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Flower Development

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Flowers are the most complex structures of plants. Studies of *Arabidopsis thaliana*, which has typical eudicot flowers, have been fundamental in advancing the structural and molecular understanding of flower development. The main processes and stages of Arabidopsis flower development are summarized to provide a framework in which to interpret the detailed molecular genetic studies of genes assigned functions during flower development and is extended to recent genomics studies uncovering the key regulatory modules involved. Computational models have been used to study the concerted action and dynamics of the gene regulatory module that underlies patterning of the Arabidopsis inflorescence meristem and specification of the primordial cell types during early stages of flower development. This includes the gene combinations that specify sepal, petal, stamen and carpel identity, and genes that interact with them. As a dynamic gene regulatory network this module has been shown to converge to stable multigenic profiles that depend upon the overall network topology and are thus robust, which can explain the canalization of flower organ determination and the overall conservation of the basic flower plan among eudicots. Comparative and evolutionary approaches derived from Arabidopsis studies pave the way to studying the molecular basis of diverse floral morphologies.

1. INTRODUCTION: WHEN DID THE FLOWER EVOLVE?

The flower is the most complex structure of plants. Flowers distinguish the most recently diverged plant lineage, the angiosperms or flowering plants, from the other land plants (Figure 1). Embryophytes originated approximately 450 million years before present (MYBP) and have as distinctive features a thick cuticle resistant to desiccation, sporopollenin, pores or true stomata that aid in gas exchange, a glycolate oxidase system that improves carbon fixation at high oxygen tensions, and importantly, distinctive multicellular diploid (sporophytic) and haploid (gametophytic) stages within their life cycles (Judd et al., 2002). The major extant land plant lineages are Bryophytes (Liverworts, Hornworts and Mosses), which do not have a vascular system, and Tracheophytes, vascular plants. Within the large latter group, Lycophytes, ferns, and seed bearing plants (Spermatophytes) can be distinguished. The Spermatophyte group has been further divided into Gymnosperms (originating 380-325 MYBP) and Angiosperms. According to the fossil record, flower-like structures originated 160-147 MYBP (Frohlich, 2006). A general trend within land plant evolution is the appearance of heterospory: the existence of a megagametophyte, including the female gametes, and a microgametophyte, including the male gametes, a progressive reduction in gametophyte size (sexual reproductive structures), and within the seed plants, the presence of a diploid embryo. While these characteristics are shared among both extant and extinct seed plant lineages, the defining features of the angiosperm flower are: (1) a closed carpel bearing the ovules, which are each generally comprised of two integuments and (2) a nucellus that contains the embryo sac within which, after double fertilization, a diploid embryo and a triploid endosperm (nutritional tissue for the embryo) will develop to form a seed (Judd et al., 2002). Another character-

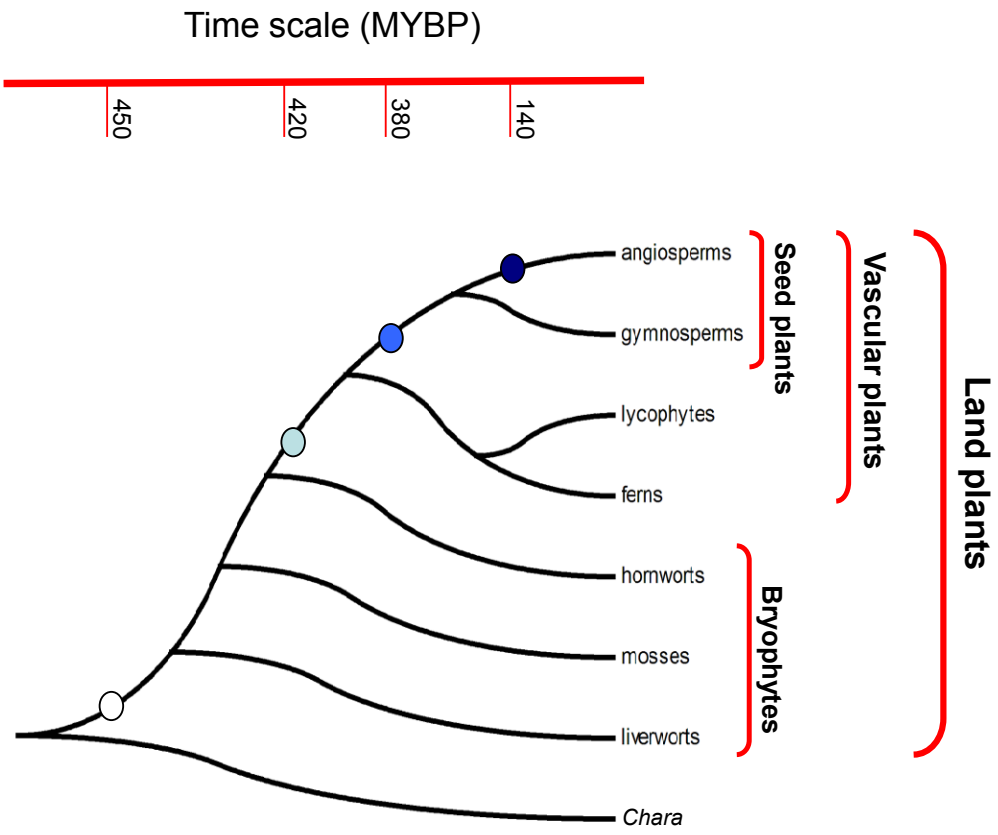


Figure 1. Phylogenetic context of *Arabidopsis thaliana*: Evolutionary history of land plants.

Phylogenetic tree of land plant evolution with some speciation events shown as colored nodes. White node, origin of land plants; light blue node, origin of vascular plants; blue node, origin of seed plants; dark blue node, origin of flowering plants. Here, *Chara* spp. from the green algae order Charales is the outgroup, since it has been used to root several recent molecular land plant phylogenies. The topology of this tree is based on studies by Soltis et al. (1999) and Nickrent et al. (2000). Time references in million years before present (MYBP) were taken from Judd et al. (2002).

istic of angiosperms is true hermaphroditism (Judd et al., 2002; Frohlich, 2006).

Flower structure has been studied in a variety of ways. Studies of the natural history and evolutionary biology of flowers have emphasized understanding the ultimate (evolutionary) causes of the wide range of variants such as color, symmetry, meristic arrangements (e.g. flower organ number), size, pollination syndrome, etc. Other studies have addressed the cellular, tissue type, morphological and physical factors that can account for both the phenotypic plasticity and developmental constraints in flower form (for a review of the developmental framework of angiosperm morphology, see Endress, 2006). A different approach flourished in the late 1980s and early 1990s, the molecular genetics of flower development in two model eudicot species: *Arabidopsis thaliana* and *Antirrhinum majus* (see reviews in: Jack, 2004; Kaufmann et al., 2005; Krizek and Fletcher, 2005; Theissen and Melzer, 2007).

Genetic studies of floral homeotic mutants in both plant species yielded the now classic combinatorial ABC developmental model for floral organ determination (Bowman et al., 1989; Coen and Meyerowitz, 1991). While much work has been and continues to be done in *Antirrhinum* and other eudicot species, including *Petunia hybrida*, the genomic and life-cycle characteristics of Arabidopsis make it the preferred experimental system for in-depth studies on the molecular components underlying cell differentiation and morphogenesis during flower development.

The basic floral architecture is mostly conserved among the so-called core eudicots, that make up over 73% of extant flowering plants (Drinnan et al., 1994) including Arabidopsis. Flowers within this group generally have four concentric whorls of organs that are specified, from the outside to the center of the flower, in the sequence: sepals, petals, stamens, and carpels. Arabidopsis has this typical floral architecture. An interesting exception to the conserved floral ground plan of eudicots is found in a Mexican rainforest monocotyledon, *Lacandonia schismatica* (Triuridaceae), which bears central stamens surrounded by carpels (Martínez and Ramos, 1989; Vergara-Silva et al., 2003; Ambrose et al., 2006).

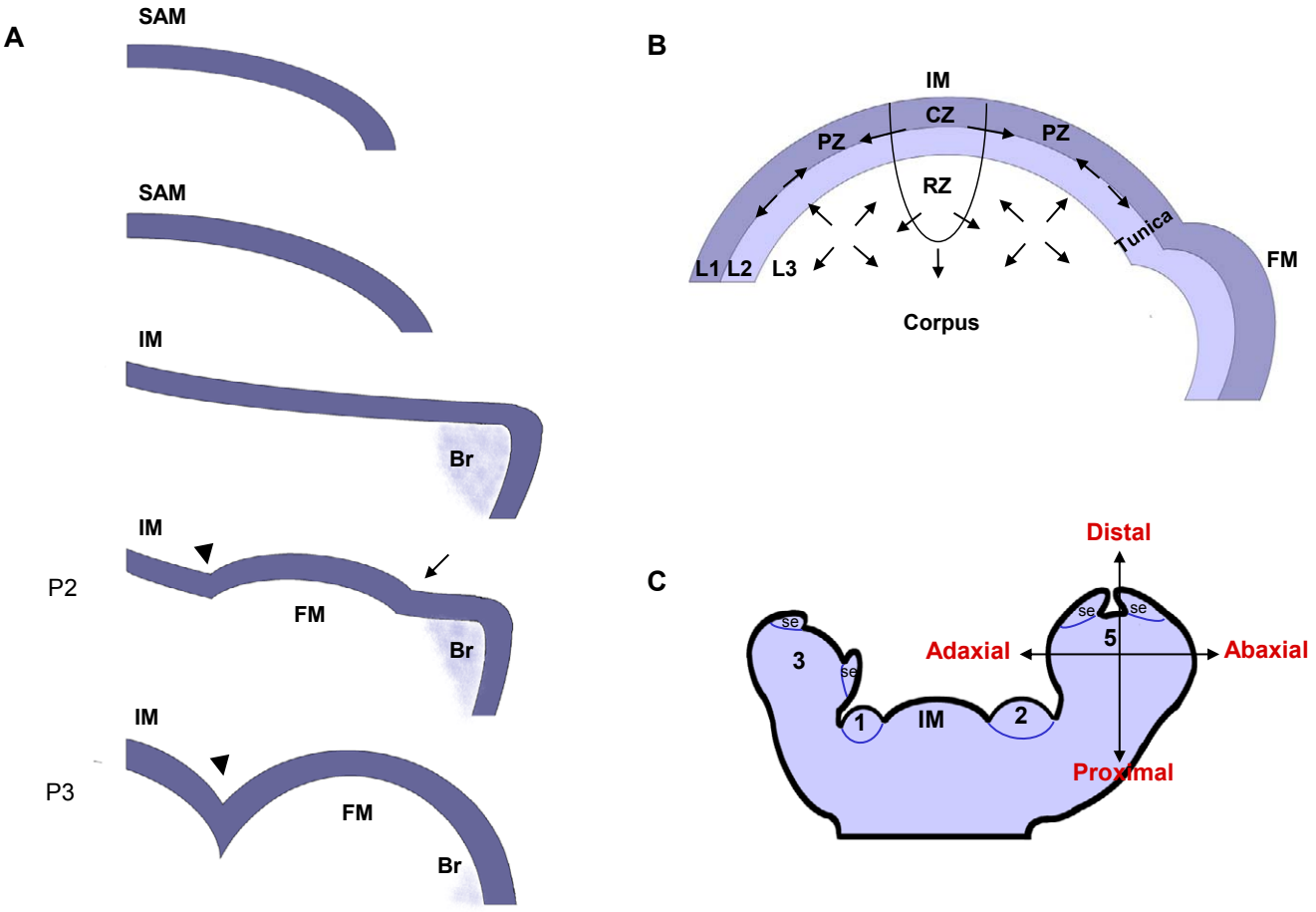


Figure 2. Schematic representation of the shoot apical meristem (SAM): the inflorescence shoot apical meristem and floral meristem.

(A) Diagram outlining the geometry of the inflorescence shoot apical meristem (IM) and flower meristem (FM) during the first stages of development of the latter. On the flank of the IM a first bulge that corresponds to the rudimentary bract (Br) appears. In its axil, a second bulge forms and this continues to grow engulfing the first one and forming the FM proper. Theses stages of FM development correspond to P2 and P3 according to Reddy et al. (2004). The arrow and arrowhead indicate the first and second visible grooves respectively (see section 2.3 for further detail).
(B) Three distinctive zones make up the IM: the central zone (CZ) which contains the stem cells; the peripheral zone (PZ) on the flanks of the CZ that gives rise to the bract and floral primordia; and the rib zone (RZ) underneath the CZ that yields stem tissue. Three cell layers are distinguished: L1 and L2 layers constitute the tunica and include portions of both the CZ and the PZ. The rest of the cells form the L3 layer or corpus. In L1 and L2, cell divisions are anticlinal, while in L3 they occur in all directions (arrows, direction of cell division). The structure is maintained in the FM.
(C) Schematic representation of the boundary zones (blue lines) and axes of polarity during floral development with the differentiation of sepals (se) from the floral primordium illustrated.

Even though the basic floral architecture is overall conserved among core eudicots, variation in the symmetry and size of flowers, the number of whorls of each organ type, the number of organs per whorl, and their arrangement, size, shape and color is common (e.g., Judd et al., 2002).

The overall conservation of the flower plan suggests that robust gene regulatory network (GRN) modules controlling the basic features of flower development were established early in the evolution of angiosperms and have persisted in the great majority of lineages throughout 140 million years of flower evolution. Recent integrated approaches to study the concerted action of the molecular components in flower development (Mendoza and Alvarez-Buylla, 1998; Espinosa-Soto et al., 2004), have led to a hypothesis that helps explain such robustness and conservation at the level of the GRN underlying floral organ specification. However structural (e.g., mechanical) constraints could also be involved in conserving floral architecture (see section 4). Approaches that integrate genetic and structural aspects of flowers should be pursued further to understand flower development in Arabidopsis and other angiosperms.

2. STRUCTURAL ASPECTS OF ARABIDOPSIS FLOWER DEVELOPMENT

In this section, we provide a summary of structural features of Arabidopsis flower development. This is essential background to the molecular genetics reviewed in section 3.

2.1 Structural Organization of the Inflorescence Meristem and Origin of the Flower Meristem

During the vegetative phase of the Arabidopsis life cycle, the shoot apical meristem (SAM) produces leaves on its flanks and on transition to flowering, the shoot bolts and the SAM becomes the inflorescence shoot apical meristem (IM). On bolting, some of the pre-existing leaf primordia become cauline leaves subtending lateral inflorescence shoots (paraclades) and the shoot apex starts to produce flowers (Hempel and Feldman, 1995). A primary IM produces lateral meristems that may go on to produce flowers or secondary inflorescences. Arabidopsis inflorescences are subtended by fully developed bracts, but flowers only by rudimentary ones. It is generally said that the IM generates the floral meristems (FM) on its flanks, but to be more precise, Arabidopsis FM are formed in the axils of the rudimentary bracts (Figure 2A; Long and Barton 2000; Hepworth et al., 2006; Kwiatkowska, 2006; reviewed in Kwiatkowska, 2008).

The SAM of the Arabidopsis inflorescence consists of a small dome of cells organized into different regions (Figure 2B) with different gene expression profiles (see section 4.1), cellular behaviors and structures. The tunica at the SAM surface and corpus are distinguished on the basis of cell division planes. In Arabidopsis, the tunica consists of two clonally distinct cell layers called L1 and L2 (Vaughan, 1952; Steeves and Sussex, 1989). Cell divisions within these meristem layers are exclusively anticlinal and the new cell walls are formed perpendicular to the surface of the meristem. The progeny of cells in the L1 will therefore remain in this same layer within the meristem similar to the underlying L2 progeny. Since outside the meristem the L1 derived cells continue to divide only anticlinally the L1 eventually gives rise to epidermal cells. The cells originating from L2 also divide periclinally (outside the SAM) and contribute for example to the leaf mesophyll or stem ground tissue formation during organogenesis. This is also the germ line in the angiosperm SAM (Ruth et al., 1985, Klekowski, 1988; Kwiatkowska, 2008). Below the tunica, cell divisions are both anticlinal and periclinal. This region of the SAM is the corpus or L3 from which the innermost tissues, like vascular tissues, are formed (Figure 2B; Brand et al., 2001).

The SAM is also organized into three different cytohistological zones each with characteristic cytoplasmic densities and cell division rates: the central zone (CZ), the peripheral zone (PZ) surrounding the CZ and the rib zone (RZ) underneath the CZ (Figure 2B; Bowman, 1994; Bowman and Eshed, 2000).

