondary players in the game, further complicating this already intricate picture. Hopefully, the recent identification of many plant hormone receptors and the rapid advances in this field will open ways for us to understand the logic and logistics of gynoecium morphogenetic pathways in the near future.

Making up a nice gynoecium is not easy, even if you start from a really well-designed leaf

It is evident that tremendous progress has been made in unravelling the way in which the Arabidopsis gynoecium is patterned. Many players have been identified, and a start has been made on discovery, but the story is not nearly complete. The gap has to be bridged between genetic data that point to interactions between players and the actual molecular mechanisms that underlie these interactions, as well as understanding how morphogenetic cues orchestrate them. As the exploration continues into what is common or specific in the complex genetic pathways directing development of the different types of lateral organs, and the extent of conservation of these pathways in the angiosperms, we continue to face the old challenge: understanding the mystery of life.

Acknowledgements

We thank Francisco Madueño for critical reading of the manuscript and helpful discussions. Our work is supported by research grants BIO2002-01301 and BIO2005-01541 from the Ministerio de Educación y Ciencia of Spain and GV04B-262 from the Generalitat Valenciana to CF, VB, MN, and MT are supported by doctoral fellowships of the Generalitat Valenciana. We apologize to those whose work we failed to address in this review because of lack of space.

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