Flower primordia are derived from the PZ of the IM and are initiated from a block of four so-called founder cells (Bossinger and Smyth, 1996; Reddy et al., 2004). This estimate was based on sector boundary analysis. However, using a non-invasive replica method and a 3-D reconstruction algorithm, Kwiatkowska (2006; 2008) argues that more cells are assigned to the flower primordium, and this is consistent with the observations by Grandjean et al. (2004). The difference could be due to the fact that not all of the cells estimated to be involved in the latter approaches are incorporated into the flower meristem proper. Some of them may form a part of the subtending rudimentary bract (Figure 2A; see next section for further discussion).

The first cells produced by the RZ following the transition to flowering are rectangular with their long axis perpendicular to the major axis of the stem, but the subsequent elongation of these cells reverses this situation (Vaughan, 1955). The RZ gives rise to stem tissue. The CZ encompasses the reservoir of stem cells that divide less frequently than cells at the periphery (Grandjean et al., 2004; Reddy et al., 2004). The CZ maintains itself and yields daughter cells that form both the PZ and RZ (Bowman and Eshed, 2000). Fifteen stages of Arabidopsis flower development have been distinguished (Smyth et al., 1990). The first stages of flower meristem development are: stage 1, when a flower buttress arises, stage 2 when the flower meristem is formed and stage 3 when sepal primordia appear. Recently researchers have been able to study early flower meristem development in greater detail (Reddy et al., 2004; Kwiatkowska, 2006; reviewed by Kwiatkowska, 2008) and have proposed subdividing stage 1 (see section 2.3).

2.2 Floral Organ Primordia

Once a flower primordium is initiated, the geometry changes and a rapid and coordinated burst of cell expansion and division occurs in three dimensions generating a concentric group of cells as an almost spherical flower primordium, from which all floral tissues are derived (Bossinger and Smyth, 1996; Reddy et al., 2004; Kwiatkowska, 2006). Jenik and Irish (2000) found that the regulation of cell divisions during early and late stages of flower development seems to depend upon different mechanisms. Early in flower development, when the floral meristem of Arabidopsis is divided into four concentric rings (each with a characteristic multigenic expression profile; see section 3.3), cell division patterns depend upon the cell's radial position in the floral meristem, and not on the future identity of the floral organ to be formed in each ring. After stage 6, during organogenesis, the ABC homeotic genes (see section 3.3) seem to control the rate and orientation of cell divisions. As a result, the continuity of the concentric rings is broken giving distinct floral organ primordia within each whorl, then cells subdifferentiate into distinct types within each organ. The initiation and identity of floral organs are also regulated by different and largely independent molecular modules. This is suggested, for example, by the fact that conversion of petals into sepal-like organs in mutant plants does not alter the number of cells involved in their initiation (Crone and Lord, 1994; Bossinger and Smyth, 1996).

Tissues of floral organs are organized according to coordinated patterns and rates of cell division in the different cell layers of the meristem that dynamically acquire distinct fates. Clonal analysis shows that L1 contributes to the epidermis, the stigma, part of the transmitting tract and the integument of the ovules, while L2 and L3 contribute to the mesophyll and other internal tissues (Jenik and Irish, 2000).

Sector boundary analysis of surface cells has shown that sepals and carpels are initiated from eight cells, stamens from four

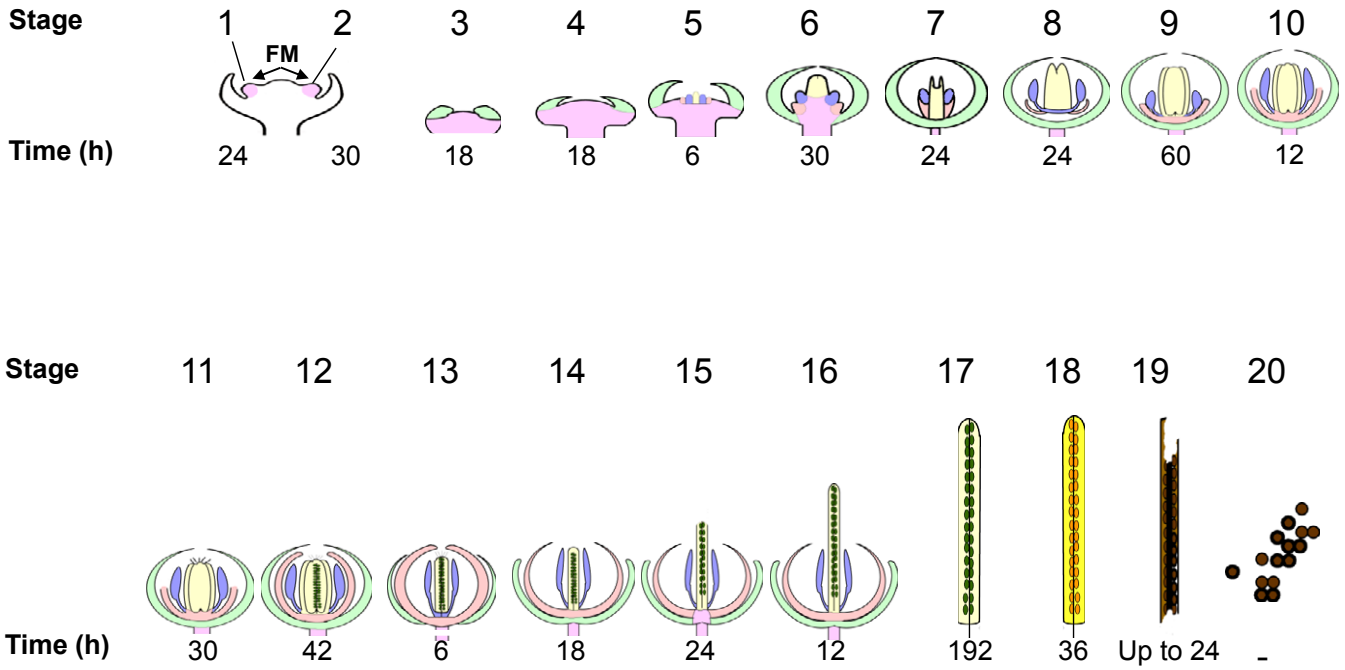


Figure 3. Summary of the 20 stages of flower development.

Schematic representation of developmental stages of Arabidopsis flowers. Briefly, the flower primordium is formed at stages 1 and 2. At stage 3, sepal primordia are already visible and continue growing until they enclose the flower meristem (from stage 4 to 6). Meanwhile, at stage 5, petal and stamen primordia are beginning to be visible, and the gynoecium starts to form (stage 6). Organ development continues and by stage 9, stigmatic papillae arise at the top of the gynoecium. At stage 12, petals are similar in length to stamens. Anthesis occurs at stage 13, fertilization occurs, and the flower opens at stage 14. Siliques reach their maximum size and are green by stage 17, then they loose water and turn yellow (stage 18) until valves separate from dry siliques (stage 19) and seeds fall (stage 20). Floral meristems (FM), pink; sepals, green; petals, bright pink; stamens, blue; gynoecia, yellow; ovules, dark green; seeds orange and brown. Duration of each stage in hours (h) is given under the figures (from Smyth et al., 1990).

cells, and petals from two cells (Bossinger and Smyth, 1996). Each organ primordium arises as a set of cells separated by boundary regions of slow-dividing cells (Figure 2C and section 3.4.2; Breuil-Broyer et al., 2004). Flower development ends when mature organs are formed and all the flower meristem cells are used up (Takeda et al., 2004; Krizek and Fletcher, 2005).

2.3 Stages of Flower Development

We provide an illustrated description of 20 states of floral development and fruit formation (Figures 2-7), mostly based on Bowman (1994), Smyth et al. (1990), Ferrándiz et al. (1999) and Roeder and Yanofsky (2006), with updates and substages as proposed by Long and Barton (2000), Reddy et al., 2004; Hepworth et al. (2006), Kwiatkowska (2006) and Kwiatkowska (2008).

STAGE 1: The first sign of flower primordium formation is the bulging of the peripheral surface of the IM in a lateral direction. This stage was referred to as P1 by Reddy et al. (2004). It is hypothesized that a lateral protrusion formed during bulging is a rudimentary bract (Figure 2A; Kwiatkowska, 2006). At this early stage, growth is fast and strongly anisotropic, with maximal growth in a meridional (i.e. radial when viewed from the top of the meristem) direction (Kwiatkowska, 2006) eventually leading to formation of a shallow crease, which corresponds to the first visible groove and to the P2 stage (according to Reddy et al., 2004) of flower development (Figure 2A). This shallow crease corresponds to the axil of the putative rudimentary bract (Kwiatkowska, 2006, 2008). Soon after the bract is formed, another bulge occurs in its axil in an upward direction. This second bulging corresponds to the formation of a flower primordium proper and to stage P3 according to Reddy et al. (2004). This stage corresponds to stage 2 according to Smyth et al. (1990). Hence, during early stages of flower development in Arabidopsis, two types of primordia (bract and flower primordium proper) and organ boundaries are observed. The first boundary is the adaxial boundary of the rudimentary bract, while the second is the boundary between the IM and the flower primordium proper (Figure 2A). The expression patterns of several genes confirm the developmental stages distinguished here (see more data on gene expression in the next section).

A significant increase in mitotic activity is observed upon formation of the primordium. The mitotic activity can be estimated as the increase in the number of cells per 24 h or the accompanying area growth rates on the condition that the mean cell size does

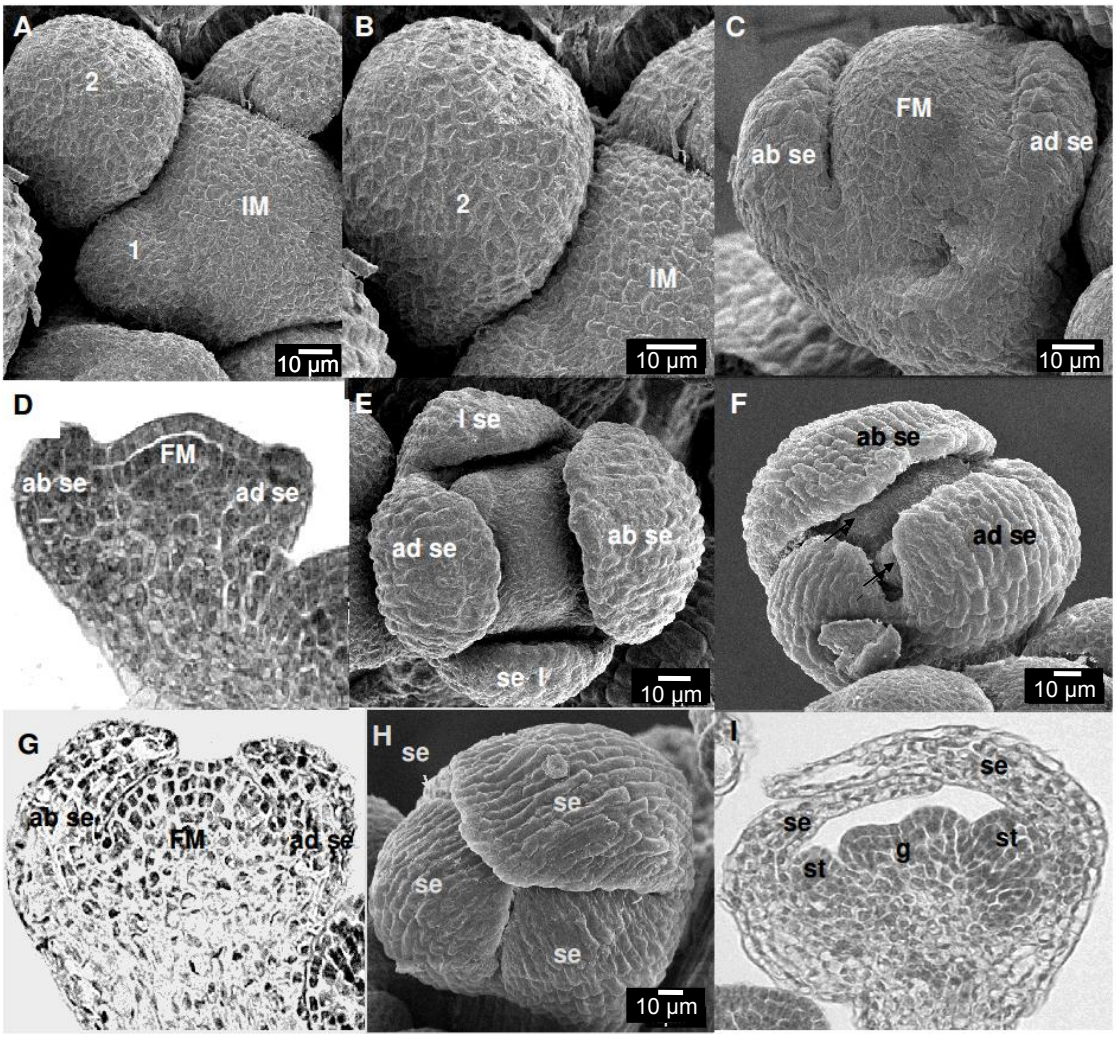


Figure 4. Stages 1 to 6 of Arabidopsis flower development.

(A) and **(B)** Inflorescence shoot apical meristem (IM) and floral meristem (FM) at stage 1 and 2 as indicated.
(C) and **(D)** Stage 3 FM showing abaxial (ab) and adaxial (ad) sepals (se).
(E) At stage 4, lateral sepals **(l)** shown growing perpendicularly to the abaxial and adaxial ones.
(F) and **(G)** At stage 5, stamen primordia are visible (arrows) and sepals almost cover the rest of the meristem.
(H) Flower bud where sepals are covering the stamens and the gynoecium primordium.
(I) Section through a stage-6 flower primordium where the gynoecium (g), stamens (st), and sepals (se) are apparent.
Pictures are scanning electron micrographs (SEM), except **(D)**, **(G)** and **(I)** which are optical images of histological sections. All pictures are of Columbia-0 wild-type plants.

not increase (Grandjean et al., 2004; Kwiatkowska, 2006; Reddy et al., 2004). During these early stages of flower development, periclinal cell divisions occur in the corpus while L1 and L2 cells only divide anticlinally (Vaughan, 1955). Hence, the two-layered tunica organization is maintained in the flower meristem, but all of its cells are mitotically active.

STAGE 2: During this stage, the hemispherical primordium continues to grow forming almost a right angle with the surface of the SAM, which itself lengthens and widens rebuilding the portion of the periphery that has been used for primordium formation (Figures 3 and 4A-B). At this stage the flower primordium becomes clearly delimited from the IM, and starts to grow larger very quickly in all directions (Figures 3 and 4A-B; Reddy et al., 2004; Kwiatkowska, 2006).

STAGE 3: This stage begins when sepal primordia become visible. By now the flower primordium is 30-35 µm in diameter and is becoming stalked with an incipient pedicel. It has also started to grow vertically. The two lateral (l) sepal primordia appear first, but are soon outgrown by the abaxial (ab) then the adaxial (ad) sepal primordia. Sepal primordia arise initially as ridges that lengthen and curve inwards until they begin to overtop the remaining dome-shaped portion of the flower primordium (Figures 3 and 4C-D).

STAGE 4: During this stage, the elongation of the pedicel continues concurrently with an increase in the diameter of the developing flower primordium to 65-70 µm. The medial sepal primordia have already partly overtopped the remaining floral meristem (Figure 4E).

STAGE 5: This stage is when the petal and stamen primordia become visible. Primordia of the four medial (long) stamens are first seen as wide outgrowths on the flanks of the central dome of the FM. The four petal primordia that arise between the sepals close to their base are just visible during this stage. The two lateral (short) stamens develop from primordia that appear later during this stage (Figures 3 and 4F-G).

STAGE 6: The sepals grow to completely cover the floral bud and the primordia of the four long stamens bulge out and become distinct from the central dome of cells that comprise the FM. The two lateral stamen primordia arise slightly lower on the dome and develop later. The petal primordia grow somewhat but are still relatively small. A rim around the central dome of the flower primordium now begins to grow upward to produce an oval tube that will become the gynoecium (Figures 3 and 4H-I).

STAGE 7: This stage begins when the growing primordia of the long stamens become stalked at their base. The stalks give rise to the filaments, and the wider upper region to the anthers. By this stage, petal primordia have become hemispherical although they are still relatively small (ca. 25 µm in diameter; Figures 3 and 5A-B).

STAGE 8: The beginning of stage 8 is defined by another landmark in stamen development: anther locules are visible as convex protrusions on the inner (adaxial) surface of the long stamens. At this stage stamens are 55-60 µm long most of which is the developing anther. Locules also appear soon after in the short stamens. Petal growth now accelerates and petal primordia become apparent (Figures 3 and 5C-E).

STAGE 9: This stage begins when the petal primordia elongate. There is a rapid lengthening of all organs especially of petals that acquire a tongue-like shape and increase in length from about 45 µm to up to 200 µm. Nectary glands appear and the stamens grow rapidly. By the end of stage 9, the medial stamens are around 300 µm long. Most of this growth occurs in the anther region, which still accounts for over 80% of their total length. At this stage the floral bud remains completely closed (Figures 3 and 5F-G).

STAGE 10: The rapidly growing petals reach the top of the lateral stamens. The cap of papillae that will constitute the stigma starts to form at the top of the gynoecium (Figures 3 and 5H-I).

STAGE 11: This stage begins when the upper surface of the gynoecium develops stigmatic papillae (Figures 3 and 6A-C) although their outward growth is limited at first to regions not in contact with the overlapping sepals. By the end of this stage petal primordia reach the top of the medial stamens.

STAGE 12: Petals continue to lengthen relatively rapidly. Lateral sepals continue to grow while the stamens and gynoecium lengthen coordinately. The anthers have almost reached their mature length of 350-400 µm and the filaments now lengthen rapidly. The upper part of the gynoecium differentiates into the style (Figure 6D) and a sharp boundary separates it from the cap of stigmatic papillae. Stage 12 ends when the sepals open (Figures 3 and 6D-F).

STAGE 13: Petals become visible between the sepals and continue to elongate rapidly. The stigma is receptive at this stage (Figures 3 and 6G-H). Stamen filaments extend even faster so the stamens outstrip the gynoecium in length and self pollination takes place. The gynoecium is now mature and its three distinct regions can be distinguished: an apical stigma, a style, and a basal ovary. After pollination, pollen tubes grow to fertilize the ovules, the stamens extend above the stigma, and furrows at both valve/replum boundaries appear.

STAGE 14: This is also defined as the stage zero hours after flowering (0 HAF), and it marks the beginning of silique (the fertilized pistil or fruit) and seed development. Cells in the exocarp continue to divide anticlinally and expand longitudinally in the replum and the valve, where there is also some expansion in other directions. There is also division and expansion in the mesocarp and many chloroplasts develop (Figures 3 and 6I).

STAGE 15: The stigma extends above the long anthers. In the carpel walls, cell division and expansion continue. The medial vascular bundles continue to grow and xylem lignifies, while the lateral bundles branch out through the mesocarp (Figures 3 and 6J-K).

STAGE 16: At this stage the silique is twice as long as a stage-13 pistil. Petals and sepals wither and tissues in the silique continue expanding (Figures 3 and 6L).

STAGE 17: This stage is defined by the abscission of the senescent floral organs from the silique, ~2 days after fertilization. The green silique grows to reach its final length and matures, a phase lasting about 8 days making this the longest stage. The dehiscence zone also differentiates (Figures 3, 7A and 7E; Substages 17A and 17B, see Roeder and Yanofsky, 2006).

STAGE 18: The silique begins to yellow from the tip to the base. One of the endocarp cell layers (the second from the inside) lignifies further, and the inner endocarp cell layer disintegrates, while the mesocarp begins to dry out. It has been suggested that lignification may contribute to the silique shattering process, acting in a springlike manner to create mechanical tensions (Figures 3 and 7B).

STAGE 19: The valves begin to separate from the dry silique, apparently owing to the lack of cell cohesion at the separation layer.(Figures 3 and 7C).

STAGE 20: At this stage the valves become separated from the dry silique and the mature seeds are ready to be dispersed (Figures 3, 7D and 7F).

2.4 Morphology, Histology and Development of Floral Organs

Sepals: In sepals L1-derived cells form the epidermis, the mesophyll originates from the L2, and the L3 contributes to the vasculature in the basal part (Jenik and Irish, 2000). Sepals and petals together form the perianth. Both organ types have a simple laminar structure, consisting of an epidermis, mesophyll and rather delicate vascular bundles (veins). The four sepal primordia (the abaxial, adaxial, and two lateral sepal primordia) are the first floral organ primordia to appear. They arise at stage 3 of flower development in a cruciform pattern (Figures 4C-D; Smyth et al., 1990; Bowman, 1994). Whether all four sepals occupy one whorl or the two lateral sepals occupy a separate outer whorl, has been the subject of discussion (Figure 4E; Smyth et al., 1990; Bowman, 1994; Choob and Penin, 2004), but all sepal primordia are formed at around the same time, shortly after they are specified (Figure 4E; Bowman, 1994).

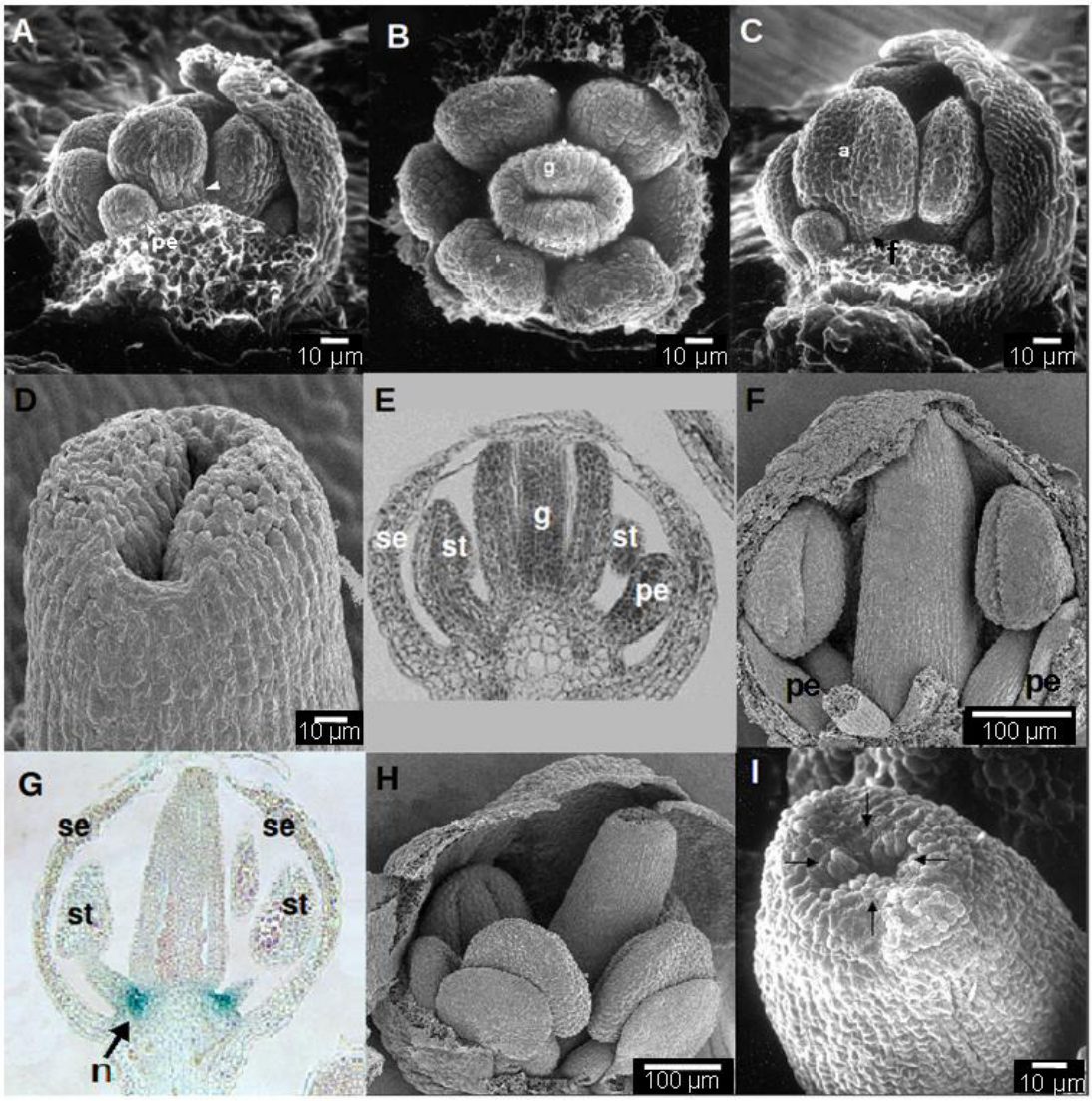


Figure 5. Stages 7 to 10 of Arabidopsis flower development.

(A) Stage 7 in which petal (pe) and stamen (arrowhead) primordia are indicated.
(B) Vertical view of the gynoecium (g) in a stage 7 floral primordium.
(C) to **(E)** Carpels and stamens at stage 8 of floral development are shown. Filament (f) and anther (a) regions of the stamen are differentiated **(C)** and a slot is formed at the tip of the style in the gynoecium **(D)**. Section through the floral bud with sepals (se), petals (pe), stamens (st) and gynoecium (g) indicated **(E)**.
(F) and **(G)** Floral bud at stage 9 in which petal primordia (pe) are indicated **(F)**. Section through flower primordium **(G)** in which *XAL1:GUS* is shown staining nectaries (n).
(H) and **(I)** Stage10 flowers. Flower bud showing the enlarged sepals which cover other floral organs, stalked petals and stamens, and developing carpels in the center **(H)**. Stigma starts to be formed at the top of the gynoecium (**I**, arrows)

Bars = 10 µm except in **(F)** and **(H)**. Images **(A)**, **(B)**, **(C)**, **(G)** and **(I)** are of Lansberg *erecta* ecotype, from Smyth et al. (1990) provided by Dr J. Bowman. Some sepals were removed from flower buds shown in **(A)**, **(B)**, **(C)**, **(D)**, (F), **(H)** and **(I)**. All images except **(E)** and **(G)** are SEM. **(D)**, **(E)**, **(F)**, **(H)** are of Columbia-0 ecotype.

The adaxial and abaxial surfaces of the sepal epidermis are different (Figures 8B and 8D-E). On the abaxial surface, cells have irregular shapes and sizes with some quite long cells (with nuclei of various sizes) and fringes of smaller cells. Unlike the adaxial surface, the abaxial surface has stomata and may have unbranched trichomes (Figure 8E; Smyth et al., 1990; Bowman, 1994; Hase et al., 2000; Krizek et al., 2000).

Petals: In the petal primordium the meristematic layer L1 contributes to the epidermis and L2 to the mesophyll; as yet cells originating from L3 have not been found to form part of the petal

(Figure 2B; Jenik and Irish, 2000). These primordia become apparent almost at the same time as stamen primordia at stage 5 of flower development. Visible signs of petal differentiation are seen by stage 9 (Figure 5F; Smyth et al., 1990; Bowman, 1994). The four petals of Arabidopsis are white and flat and approximately the same size and shape. They are narrower and greenish toward the base (Figure 8C; Takeda et al., 2004).

Cells on the adaxial surface are conical with epicuticular thickenings running from the cell base to the apex, whereas those on the abaxial surface are flatter and more cobblestone-like with cuticular thickening (Figures 8F-G). Stomata are absent from both petal surfaces (Bowman, 1994; Krizek et al., 2000). Cells toward the base of petals resemble those of stamen filaments (Bowman, 1994).

Stamens: Primordia appear at stage 5 of flower development (Figure 4F) due to periclinal divisions in the subprotodermal cell layer (L2) and sometimes in L3 (Crone and Lord, 1994; Jenik and Irish, 2000). Stamen primordia are visible at stage 6. By stage 7, differentiation can be observed and long stamen primordia appear stalked at their bases (Figures 4I and 5A-B; Bowman, 1994; Smyth et al., 1990). At this stage stamen primordia are composed of an L1-derived epidermis, one layer of L2-derived subepidermis, and an L3-derived core (Figure 2B; Jenik and Irish, 2000). Locules appear in the anthers by stage 8 (Figure 5C). Growth of the internal anther tissue at this stage is due to divisions of L2-derived cells (Jenik and Irish, 2000). At stage 14, anthers extend above the stigma (Figure 5I; Bowman, 1994). In the mature anther, the L3 cells contribute only to the vasculature (Jenik and Irish, 2000). Stamens of the Arabidopsis flower are not formed simultaneously: four long medial stamens arise a little earlier than the two short lateral ones (Smyth et al., 1990).

Each stamen consists of two distinct parts, the filament and the anther. At the tip of the filaments, the anther develops both reproductive and non-reproductive tissues that produce, harbor, and release pollen grains upon maturity (Goldberg et al., 1993). The anther is a bilocular structure with longitudinal dehiscence (Figure 6G; Bowman, 1994). Each locule develops from successive divisions of subprotodermal archesporial cells formed in the anther primordium that gives rise to three morphologically distinct layers: the endothecium, the middle layer, and the tapetum which surrounds the pollen mother cells (PMCs). The PMCs undergo meiosis and form the haploid microspores. The tapetum is a source of nutrients and is indispensable for microspore maturation (Xing and Zachgo, 2008). Anther development and microspore formation in Arabidopsis is a complex process that has been divided into 14 stages (See also section 3.4.5; Sanders et al., 1999).

Once formed, PMCs are surrounded by a layer of callose. After meiosis, the anther contains most of its specialized cells and tissues, and tetrads of microspores are present within the pollen sacs; with microspores in each tetrad surrounded by a callose wall. Callose dissolves and microspores are released. As pollen grains develop, the anther enlarges and is pushed upward in the flower by the elongating filament (Scott et al., 2004).

Carpels: The fourth and innermost whorl is occupied by the gynoecium that is composed of two fused carpels. Carpel primordia start to form at stage 6 of flower development (Figure 4I) due to periclinal cell divisions in the L3 layer (Jenik and Irish, 2000). Carpels enclose and protect the developing ovules, mediate pollination, and after fertilization develop into a fruit within which fertilized ovules develop into seeds (Bowman et al., 1999). The gynoecium consists of two valves separated by a false septum with ovules arising from parental placental tissue on each side of the septum (Bowman, 1994). The valves grow upward from the flower meristem to form a closed cylinder. At early stage 8, the walls of the cylinder are composed of an L1-derived epidermis, one L2-derived subepidermal layer and a two-cell thick, L3-derived core. At this stage the distal L2 cells start to divide periclinally (with respect to the top surface of the cylinder), contributing to the longitudinal growth of the carpel (Figure 2B; Jenik and Irish, 2000). Later the inner surfaces of septal outgrowths within this cylinder will fuse, the tip will close and ovules will develop along the margins of the fused walls (placenta) of the bilocular chamber (Bowman, 1994; Sessions and Zambryski, 1995). The gynoecium is oriented in the flower so that the septum coincides with the medial plane (Figures 4D-E and 4G; Sessions and Zambryski, 1995).

At the distal end of the gynoecium, the stigma, an epidermal structure composed of stigmatic papillae (bulbous elongated cells), functions in pollen binding and recognition and participates in the induction of pollen germination (Figures 6A-B). After germination, the pollen tubes will grow between the papillar cells into the transmitting tract at the center of the style and the septum of the ovary (Bowman, 1994; Sessions and Zambryski, 1995).

At about stage 11, the inner and outer integuments of the ovule are formed. By stage 12, the integuments of the developing ovule grow to cover the nucellus and megagametogenesis occurs (Figures 6E-F; Bowman, 1994).

Nectaries: These organs produce and secrete nectar. Nectar is a protein- and carbohydrate-rich solution, which varies in composition among different plant species (Davis et al., 1998). Nectar may be a reward for pollinators or for insects that protect the plant against herbivores, or even a lure for animal prey in carnivorous plants (Davis et al., 1998; Baum et al., 2001; Lee et al., 2005a).

In Arabidopsis, the nectarium (multiple nectary) found in individual flowers (Davis et al., 1998) is composed of two parts: nectary glands that form below the stamen filament, and the connective tissue linking the glands in a continuum around the androecium (Bowman, 1994; Baum et al., 2001). The nectarium is always situated in the third whorl of the flower and its location is independent of the identity of the other organs occupying this whorl. These glands are formed from stage 9 to 17 of flower development (Figure 5G; Bowman, 1994; Bowman and Smyth 1999; Baum et al., 2001; Tapia-López et al., 2008).

3. MOLECULAR GENETICS OF ARABIDOPSIS FLOWER DEVELOPMENT

Plant organogenesis, including flower formation, occurs from actively proliferating meristems over the entire life cycle. In the next section we provide a very brief summary of the molecular mechanisms that maintain an active SAM. In section 3.2, we explain how the flower meristem is specified and becomes determinate after the flower organs are formed.

3.1 Shoot Apical Meristem Proliferation and Maintenance

The balance between cell proliferation and cell recruitment to differentiated tissues in the SAM is dependent on mechanisms

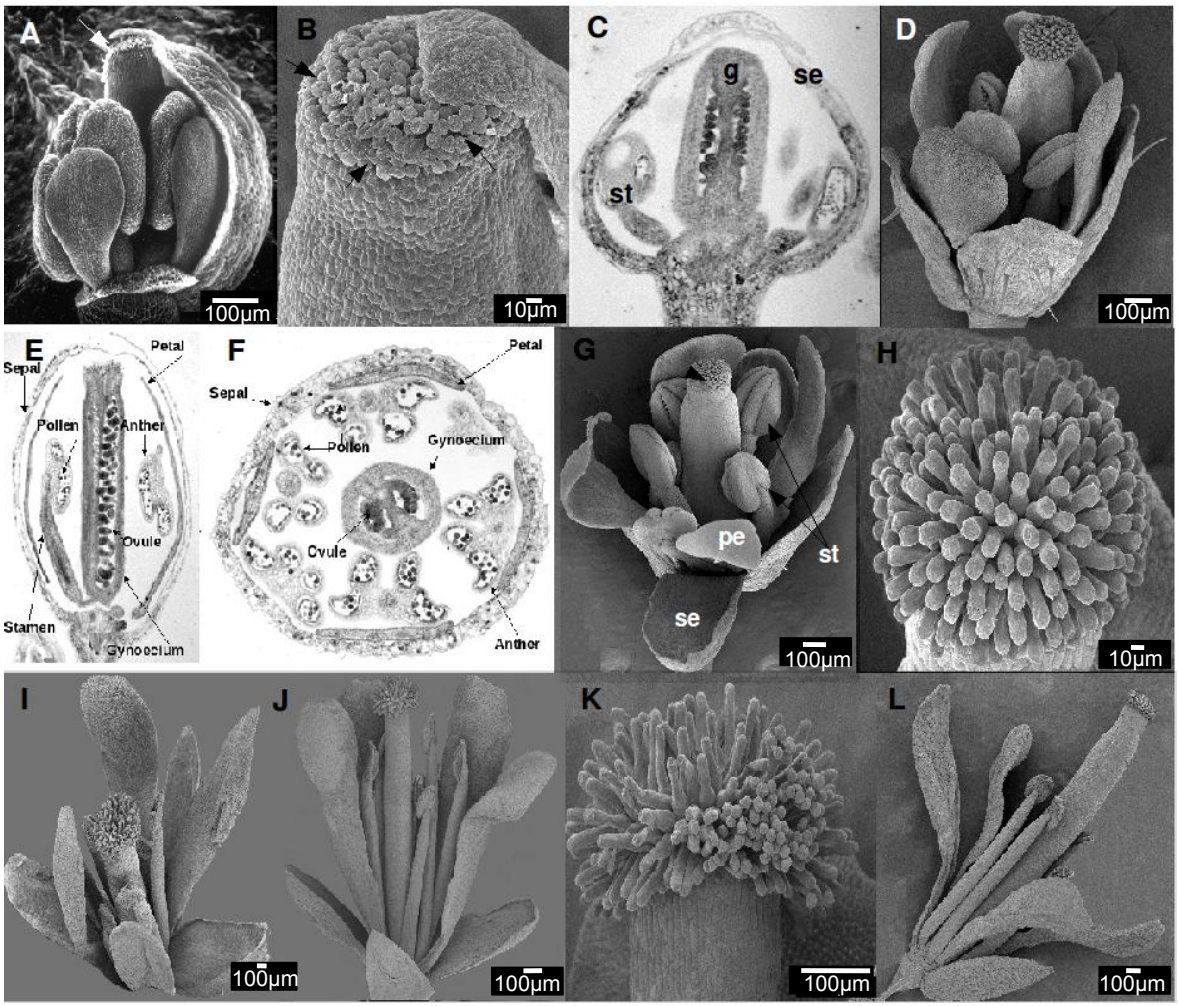


Figure 6. Stages 11 to 16 of Arabidopsis flower development.

(A) to **(C)** Stage 11 of flower development where the gynoecium develops stigmatic papillae (arrows) **(A)** and **(B)**. Longitudinal section where sepals (se), stamen (st), and gynoecium (g) are indicated **(C)**.
(D) to **(F)** Flower primordium at stage 12. Longitudinal **(E)** and transverse **(F)** sections showing all the organs as well as ovules and pollen grains.
(G) and **(H)** Flower anthesis at early stage 13 when the stigma (arrowhead) is already receptive **(G)**; a close-up view of the stigma **(H)**.
(I) to **(L)** Flower primordium at stages 14 **(I)** and 15 where the gynoecium has begun to enlarge to form the silique **(J)**. Close-up of a stage-15 stigma **(K)** and stage-16 flowers where sepals and petals are beginning to wither **(L)**.
Bars = 100 µm. All images except **(C)**, **(E)** and **(F)** are SEM. Images are of Columbia-0 ecotype, except **(A)** that is of Landsberg *erecta* (from Smyth et al.,1990, provided by Dr. J. Bowman).

regulated by *WUSCHEL* (*WUS*; Laux et al., 1996; Sablowski, 2007). The homeodomain-containing WUS transcription factor has the role of the maintaining the identity of stem cells in the organizing center of the CZ; *wus* mutants lack stem cells in the SAM (Mayer et al., 1998). *WUS* expression is limited to the cells immediately below the stem cells, an expression domain regulated by the receptor-kinase signaling system that includes the *CLAVATA1*, *2* and *3* (*CLV1, 2, 3*) gene products (Mayer et al., 1998; Brand et al., 2000; Schoof et al., 2000). *CLV1* is expressed in most L3 stem cells while *CLV3* is expressed in all three stem cell layers but mostly in L1 and L2 stem cells (see Figure 2B; Clark et al., 1997; Fletcher et al., 1999). In *clv* mutants, there is an imbalance between cells retained within meristems versus those recruited to form lateral organs. *clv* mutations cause an expansion of the *WUS* expression domain resulting in an enlarged stem cell niche. *CLV3* expression is, in turn, positively regulated by *WUS*, suggesting that meristem size depends greatly on a *WUS-CLV* regulatory loop (Clark et al., 1993, 1995; Kayes and Clark, 1998; Brand et al., 2000). Overexpression of *CLV3* represses *WUS* expression and decreases meristem activity, suggesting that CLV3, a secreted CLE-domain peptide, is the signal that regulates *WUS* expression via the CLV1/CLV2 LRR protein-kinase transduction complex (Fletcher et al., 1999; Jeong et al., 1999; Trotochaud et al., 1999; Clark, 2001a

and 2001b; Ni and Clark, 2006). It has been shown that other LRR-protein kinases closely related to CLV1 like BARELY ANY MERISTEM1 and 2 (BAM1, 2) are also involved in meristem maintenance possibly by sequestering CLV3 on the flanks of the meristem where they are expressed (DeYoung et al., 2006; DeYoung and Clark, 2008).

SHOOT MERISTEMLESS (*STM*) is a *KNOTTED1-like homeobox* (*KNOX*) gene that encodes a protein expressed in the SAM's CZ, RZ and regions of the PZ that have not been assigned to a primordium, i.e. it is expressed throughout the meristem except for anlagen, the sites of primordium formation (Figure 2B). STM promotes the profileration of stem cell derivatives until a critical cellular mass is attained sufficient to form either leaves or floral primordia. It also inhibits the expression of *ASYMMETRIC LEAVES1* and *2* (*AS1, 2*) genes in the SAM, preventing these cells from undergoing premature differentiation (Byrne et al., 2000; Byrne et al., 2002). Thus, the *STM* gene is considered to play a pivotal role in meristem maintenance (Long et al., 1996; Carles et al., 2004). *ULTRAPETALA1* (*ULT1*) encodes a cysteine-rich protein with a B-box like domain that restricts the size of shoot and floral meristems. It functions antagonistically to the proliferative roles of WUS and STM during most of the Arabidopsis life cycle but it in an independent genetic pathway (Carles et al., 2004).

3.2 Floral Meristem Specification and Determination

The changes in cellular characteristics, growth and geometry observed in the transition of the SAM to an IM (Kwiatkowska, 2006) are correlated with dynamic changes in the spatial and temporal expression of certain genes. The Arabidopsis IM produces rudimentary bracts in whose axils flower meristems emerge. *STM* and *AINTEGUMENTA* (*ANT)* expression patterns correlate with the development of this rudimentary bract primordium (Long and Barton, 2000).

The expression of *LEAFY* (*LFY*), a transcription factor found only in plants (Schultz and Haughn, 1991; Weigel et al., 1992; Maizel et al., 2005; Weigel, 2005) and *ANT* has been used in order to trace the cells that form the flower primordium (Grandjean et el., 2004). First, tens of cells are rapidly recruited to those already committed to become part of the flower meristem. This stage may correspond to the upward bulging at the shallow crease formed between the rudimentary bract and the IM described by Kwiatkowska (2006). These cells which express *LFY* then continue to proliferate. Interpretating this, the first cells that express *LFY* would correspond to the rudimentary bract (but not its axil or shallow crease), and later the domain of *LFY* expression would expand to include the cells committed to the flower primordium proper (Kwiatkowska, 2006). This interpretation can explain the discrepancy in the number of founder cells estimated using sector boundary analysis (Bossinger and Smyth, 1996) and using *in vivo LFY* expression patterns (Grandjean et al., 2004). Bossinger and Smyth (1996) concluded that a FM derives from four founder cells directly on the surface of the IM (or SAM). In support of this, evidence from confocal laser scanning microscopy indicates that flower primordia are formed from two rows of cells in a radial arc (Reddy et al., 2004). In contrast, the number of cells expressing *LFY* at these early stages (Reddy *et al.,* 2004) suggest that

Figure 7. Stages 17 to 20 of Arabidopsis flower development.

(A) to **(D)** Photographs of developing and mature siliques at stages 17 **(A)**, 18 **(B)**, 19 **(C)**, and 20 **(D)** of flower development.
(E) SEM of seeds from a silique at stage 17.
(F) Close-up view of a seed from a stage-20 dehiscent silique.
All photographs are of Columbia-0 ecotype.

a flower meristem has more founder cells. An explanation that resolves the discrepancy is that the *LFY*-expressing cells could include those that eventually form the rudimentary bract, as well as those which form the flower primordium (Kwiatkowska, 2006; reviewed in Kwiatkowska, 2008)

The gene *CUP-SHAPED COTYLEDON2* (*CUC2*) is expressed in the slow-dividing cells that expand in a latitudinal direction (Reddy et al., 2004) to define the second boundary between the floral primordium proper and the IM (Breuil-Broyer et al., 2004). Several regulators of *CUC* including a miRNA have been described as important components of the GRN involved in this developmental process (Laufs et al., 2004; Aida and Tasaka, 2006a).

Flower versus inflorescence meristem identity is controlled by a complex GRN that integrates environmental and internal

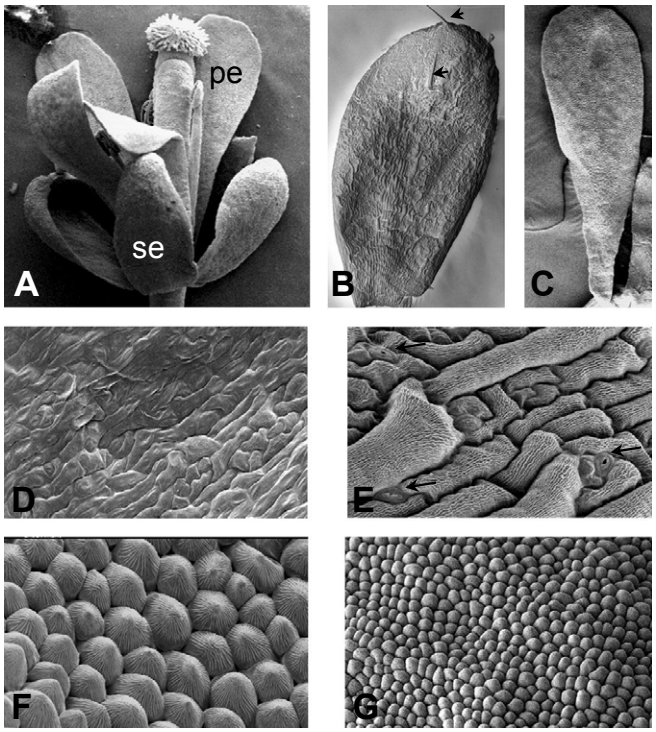


Figure 8. Sepal and petal cell types.

Scanning electron micrographs (SEM) of wild-type flowers and flower organs.

(A) A mature flower with sepals (se) and petals (pe) fully expanded and the stigma extending above the long stamens.

(B) Sepal blade showing simple unbranched trichomes (arrowheads) characteristic of the abaxial surface.

(C) Mature petal blade consisting of a basal claw and a distal blade.

(D) Adaxial sepal surface with irregular sizes and shapes of cells, some elongated (800x).

(E) Abaxial sepal surface bearing stomata (arrows) and characteristic elongated cells (500x).

(F) Adaxial surface of a mature petal blade showing conical cells with epicuticular thickenings running from the base to the apex (800x).

(G) Abaxial petal surface showing flatter, cobblestone-shaped cells with cuticular thickenings. Both petal surfaces lack stomata.

cues (Figure 9). On induction of flowering, the IM genes, such as *TERMINAL FLOWER 1* (*TFL1*; Alvarez et al., 1992; Shannon and Meeks-Wagner, 1991 and 1993, Ohshima et al., 1997) and *EMBRYONIC FLOWER 1 and 2* (*EMF1, 2*; Chen et al., 1997; Aubert et al., 2001), are repressed in the FM, while the floral meristem identity (FMI) genes, mainly *LFY*, *APETALA1* (*AP1*), *APETALA2* (*AP2*), and *CAULIFLOWER* (*CAL*), are upregulated (Figure 10; Blazquez et al., 1997; for review Mandel et al., 1992; Weigel et al., 1992; Bowman et al., 1993; Kempin et al., 1995; Mandel and Yanofsky, 1995a; Blazquez et al., 2006).

Mutual repression of the IM and FMI genes seem to underlie the co-existence, identity and boundaries of both types of meristem in the SAM in the transition to flowering (Chen et al*.,* 1997; Liljegren et al*.,* 1999; Ratcliffe et al*.,* 1999). For example, if genes such as *TFL1* or *EMF1* or *2* are mutated, *LFY* and/or *AP1* are ectopically expressed in the IM that is then transformed into a FM (Shannon and Meeks-Wagner, 1991; 1993; Weigel et al*.,* 1992, Bowman et al*.,* 1993; Gustafson-Brown et al*.,* 1994; Bradley et al*.,* 1997; Chen et al*.,* 1997; Moon et al*.,* 2003). On the contrary, if *AP1, CAL* and *LFY* are repressed, the FM attains IM identity (Ratcliffe et al*.,* 1998; Ratcliffe et al*.,* 1999). *TFL1* is an important regulator of inflorescence development (Alvarez et al., 1992; Ratcliffe et al., 1998; Parcy et al., 2002). It encodes a phosphatidyl ethanolamine-binding protein (PEBP) that is transcribed in the center of the IM but the protein moves to other cells where *AP1* and *LFY* are downregulated (Bradley et al., 1997; Conti and Bradley, 2007). *EMF* genes are required for vegetative growth, but they seem to regulate flowering time and inflorescence development too (Sung et al*.,* 1992; Aubert et al*.,* 2001; Yoshida et al*.,* 2001). Loss-of-function mutants in these genes produce flowers immediately after germination skipping the vegetative phase (Yang et al., 1995; Chen et al., 1997). *EMF1* encodes a transcription factor that represses *AP1* but not *LFY*, and *EMF2* encodes a novel zinc finger protein related to the polycomb group (Aubert et al., 2001; Yoshida et al*.,* 2001).

LFY is necessary and sufficient to specify FMI (Weigel et al., 1992; Weigel and Nilsson, 1995). In *lfy* mutants, leaves and secondary shoots are produced instead of flowers (Schultz and Haughn, 1991; Weigel et al., 1992) and *LFY* overexpression causes the conversion of leaves and axillary meristems to flowers (Weigel and Nilsson, 1995). *LFY* is expressed in the leaf primordia during vegetative growth, but when induced by external (vernalization and light) and/or internal (gibberellins) signals, it is strongly expressed and relocates to the SAM flanks where floral meristems are formed (Figure 9; Blazquez et al*.,* 1997; Hempel et al*.,* 1997; for *LFY* regulation see: Nilsson et al., 1998; Blazquez and Weigel, 2000; Liu et al., 2008). *LFY* expression persists at high levels in the FM until stage 3 of development and then diminishes in the center of the flower (Figure 10; Blazquez et al*.,* 1997; Wagner et al., 2004). LFY protein abundance, however, is homogenous in the FM, probably because it moves between cells (Parcy et al*.,* 1998; Sessions et al*.,* 2000; Wu et al*.,* 2003).

LFY and *AP1* have overlapping functions in establishing the FM; while the *ap1* mutant has shoots with inflorescence characteristics, the *lfy ap1* double mutant has an almost complete conversion of flowers into shoots (Huala and Sussex, 1992; Bowman et al*.,* 1993). Both genes when overexpressed cause a terminal flower phenotype suggesting that each one is sufficient to determine the IM (Mandel and Yanofsky, 1995a; Weigel and Nilsson, 1995). *CAL*, the closest paralogue of *AP1*, and *FRUITFULL* (*FUL*) from the same gene clade within the MADS-box phylogenetic tree (Alvarez-Buylla et al., 2000; Martínez-Castilla and Alvarez-Buylla, 2003; Parenicová et al., 2003), may also act redundantly to *AP1* in FM specification. Single *cal* and *ful* mutants do not show any FMI disorders, but in combination with *ap1* in double or triple mutants, the *ap1* phenotype is greatly intensified (Bowman et al*.,* 1993; Kempin et al*.,* 1995; Ferrándiz et al*.,* 2000a). *FUL* is expressed at the same time as *LFY* during the establishment of the FMI (Mandel and Yanofsky, 1995b; Hempel et al*.,* 1997), but is mostly localized in the IM (Figure 10). Later during flower development, *FUL* is expressed again during carpel and silique development where it plays an important role (Gu et al*.,* 1998). Despite its close similarity to *AP1*, overexpression of *CAL* is not able to determine the IM as does overexpression of *AP1*, indicating that CAL does not interact with the same partners as AP1. The unique functions

of AP1 rely on residues within the K and COOH domains that are not found in CAL (Alvarez-Buylla et al., 2006).

LFY directly regulates *AP1* and *CAL* transcription by binding to the consensus sequence CCANTG (CArG-box; Parcy et al., 1998; Wagner et al., 1999; Wagner et al., 2004; William et al., 2004). However, expression reminiscent of *AP1* is seen in the *lfy* mutant, while it is completely abolished in the double mutant *lfy ft* (*flowering locus t*; Ruiz-Garcia et al., 1997; Schmid et al., 2003; Wigge et al., 2005). Thus FT, a homolog of TFL1 (Koornneef et al., 1991; Kardailsky et al., 1999), together with FD, a bZip transcription factor (Abe et al., 2005), redundantly regulate *AP1* with LFY. AP1 and CAL in turn regulate *LFY* by positive feedback, allowing it to exert its transcriptional regulation during flower development (Bowman et al., 1993; Liljegren et al., 1999). Recently, additional LFY targets have been found (William et al., 2004), among them *LATE MERISTEM IDENTITY1* (*LMI1*), which encodes a homeodomain leucine-zipper transcription factor and functions as a FMI gene. Interestingly, LMI1 acts together with LFY to activate *CAL* expression (Figure 9; Saddic et al., 2006).

AP2 encodes a putative transcription factor of a plant-specific gene family (*AP2/EREBP*) with diverse functions (Riechmann and Meyerowitz, 1998). Mutations in *AP2* enhance both *ap1* and *lfy* mutant phenotypes, indicating that *AP2* also plays a role in specifying FMI (Huala and Sussex, 1992; Schultz and Haughn, 1993; Shannon and Meeks-Wagner, 1993; Simpson et al., 1999).

MADS-box genes are key components of the regulatory module that integrates flowering transition signaling pathways (for review see Jack, 2004; Parcy, 2005, Blazquez et al., 2006), IM and FM identities (Mandel et al., 1992; Bowman et al., 1993; Mandel and Yanofsky, 1995a, 1995b), and floral organ specification (see section 3.3; Coen and Meyerowitz, 1991). To specify the FM, LFY and/or AP1 are also required to downregulate flowering induction genes such as *AGAMOUS-LIKE 24* (*AGL24*), *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*), *SHORT VEGETATIVE PHASE* (*SVP*), and *FUL* (Figures 9 and 10). Overexpression of any of these genes causes FM to revert to IM-like structures as when LFY and/or AP1 are mutated (Mandel and Yanofsky 1995b; Yu et al., 2004a; Liu et al., 2007).

Floral reversion is often found in plants heterozygous for *lfy-6* (*LFY/lfy*) and homozygous for *agamous-1* (*ag-1*), suggesting a key role for *LFY* and *AG* in the maintenance of determinate floral meristems (Okamuro et al., 1996). The reason for this is that late in floral organogenesis *AG*, induced by *WUS*, *LFY* and *PERIANTHIA* (*PAN*) among others, positively regulates *KNUCKLES* (*KNU*) which in turn represses *WUS* expression to terminate the stem cell niche after a limited number of organs have been formed (Parcy et al., 1998; Busch et al., 1999; Lenhard et al., 2001; Lohmann et al., 2001; Das et al., 2009; Maier et al., 2009; Sun et al., 2009). In fact, while *WUS* expression declines after stage 6 in wild-type flowers, it persists in *pan* or *ag* flowers (Lenhard et al., 2001; Lohmann et al., 2001; Das et al., 2009; Maier et al., 2009). *ULT* also participates in meristem determinancy together with *AG* downregulating *WUS* (Carles et al., 2004).

Although it is very rare to observe spontaneous or induced reversion from FM to IM, a set of genes that actively maintain FM identity could conform to a "flower developmental module" that prevents reversion. The genetic mechanisms involved in maintaining FMI are closely linked to hormone balance and environmental factors (Tooke et al., 2005). For example, we now know that *STM* is a positive regulator of local cytokinin (CK) biosynthesis and accumulation (Jasinski et al., 2005; Yanai et al., 2005), and a repressor of gibberellin (GA) production (Jasinski et al., 2005). On the other hand, *WUS* enhances CK activity by repressing ARABIDOPSIS *TYPE A RESPONSE REGULATORS* (*ARRs*) (Leibfried et al., 2005). The resulting high CK:auxin ratio and low GA levels promote indeterminate growth (Shani et al., 2006). While a high auxin concentration restricts *STM* and *CUC* expression (see section 3.4.2), it also downregulates CK biosynthesis and activity, thus yielding a high auxin:CK ratio and high levels of GA, which induce floral meristem formation. Raising GA levels or response, for example by crossing with the *spindly* (*spy*) mutant, is sufficient to suppress FM reversion to IM in *lfy*, *ap1*, *ap2* and *ag* mutants. This demonstrates the importance of GA in the maintenance of FM identity (Okamuro et al., 1996; Okamuro et al., 1997).

Light signal transduction pathways are also involved in FM maintenance. Spontaneous floral reversion in wild-type Arabidopsis has only been observed at low frequencies in the first flowers of Landsberg *erecta* grown in short days. However, *long hypocotyl* (*hy1-1*), a mutant in which phytochrome activity is blocked, suppresses floral reversion of both *lfy and ag* single mutants in short days (Okamuro et al., 1996). Floral reversion seems to be a developmental abnormality with no apparent adaptative significance, unless plant resources are somehow saved under certain conditions if flowering is reversed. Further ecological and evolutionary developmental studies of Arabidopsis ecotypes will continue to elucidate the genetic, epigenetic, physiological, and environmental mechanisms involved in the maintenance of the FMI.

3.3 Specification of Floral Organs: The ABC Genes

Very soon after FM specification (11-13 days after germination in Landsberg *erecta* ecotype), the flower meristem is subdivided into four regions. Each one will give rise to the primordia of the different floral whorls, which from the outside to the inside are: sepals, petals, stamens, and carpels. The genes responsible for floral whorl specification attain their spatio-temporal pattern as a result of regulatory interactions among themselves, interactions with meristem identity genes and with some other genes, such as *WUS* and *UNUSUAL FLORAL ORGANS* (*UFO*; Levin and Meyerowitz, 1995). The complexity of the interactions involved is shown in the 'floral organ specification gene regulatory network' (FOS-GRN) model, analyzed in Section 4.1. This model includes a set of interacting genes sufficient to pattern the IM and FM during the first stages of flower development.

One of the key FM identity genes is *LFY*. The protein encoded by this gene requires co-factors to set the spatial limits of expression of the floral organ identity genes *AP3*, *PI*, and *AG*. For example, LFY participates with UFO in the regulation of *AP1* and *AP3* transcription (Lee et al., 1997; Chae et al., 2008), and with WUS co-regulates the expression of *AG* (Lenhard et al., 2001; Lohmann et al., 2001). LFY also regulates the expression of the *SEPALLATA* (*SEP*) genes *SEP1*, *SEP2* and *SEP3*, additional MADS-box genes required for organ identity specification (Krizek and Fletcher, 2005).

UFO is expressed in the second and third whorls during floral stage 3, probably restricting the B-gene expression domain to these whorls, together with LFY (Lee et al., 1997; Traas and

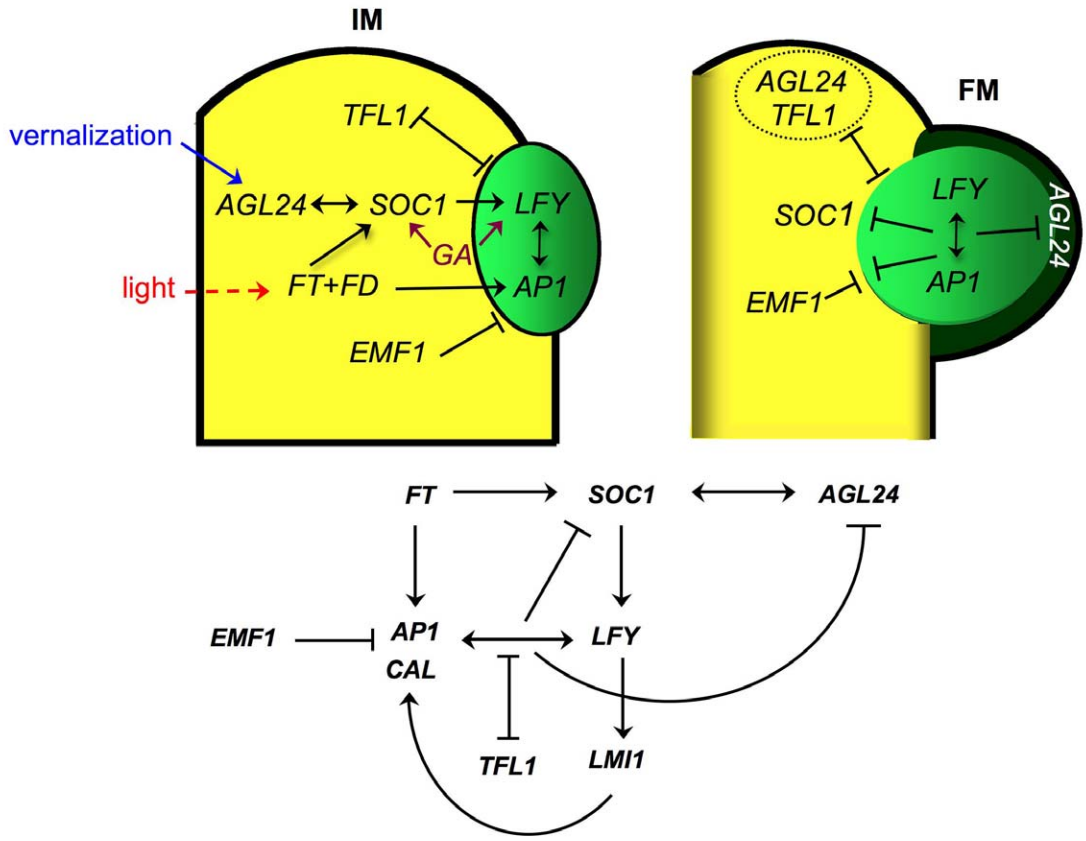


Figure 9. Inflorescence shoot apical meristem (IM) versus flower meristem (FM).

Simplified model of a gene regulatory network (GRN) that induces and maintains the FM. Flowering induction genes like *FT, SOC1* and *AGL24* are highly expressed in the IM in response to external (vernalization and light) and internal (gibberellins; GA) signals. These proteins in turn promote the expression of flower meristem identity (FMI) genes, *LFY* and *AP1*. Paradoxically, during the establishment of the FM, genes like *TFL1* and *EMF1* that help to maintain the IM identity are also expressed, keeping the expression of the FMI genes out of the IM. Later in development, LFY and AP1 repress the expression of *TFL1* and flowering genes *SOC1* and *AGL24*, among others, thus maintaining the FMI. Arrows and bars indicate positive and negative regulatory interactions respectively. (See references in main text).

Doonan, 2003). The *UFO* gene encodes a protein containing an F-box domain, which is a characteristic of E3 ubiquitin ligases that are components of SCF (Skp Cullin F-box containing) complexes and mark proteins for proteosome-dependent degradation (Deshaies, 1999). It was recently shown that LFY interacts with UFO in order to directly bind the *AP3* promoter. Furthermore, the proteosome activity mediated by UFO is required for the transcriptional activation of *AP3* by LFY (Chae et al., 2008).

Key components of the GRN that underlies the early patterning of the flower meristem are the so-called ABC homeotic genes, *AP1*, *AP2*, *AP3*, *PI*, and *AG*, which are all transcription factors belonging to the MADS-box gene family, except *AP2* (Coen and Meyerowitz, 1991; Wagner et al., 1999; Ng and Yanofsky, 2001; Lamb et al., 2002).

The classic ABC model was inferred using Arabidopsis and *Antirrhinum* homeotic flower mutants (Coen and Meyerowitz, 1991). In these mutants two floral organ types are replaced by two other floral organ types as follows: A- class mutant flowers have carpels-stamens-stamens-carpels (from the outermost to the innermost whorl), B-class mutant flowers have sepals-sepals-carpels-carpels, and C-class mutant flowers have sepals-petals-petals-sepals (Coen and Meyerowitz, 1991). It was shown that mutations in all three functions lead to the transformation of all floral organs into leaf-like organs, suggesting that flowers are modified leaves (reviewed in Robles and Pelaz, 2005). The Arabidopsis ABC mutants are shown in Figure 11.

Hence, three different classes of homeotic genes with overlapping activities were proposed to be necessary for floral organ specification. The A function specifies sepals, the A and B functions specify petals, the B and C functions specify stamens and the C function specifies carpels (Figure 12; Bowman et al., 1991). The A and C functions negatively regulate each other and the B function is restricted to the second and third whorls. The latter was originally thought to be independent of A and C functions (Bowman et al., 1991; Drews et al., 1991), but it was later shown that the A function gene *AP1* regulates the B genes. AP1 binds to the promoter of *AP3* (Hill et al., 1998; Tilly et al., 1998). AP1 can also specify petals by regulating the spatial domain of B genes together with UFO in the first flowers to arise, and independently of UFO in later flowers (Ng and Yanoksky, 2001).

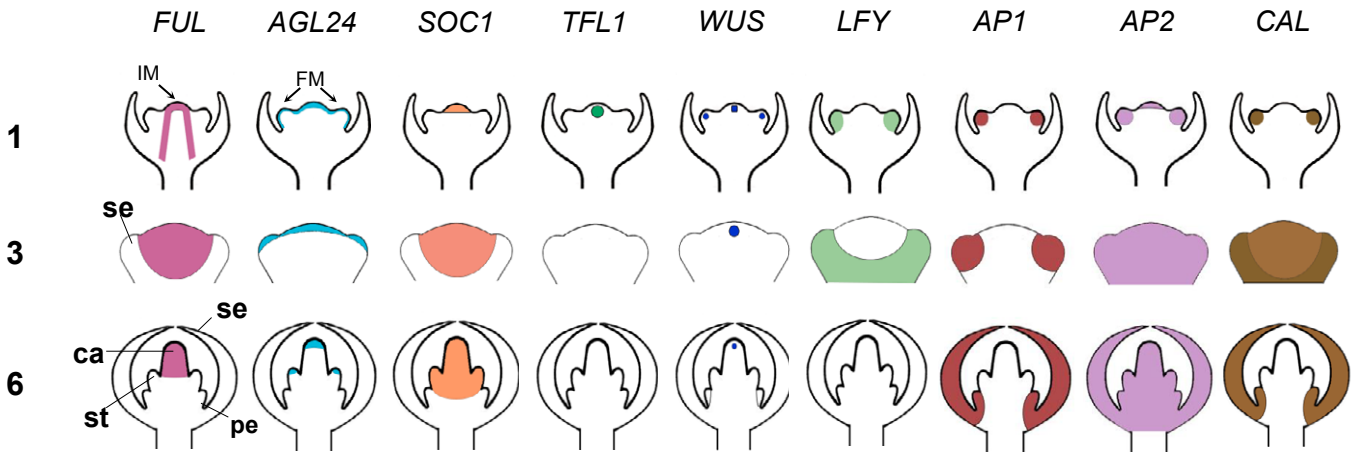


Figure 10. Schematic representation of some inflorescence shoot apical (IM) and flower (FM) meristem gene expression patterns at stages 1, 3 and 6.

Flowering (*FUL, AGL24* and *SOC*), indeterminate (*WUS* and *TFL1*), and FMI (*LFY, AP1, AP2* and *CAL*) gene expression patterns based on in situ hybridization data during floral primordium developmental stages 1, 3 and 6. At stage 1, expression patterns correspond to their functions in IM and FM identities. Sepal (se), petal (pe), stamen (st) and carpel (ca) primordia are indicated. At stages 3 to 6, all with the exception of *TFL1* are expressed in the FM, probably because their respective proteins also affect organ development. FUL will participate in fruit development, LFY will induce all the ABC genes and AP1 and AP2 are fundamental in sepal and petal formation (see references in main text).

Once identified at the molecular level, the mRNA expression patterns of the ABC genes were shown to overlap with the floral regions where the corresponding mutants had a phenotype (Yanofsky et al., 1990; Mandel et al., 1992; Goto and Meyerowitz, 1994; Jack et al., 1994). *AP1* and *AP2* are A-function genes. *AP1* is expressed in the two outer whorls of the floral meristem (Figures 10, 12, 13A; Mandel et al., 1992) and is important for the establishment of sepal and petal identity as well as the FM (section 3.2). *AP1* expression is first up-regulated by LFY and FT/FD (section 3.2), but later is maintained by the B class genes in a positive feedback loop (Sundström et al., 2006). Strong *ap1* alleles (*ap1-1*) often lack petals in the second whorl, while weaker mutant alleles of this gene do not have a full homeotic conversion of floral organs (see section 3.2; Irish and Sussex, 1990).

In contrast to the MADS-box ABCs, the expression pattern of *AP2* does not correlate with the site where it exerts its function in floral organ identity. *AP2* mRNA is found throughout the flower meristem (Figures 10 and 12; Jofuku et al., 1994). Recent data has shown that *AP2* is repressed at the translational level by a microRNA (miR172), which is active only in whorls 3 and 4 (Chen, 2004), thus explaining why the function of AP2 is restricted to the first two whorls of flower organs. In a recent experiment using double mutants of *ag* and an *ap2* allele, which is insensitive to repression by miR172, it was shown that both *AG* and miR172 independently downregulate *AP2,* but miR172 is more important than *AG* (Zhao et al., 2007). *ap2* mutants rarely develop petals and their sepals are transformed into carpelloid structures due to ectopic *AG* expression (Figure 11), which is negatively regulated by AP2 itself (Drews et al., 1991). AP2 is also implicated in the upregulation of the B genes, *AP3* and *PI* (Zhao *et al*., 2007).

The B class genes (*AP3* and *PI*) are expressed in the second and third whorls and mutant flowers of any or both of these two genes lack petals and stamens, as predicted in the ABC model (Figure 11, 12 and 13; Coen and Meyerowitz 1991; Goto and Meyerowitz, 1994; Jack et al., 1994; Honma and Goto, 2000). The fact that both single mutants yield the same phenotype shows their interdependence. *AP3* and *PI* are regulated in two steps: they are first induced by LFY/UFO in response to flowering signals and they later maintain their expression in a self-regulatory loop (Honma and Goto, 2000). The proteins encoded by these two genes form heterodimers to exert their B function during petal and stamen development (Figure 14; Jack et al., 1992; Goto and Meyerowitz, 1994; Zik and Irish, 2003a) and this oligomerization is necessary for them to move into the nucleus (McGonigle et al., 1996).

Both genes are also regulated positively in a regulatory loop by *AP1* and negatively by *EARLY BOLTING IN SHORT DAYS* (*EBS*), a gene that encodes a nuclear protein that participates in petal and stamen development and regulates flowering time by repressing *FT* (Gómez-Mena et al., 2001; Piñeiro et al*.,* 2003*). ANT*, a member of the *AP2* gene family, is another regulator of the B function, positively inducing *AP3* (Klucher et al., 1996; Nole-Wilson and Krizek, 2006; see section 3.4.2).

The only C-type gene discovered up to now is the MADS-box gene *AG* (Bowman et al., 1989). *ag* mutant flowers lack stamens and carpels, and also bear indeterminate flowers with reiterating sepals and petals (Figure 11), suggesting that *AG* is important for floral meristem determinancy (see section 3.2), besides its role in stamen and carpel identity (Yanofsky et al., 1990; Mizukami and Ma, 1997). The regulation of *AG* has been much studied; at least ten proteins repress and five activate it to maintain its expression in the appropriate whorl (Figures 12 and 13A).

AG is repressed by a transcriptional co-repressor complex formed by *LEUNIG* (*LUG*) and *SEUSS* (*SEU*) (Figure 15; Franks et al., 2002). *LUG* encodes a transcription protein similar to *TUP1* from yeast and interacts with *SEU,* which encodes a plant specific protein (see Table S1; Conner and Liu, 2000; Franks et al.,

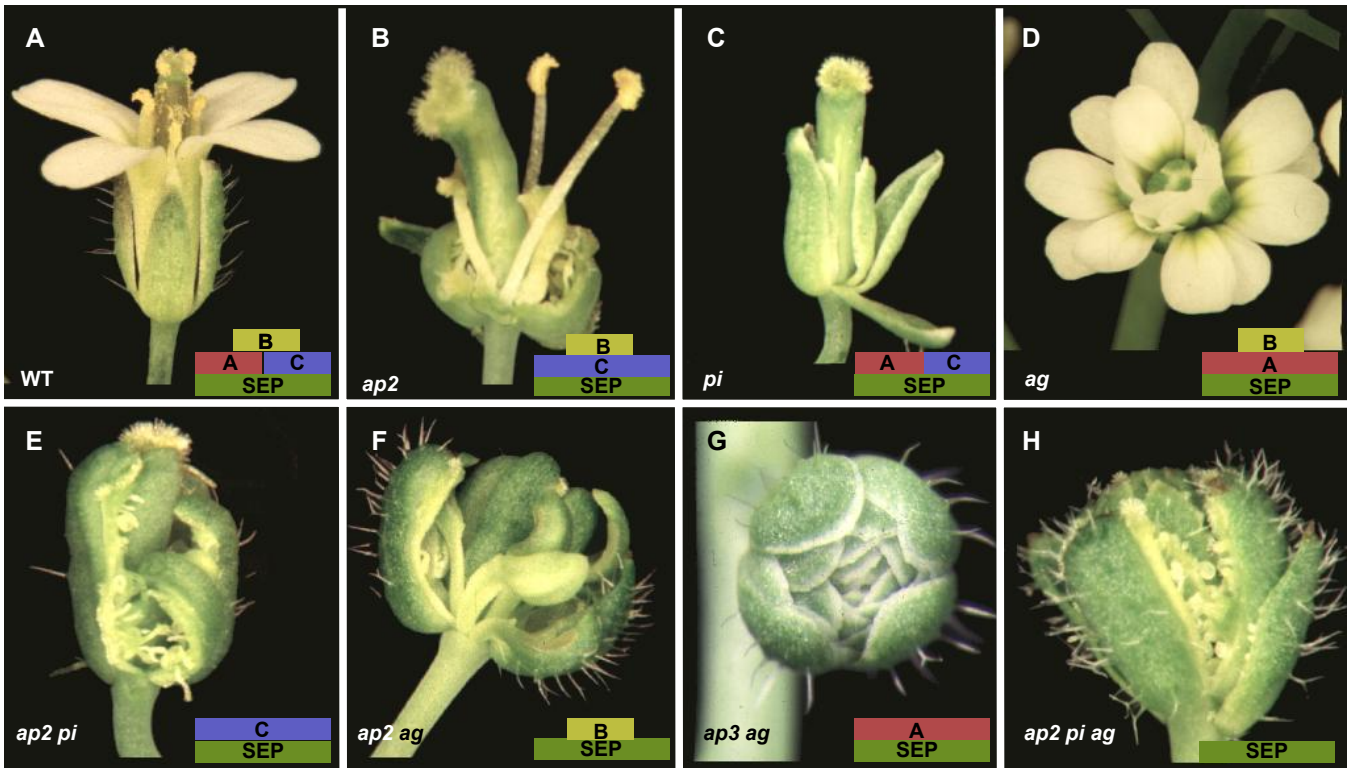


Figure 11. Arabidopsis ABC homeotic floral mutants.

Photos of single, double and triple ABC gene mutant flowers. Each photo is accompanied by a small diagram where rectangles represent the A (*AP1* and *AP2*), B (*AP3* and *PI*), and C (*AG*) combinatorial transcriptional regulatory functions and the *SEP* (*1, 2, 3, 4*) genes active in these mutants. Organs are listed below from the outer to the inner whorl unless stated otherwise.
(A) Wild-type (WT) flower.
(B) Single *ap2* mutant flower composed of carpelloid sepals, stamens, stamens and carpels.
(C) The *pi* mutant has flowers composed of sepals, sepals, carpels and carpels.
(D) The *ag* flower has the stamens transformed into petals and the carpels are replaced by another flower repeating the same pattern.
(E) The *ap2 pi* double mutant displays flowers composed only of sepalloid carpels.
(F) The *ap2 ag* flowers have leaf-like organs in the first and fourth whorls and mosaic petal/stamen organs in the second and third whorls.
(G) *ap3 ag* double mutants produce flowers composed of repeated whorls of sepals.
(H) The *ap2 pi ag* mutant has leaf-like organs with some residual carpel properties. (Photographs provided by Dr. J. Bowman).

2002; Sridhar et al., 2004). Neither of these proteins are able to bind DNA sequences and AP1 and SEP3 recruit SEU/LUG to the second intron of *AG* to perform their inhibitory function and prevent the ectopic expression of *AG* (Sridhar et al., 2006). Recently, another transcriptional repressor of *AG* was identified, *LEUNIG_HOMOLOG* (*LUH*). This gene is the closest homolog of *LUG* and its inhibitory function on *AG* is completely dependent on SEU (Sitaraman et al., 2008).

Another repressor of *AG* is BELLRINGER (BLR), a homeodomain protein that binds to regions in the second intron of *AG* and prevents ectopic *AG* expression in the two outer whorls of the flower (see Table S1; Bao et al., 2004). *AG* is also negatively regulated epigenetically by a histone acetyltransferase *GCN5* (Bertrand et al., 2003). Other genes that participate in floral organogenesis are repressors of *AG*, namely *RABBIT EARS* (*RBE*, see section 3.4.4), *ANT* and *STERILE APETALA* (*SAP*) (see Table S1). *AG* is also positively regulated at the post-transcriptional level by several *ENHANCER OF AG-4* (*HUA*) and *HUA ENHANCER* (*HEN*) genes. All of these genes play a major role in pre-mRNA processing of *AG* (Cheng et al., 2003).

The ABC proteins exert their regulatory function as multimers. In *Antirrhinum majus,* a ternary complex between A and B function proteins was found to bind CArG DNA boxes more efficiently than single proteins (Egea-Cortines et al., 1999). More specifically, a higher-order complex consisting of SQUAMOSA *(SQUA,* the *AP1* ortholog*)*, DEFICIENS, and GLOBOSA *(DEF* and *GLO* are *A. majus AP3* and *PI* orthologues, respectively) bound DNA more efficiently than DEF/GLO or SQUA alone (Egea-Cortines et al., 1999). These results suggest that transcriptional complexes that combine A and B function proteins are more stable than those formed with proteins of any one function alone.

The fact that the ABC genes are necessary but not sufficient to determine floral organ identity was later confirmed in Arabidopsis. Honma and Goto (2001) used a yeast three-hybrid method to

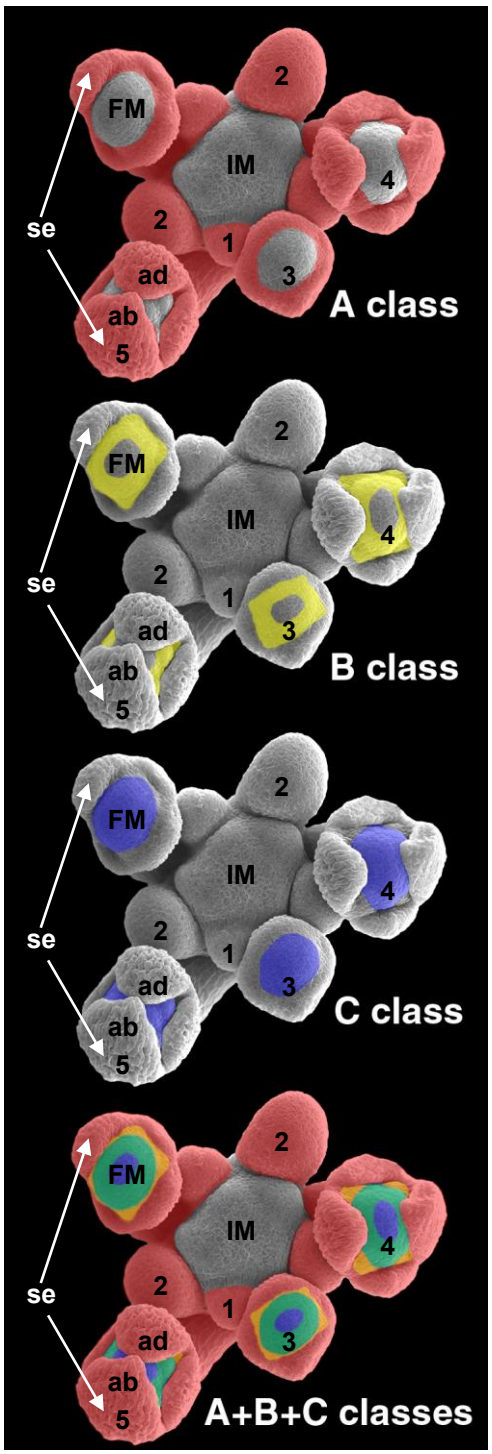


Figure 12. Expression patterns of the ABC genes during early stages of *Arabidopsis* flower development.

SEM of meristems have been colored to show expression patterns of **A class** (red, outer whorls), **B class** (yellow, petal and stamen primordia) and **C class** (blue, inner whorls) genes. Five flowers at early stages of development are marked 1 to 5 (5 being the oldest). Inflorescence shoot apical meristem (IM), floral meristem (FM) and sepals (se): adaxial (ad) and abaxial (ab) are indicated. (Photographs provided by Dr. J. Bowman).

show that SEP3 and AP1 are able to interact with the heterodimer AP3/PI but not with AP3 or PI alone. Moreover, they described this interaction as essential, since the heterodimer AP3/PI lacks the activation domain necessary for a transcription factor to function, a domain which both SEP3 and AP1 possess (Honma and Goto, 2001). These findings suggest that the inclusion of SEP3 or AP1 together with AP3/PI could result in an active tetrameric transcriptional complex (Figure 14). It was also demonstrated that the ABC proteins on their own or combined according to the ABC model (A, AB, BC, or C) were not sufficient to determine floral organs when expressed in leaves under the action of the 35S constitutive promoter (Pelaz et al., 2001). However, floral organs could indeed be recovered in leaves once appropriate combinations of genes were expressed (Honma and Goto, 2001; Pelaz et al., 2001).

The *SEP* genes received their names because the floral organs that develop in all four whorls in triple *sep* mutants resemble sepals (Pelaz et al., 2000). This *sep1 sep2 sep3* triple mutant phenotype is markedly similar to that of double mutants that lack both B and C class activity, such as *pi ag* and *ap3 ag* (Figure 11G; Bowman et al., 1989; Pelaz et al., 2000) in which the floral meristem becomes indeterminate as well. Single or double mutants for these *SEP* genes yield flowers indistinguishable from wild type, thus suggesting that the three *SEP* genes are functionally redundant and are important in determining three of the four floral organs: petals, stamens, and carpels (Honma and Goto, 2001; Pelaz et al., 2001; Robles and Pelaz, 2005).

Given that the triple *sep1 sep2 sep3* mutant does not show alterations in sepal identity, an additional gene is likely to be involved in sepal specification. Indeed, another *SEP*-like MADS-box gene, *SEP4* (previously *AGL3*), has now been characterized (Ditta et al., 2004), and the quadruple *sep1 sep2 sep3 sep4* mutants produce flowers with leaf-like organs in all whorls, thus confirming the *SEP* genes contribute to each floral organ identity (Figure 14). Coincidently, *SEP* genes are expressed in the whole floral meristem during flower development (Figure 13B; Flanagan and Ma, 1994), are important in regulating B and C gene expression (Liu et al., 2009), and encode proteins that apparently interact with the ABC proteins (Figure 14; Robles and Pelaz, 2005).

Based on data from *Antirrhinum* and yeast two-hybrid and three-hybrid protein interactions, and on the phenotypes of the ABC mutants, three models have been proposed to explain how the MADS domain proteins interact to constitute functional transcriptional complexes and bind DNA. None of the models completely explains the experimental data available, but the quartet model seems the most plausible (Jack, 2001; de Folter et al., 2005). This model proposes that MADS domain proteins form tetrameric complexes during floral organ determination (Figure 14; Theissen, 2001; Theissen and Saedler, 2001; Becker and Theissen, 2003; Jack, 2004). Within each transcriptional complex, there would be two MADS dimers, each one binding to a single CArG-binding site causing the DNA of the promoter region to bend, enabling the MADS dimers to act cooperatively in a tetrameric complex to regulate the gene. For example, binding of one dimer within the tetramer to DNA could increase the affinity of the second dimer for local DNA binding (Melzer et al., 2009). Besides, one of the dimers could function as the activation domain of the tetramer allowing for efficient transcriptional activation (Honma and Goto, 2001). Interestingly, several dimers

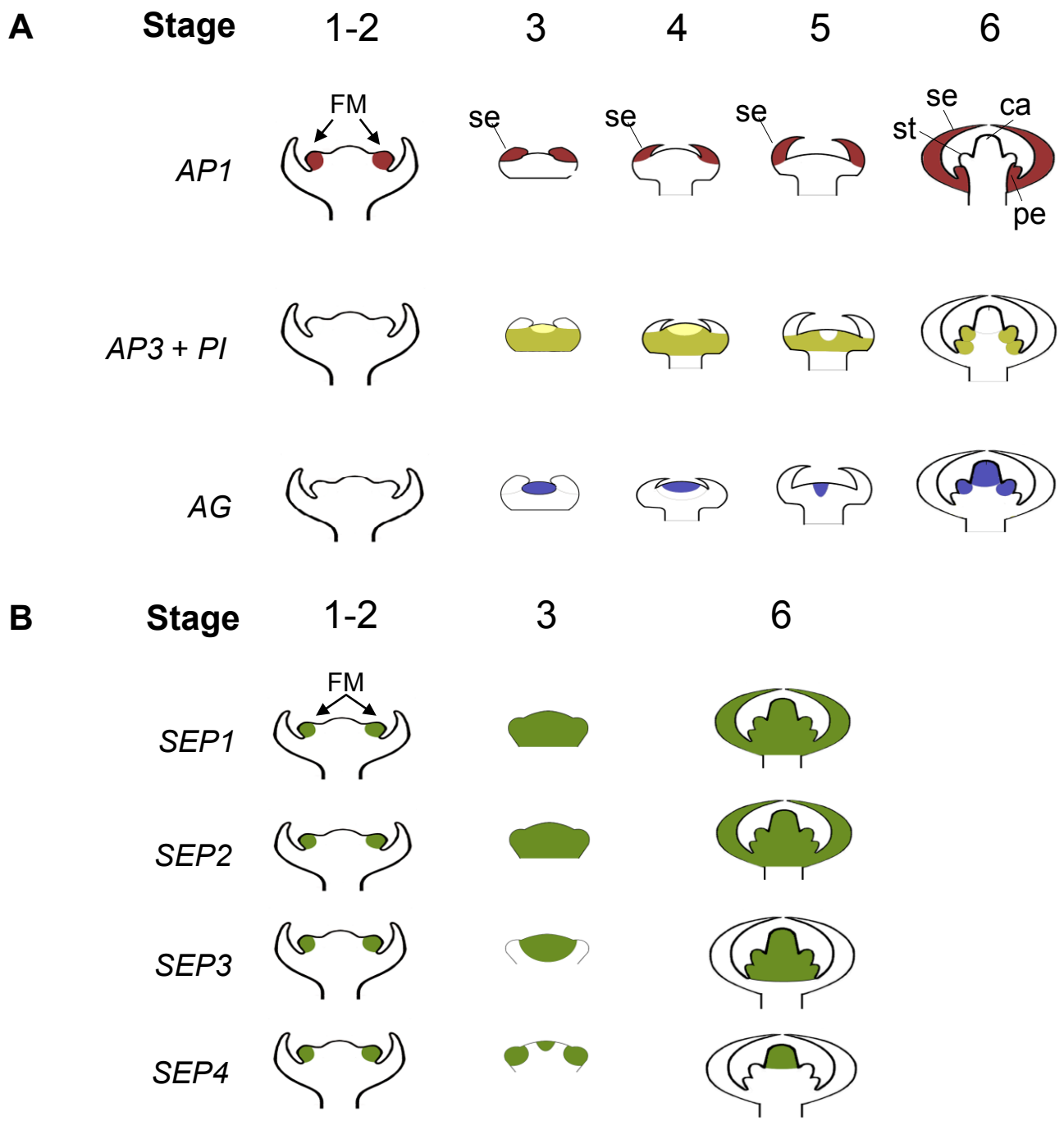


Figure 13. Diagram illustrating mRNA expression patterns of Arabidopsis ABC and SEP genes during different stages of flower development.

(A) ABC gene expression patterns illustrated from stage 1 to 6. The A function gene *AP1* is expressed (red) in the two outer floral primordia whorls that will later develop into sepals (se) and petals (pe) (Mandel et al., 1992; Gustafson-Brown et al, 1994; Parcy et al., 1998). The A function gene *AP2* is expressed in all four whorls of the flower (see figure 10; Jofuku et al., 1994). The B function genes (dark yellow) *AP3* and PI are expressed from stage 3 in the next two inner whorls of the flower (Weigel and Meyerowitz, 1993; Parcy et al., 1998). Interestingly *PI* is also expressed at stages 3 and 4 in cells that will generate the fourth whorl (light yellow). After stage 5, the pattern of PI expression largely coincides with that of *AP3*, only in petal and stamen (st) primordia (Goto and Meyerowitz, 1994). The C function gene AG is expressed (blue) in the two inner whorls that will become the stamens and carpels (ca) (Yanofsky et al., 1990; Gustafson-Brown et al., 1994; Parcy et al., 1998; Ito et al., 2004).

(B) SEP gene expression pattern during several stages (1 or 2, 3 and 6) of flower development. *SEP1* and *SEP2* are expressed in all whorls of the flower (Savidge et al., 1995). *SEP3* is first detected in late stage 2 flower primordia and afterwards in petal (pe), stamen (st), and carpel (ca) primordia. The expression pattern at stage 6 was deduced that from at stage 7 (Mandel and Yanofsky, 1998). *SEP4* is weakly expressed in sepal primordia at stage 3 and strongly expressed in carpel primordia from stage 3 to 6. (Ditta et al., 2004). Both figures have been modified and expanded from Krizek and Fletcher (2005).

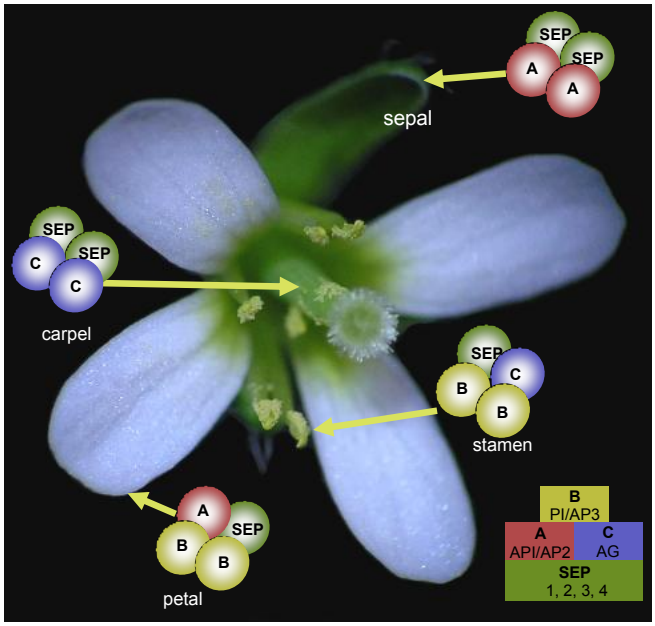


Figure 14. Schematic representation of the interaction of ABC and SEP proteins in the quartet model for *Arabidopsis* floral organ specification.

Possible MADS-domain protein complexes (circles) of the ABC model are sufficient for the specification of each of the four floral organs. In the ABC model, rectangles represent the A (*AP1* and *AP2*), B (*AP3* and *PI)*, and C (*AG*) combinatorial transcriptional regulatory functions necessary for sepal, petal, stamen and carpel primordia specification. The green rectangle below represents the SEP (1, 2, 3 and 4) proteins that interact with proteins encoded by the ABC genes (unknown for AP2 which has not been tested) to specify each floral organ (modified from Bowman et al., 1993; Robles and Pelaz, 2005).

and potential tetramers have been documented in a complete Arabidopsis MADS-domain family protein-protein interactome via yeast two-hybrid interactions (de Folter et al., 2005). This data base has been updated with a yeast three-hybrid screen for MADS-domain proteins (Immink et al., 2009). Future studies should test which of the complexes inferred from the MADS interactomes are functional and what their roles are during Arabidopsis development.

3.3.1 Target genes of the ABCs

Target genes of the ABC genes link the floral organ specification - gene regulatory network (FOS-GRN) with processes in organ primordia establishment and development (for review of MADS target genes, see de Folter and Angenent, 2006). Among the direct targets of the ABC genes, transcriptional regulators and hormone-related genes are prominent (Sablowski, 2009). But the sets of target genes change as organ development progresses; at later stages of floral organ development, several components of what could be common modules have been found that are involved in generic developmental processes (see below) during sepal, petal, stamen and carpel development. Finally, multiple genes having cell-specific roles are turned on especially during stamen and carpel development that is much more complex than perianth development (Sablowski, 2009).

The first examples of genes regulated by the ABC genes were two MADS-box genes, *AGL1* and *AGL5* (renamed the *SHATTERPROOF* genes (*SPH1* and *2*, respectively). There is virtually no expression of either gene in *ag* mutants (Savidge et al., 1995). *SHP2* is only expressed in carpels and *AG* can activate an *SPH2*:*GUS* reporter construct; furthermore, *AG* binds its promoter in vitro (Savidge et al., 1995).

The first non-ABC gene identified as a target of a MADS A, B or C protein was *NAC-LIKE ACTIVATED BY AP3/PI* (*NAP*), a target of the AP3/PI complex. It is important for the transition between cell division and cell expansion during petal and stamen development (see section 3.4.5 and Table S1; Sablowski and Meyerowitz, 1998).

A recent study identified two genes negatively regulated by AP3/PI, *GATA NITRATE INDUCIBLE, CARBON-METABOLISM-INVOLVED* (*GCN*) and *GCN-LIKE* (*GNL*), a GCN paralog (Mara and Irish, 2008). Both genes regulate chlorophyll biosynthesis in plant cells. Thus, their downregulation could be important in preventing chlorophyll accumulation in petals and anthers. The same study shows that both *GNC* and *GNL*, together with the B class genes, regulate the expression of a number of other GATA-motif-containing target genes like *HEXOKINASE1* (*HXK1*; Mara and Irish, 2008).

SUPERMAN (*SUP*; Bowman et al., 1992) is upregulated by AP3/PI and AG and by *LFY* (Riechmann et al., 1996; Sakai et al., 2000). *SUP* encodes a transcription factor with a C2H2-zinc finger motif and is involved in the maintenance of the stamen/carpel whorl boundary (Sakai et al., 2000; Dathan et al., 2002; see section 3.4.2). While the B genes and *LFY* seem to regulate early *SUP* expression, *AG* and the B genes are involved in maintaining its expression in flowers from stage 5 onward (Sakai et al., 2000).

Recent microarray experiments have proved useful in revealing new targets of the ABC MADS homeotic genes, as well as many putative components of the complex networks involved in floral organogenesis. For example, it was shown that the AP3/PI dimer regulates, directly or indirectly, 47 target genes. Only two of these are transcription factors, while most participate in basic cellular functions required for stamen and petal development (Zik and Irish, 2003a). By contrast, AG controls, directly or indirectly, the expression of 149 genes most of which are transcription factors, including other members of the MADS-box gene family. Ten of these were also shown to be direct targets of AG using chromatin immunoprecipitation (ChIP), including *AG* itself, *AP3*, *CRC* and *ATH1*, a gene that encodes a *BELL*-type homeodomain protein that participates in the development of the basal region of shoot organs (Gómez-Mena et al., 2005).

A more exhaustive experiment used four homeotic mutants (*ap1*/*ap2*, *ap3*, *pi* and *ag*) in two types of microarray assays: a whole genome microarray with approximately 26,090 gene-specific oligonucleotides and a flower specific-cDNA microarray with 5,000-6,000 genes. To summarize the assay results, transcription factors were neither over or underrepresented as being regulated by the ABC genes; on the contrary, genes involved in general cellular maintenance (DNA recombination and protein synthesis) were underrepresented. Genes specifically expressed in each of the four different whorls were identified: 13 genes for sepals, 18 for petals, 1162 for stamens, and 260 for carpels. As expected

from their structural and cellular complexity, the reproductive floral organs had many more specific target genes than the perianth organs (Wellmer et al., 2004; Sablowski, 2009).

In another genomic study of early floral stages it was found that many genes were downregulated in incipient floral primordia while many of them were activated during the differentiation of floral organs (Wellmer et al., 2006). However, some genes were overrepresented during all stages analyzed (i.e. transcription factors including the family of MADS-box genes, *PIN* dependent auxin transport genes, as well as auxin and GA metabolism genes). Even though the MADS box genes were overrepresented, the promoter regions of the genes expressed during these different stages are not enriched in CArG-box sequences compared to random samples from the whole genome. This result suggests that MADS-domain transcription factors may be able to bind sequences other than CArG motifs, or that they have few direct targets during the developmental stages analyzed (Wellmer et al., 2006).

In a different approach, an inducible post-translational version of *AG* was used in gene expression profiling to detect AG target genes. One of the genes identified that is upregulated by AG is *SPOROCYTELESS* (*SPL*). AG is able to bind *in vitro* to the 3' region (downstream of the stop codon) of the *SPL* gene (Ito et al., 2004). *SPL* has been described as a key regulator of sporogenesis later during stamen and carpel development (see sections 3.4.5, 3.4.6 and Table S1; Schiefthaler et al., 1999; Yang et al., 1999).

3.4 Floral Organogenesis

The challenge of inferring the topology of the gene regulatory network (GRN) underlying the establishment of floral organ primordia, and their development (cell differentiation, morphogenesis and growth) is still ahead. However, some key components and GRN functional modules characterized to date are summarized in Section 4. Such modules involve several functional feedback loops and underlie different generic developmental processes mainly: primordia type specification; delimitation; floral organ primordia positioning that depends on fundamentally on auxins; primordia number; inter-whorl and within-whorl boundaries; and primordia and organ adaxial-abaxial polarity (Figures 2C and 15; Irish, 2008). At later stages of floral organ development, subcellular differentiation and patterning, as well as overall organogenesis takes place and more specific regulatory modules are involved. The genes within such modules are treated separately for each organ type (Figures 16-17).

As a precursor to integrating GRN modules in the above categories, we now provide a synthesis of the molecular genetic studies of how such generic developmental processes are regulated. Several of these have also been identified as important regulators of leaf development, substantiating the proposal of Goethe that all plant organs are elaborations or modifications of a core leaf-like developmental program (for review of common pathways see Sablowski, 2009). ABC floral organ identity genes are also important in fine-tuning or coordinating the role of genes involved in some of the generic developmental modules during flower development (Figure 15; Sablowski, 2009). Some genes participate in more than one process or module and are important for making connections between different GRN modules. In such cases, they are considered in more than one category.

Regulatory modules controlling distinct components of floral organ development have been elucidated to different extents depending on available mutant phenotypes. In correlation with anatomical and morphological complexity, the size and complexity of the regulatory modules underlying stamen and carpel development are much greater than those that regulate sepal or petal development. Carpel development is covered in the "Fruit Development" chapter (Roeder and Yanofsky, 2006) in this book, and is only briefly considered here.

In the flower meristem, normal organogenesis depends upon a homeostatic equilibrium between stem cell specification and cellular differentiation (Green et al., 2005). Plant morphogenesis is influenced both by the orientation and rate of cell division, as well as by cell expansion and differentiation (see section 2 for a description of floral organ initiation and morphogenesis). How the molecular aspects of these processes are coordinated has been very difficult to elucidate. However, it is generally accepted that cells in meristematic regions respond to positional information important for inducing and controlling morphogenesis (Sussex, 1954; 1955; Meyerowitz 1997; Hauser et al., 1998). One of these positional signals is auxin (see Section 3.4.1; Reinhardt et al., 2000; Benková et al., 2003; Reinhardt et al., 2003; de Reuille et al., 2006). Several mutations that affect the number, size, and/or shape of one or several floral organs have also been characterized. Some of these phenotypes are pleiotropic consequences of mutations in genes acting from earlier steps of plant and flower development. Others are the result of alterations in organ specific genes (Figures 16-17). An extensive list of genes involved in flower organ morphogenesis with their inferred functions, mutant phenotypes and mRNA expression patterns is given in Table S1.

3.4.1. Floral meristem and organ primordia positioning: the role of auxin

The shoot apical meristem produces leaves and then flowers in a highly predictable and regular phyllotactic pattern (Tanaka et al., 2006). One of the key compounds that regulate this developmental process is the hormone auxin (Reinhardt et al., 2000). Increased auxin levels mark the initiation sites for organ primordia (including those of floral organs) and local application of auxin is sufficient to trigger leaf or flower formation in the shoot apex (Reinhardt et al., 2000; Tanaka et al., 2006). Once the primordium is established, there is a depletion of auxin around it and another peak of auxin is only able to form in cells at a specific distance from pre-existing primordia, generating a phyllotactic pattern (Reinhardt et al., 2000; Reinhardt et al., 2003; de Reuille et al., 2006; Tanaka et al., 2006; Berleth et al., 2007; Kuhlemeier, 2007). After initiation, the primordium grows by cell proliferation and cell expansion, and the organ differentiates along the apical-basal and dorsal-ventral axes (Heisler et al., 2005; Golz, 2006).

The overall distribution of auxin depends on its biosynthesis, metabolism, and directional transport. Most auxin is synthesized in young tissues of the shoot and distributed throughout the plant by two physiologically distinct pathways. One of them is passive and occurs only by diffusion through the mature phloem. The other one is an active polar auxin transport (called PAT) that mediates cell-to-cell movement of auxin through two different types of

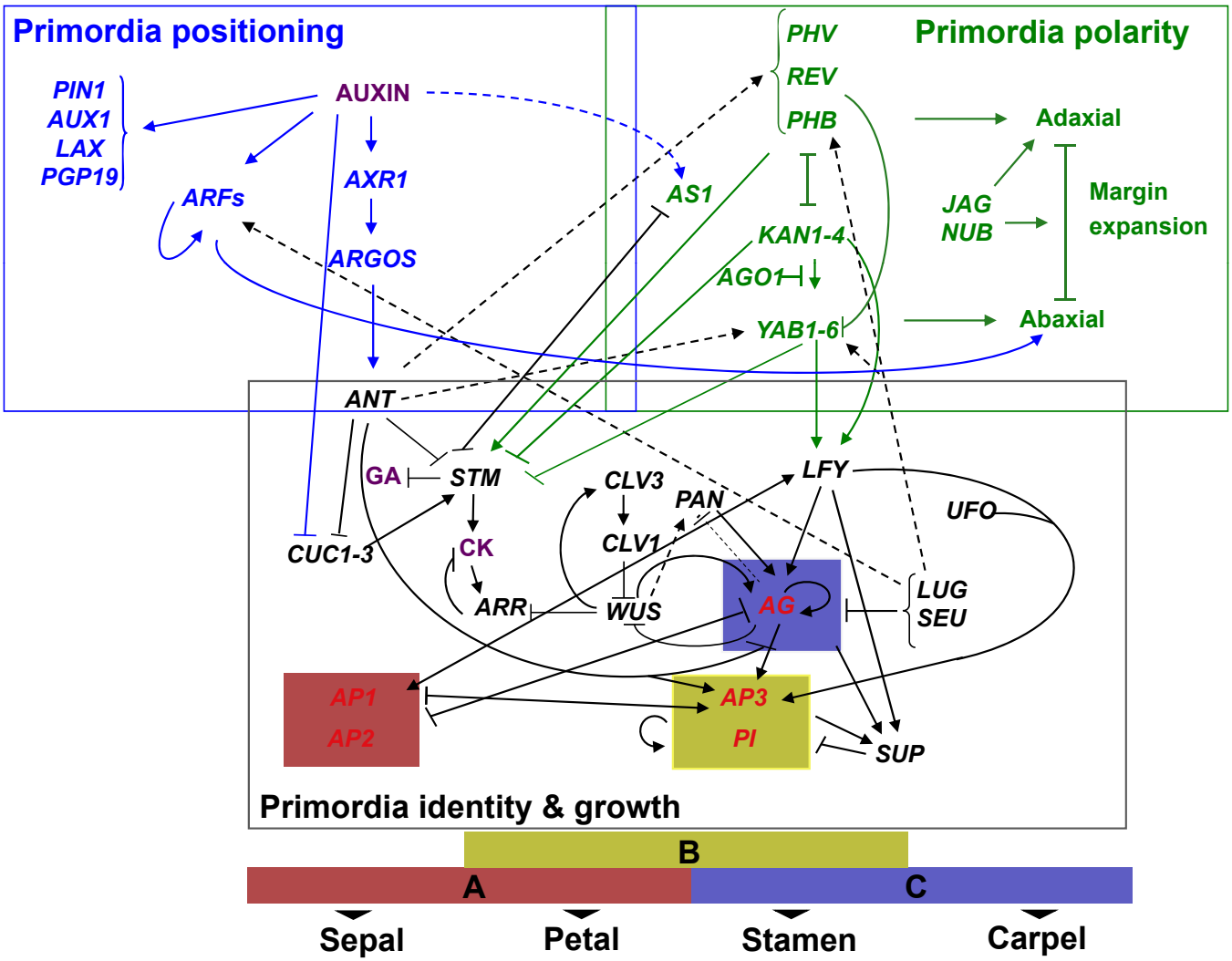


Figure 15. Functional gene regulatory modules during early flower development.

Common molecular modules act during early meristem morphogenesis from the SAM both before and after reproduction. During floral organogenesis, these modules interact among themselves and with the FOS-GRN that includes the floral homeotic genes. Anlagen positioning in the SAM flanks depends on auxin gradients. Transport and signal transduction proteins, as well as other factors regulated by auxins (letters in blue), participate in the establishment of such gradients and finally determining the position of primordia. The auxin pathway also downregulates some members of the NAC family (*CUC1* to *3* are important for organ boundary establishment), which also participate in the positive regulation of *STM* and *KNOX* genes. Since *WUS* maintains the apical meristem stem cells in a proliferating state with CLV proteins that in turn regulate its expression in a non cell-autonomous negative-positive feedback loop, and *STM* prevents meristem cell differentiation by inducing the production of cytokinins (CK) and the *ARR* transduction pathway (see text), floral primordia may emerge if cells in the anlagen are able to downregulate *STM*. This can be achieved by the action of AS1 and ANT. Upregulation of *LFY* by the flowering genes (Section 3.2; Figure 9) in conjunction with some KAN and YAB proteins, activate the expression of ABC homeotic genes (in red) for the establishment of the floral organ primordia identity and growth (gene acronyms in black, see text and Table S1 for full names). Lateral organ primordia acquire apical/basal, lateral/medial and adaxial/abaxial polarities by the action of protein families that include PHABs (PHB, PHV and REV), KANs (KANADI1-3, ATS/KAN4), YABs (FIL/YAB1, YAB2, YAB3, INO/YAB4, YAB5 and CRC/YAB6), JAG and NUB (letters in green). Some of these are organ-specific while others are shared by different floral organ primordia (see section 3.4). Not all the genes involved in each module are depicted, just some of the most representative ones, which help us to understand how they are interconnected. Arrows and bars indicate positive and negative regulatory interactions, respectively, and dashed lines a postulated interaction not yet proven. The text color used for the gene names in each module is the same as in Figures 16, 17, and 19 where specific organ developmental processes are summarized and the ABC genes are shown in boxes on the organ specified as in the model shown below. Hormones are in purple.

This figure was composed partially from information in Clark (2001b), Blazquez et al. (2006), Hord et al. (2006), Shani et al. (2006) and Feng and Dickinson (2007).

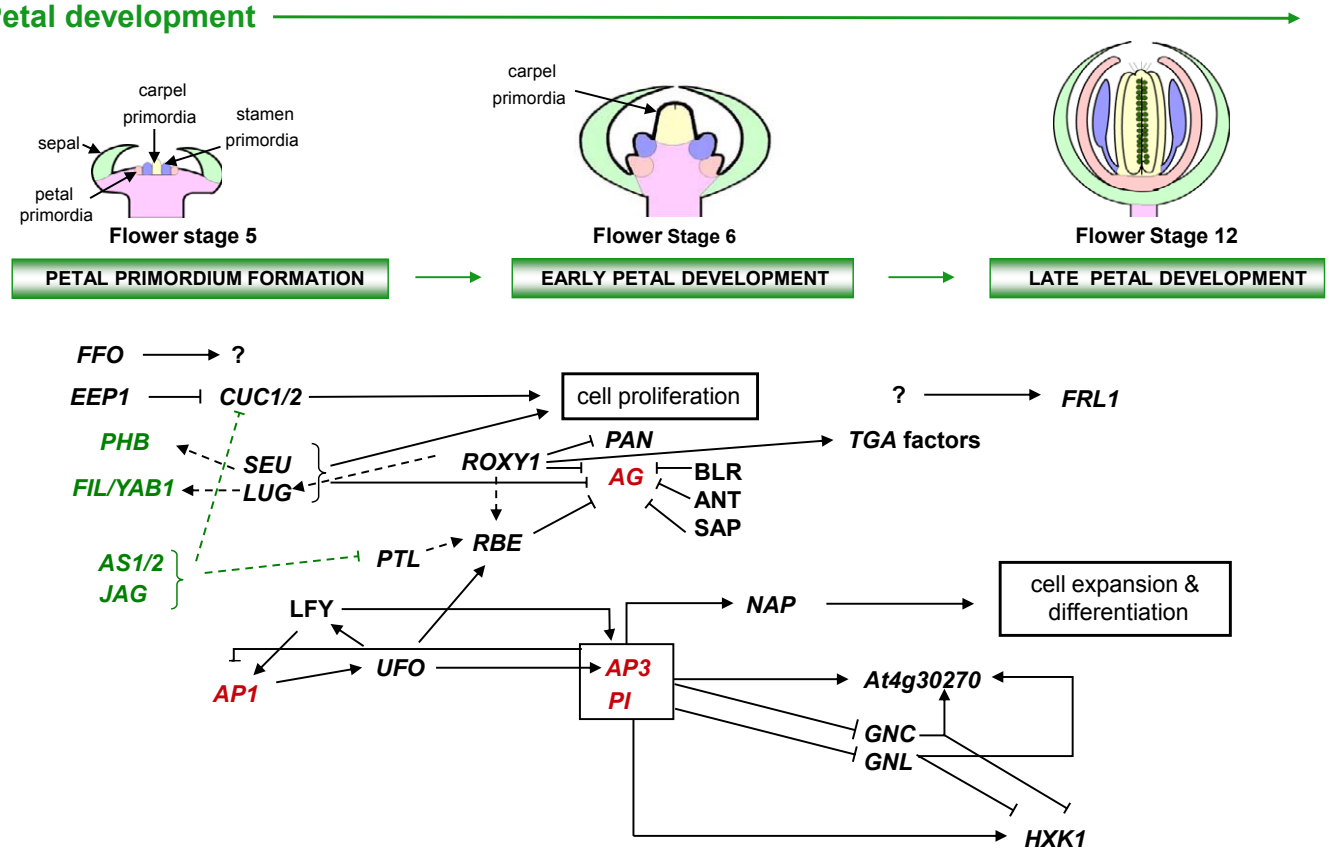


Figure 16. Main stages of petal development and some genes involved.

Schemes at the top illustrate three different stages of petal development (for details see section 2). Briefly, GRN modules (genes) in petal development include those involved in the establishment of the second whorl domain, the specification of petal identity and cell differentiation. *CUC* genes under the regulation of miR164c are involved in establishing whorl boundaries. Genes involved in polarity determination like *JAG*, *PHB* and *YAB1* are also necessary for petal development. A, B and *SEP* genes, and the absence of C genes, determine petal identity (AP2 and SEP genes are not shown here for clarity; see Figures 11 and 14). Petal blades are formed by active cell division at early developmental stages and by cell enlargement and differentiation at later stages. Some of the genes expressed early need to be continuously expressed throughout petal growth, including *ROXY1*, *SEU*, and *LUG*. Downregulation of the *GNC, GNL*, and *HXK1* genes inhibits chlorophyll accumulation and expression of photosynthetic genes. *At4g30270* might be necessary for correct cell wall dynamics during petal growth (see text section 3.4.5 and Table S1 for details; Franks et al., 2006; Irish, 2008;). Gene color code as in Figure 15; arrows and bars indicate positive and negative regulatory interactions, respectively.

proteins, efflux and influx carriers. Some of the genes that encode these transporters (or carriers) have been cloned: *PIN-FORMED* (*PIN*) and *P-GLYCOPROTEINS* (ABCB/PGP) for auxin efflux, and *AUXIN1* (*AUX1*) and its paralogs *LIKE-AUX1* (*LAX1-3*) for auxin uptake/influx (Figure 15; Bennett et al., 1996; Friml, 2003; Yang et al., 2006; Bandyopadhyay et al., 2007).

The *PIN* gene family encodes eight protein members in total; three of them (*PIN5*, *6*, and *8*) of unknown function. All of the PIN proteins characterized until now are asymmetrically distributed on the plasma membrane and some of them can be found in specific cell types with no pronounced polarity (Vieten et al., 2007). The direction of auxin flow is believed to be determined by the asymmetric cellular localization of PIN proteins (Friml, 2003). The first of these proteins to be characterized was *PIN1*, and its mutation (*pin1*) results in pin-shaped inflorescence meristems without flowers. *PIN1* expression is induced by auxin and it encodes a protein with 10-12 putative transmembrane domains and shares some similarity with bacterial transporters (Gälweiler et al., 1998). *pin1* mutant plants accumulate high amounts of auxin in vegetative meristems and a deficiency in the apical inflorescence meristem, which results in a defective organ initiation of leaves and flowers, a phenotype that can be imitated in wild type using auxin efflux inhibitors (Okada et al., 1991; Reinhardt et al., 2000). Of the other PIN proteins, only *pin3* and *pin7* loss-of-function mutants have flowers, and these bear fused petals, no stamens, and occasionally no sepals (Benková et al., 2003). *PIN3* is essentially involved in mediating differential shoot growth (Friml et al., 2002) and *PIN7* is important during early embryo development (Friml et al., 2003).

Auxin movement mediated by PIN carrier proteins determines the growth axis of the developing organ by establishing an auxin

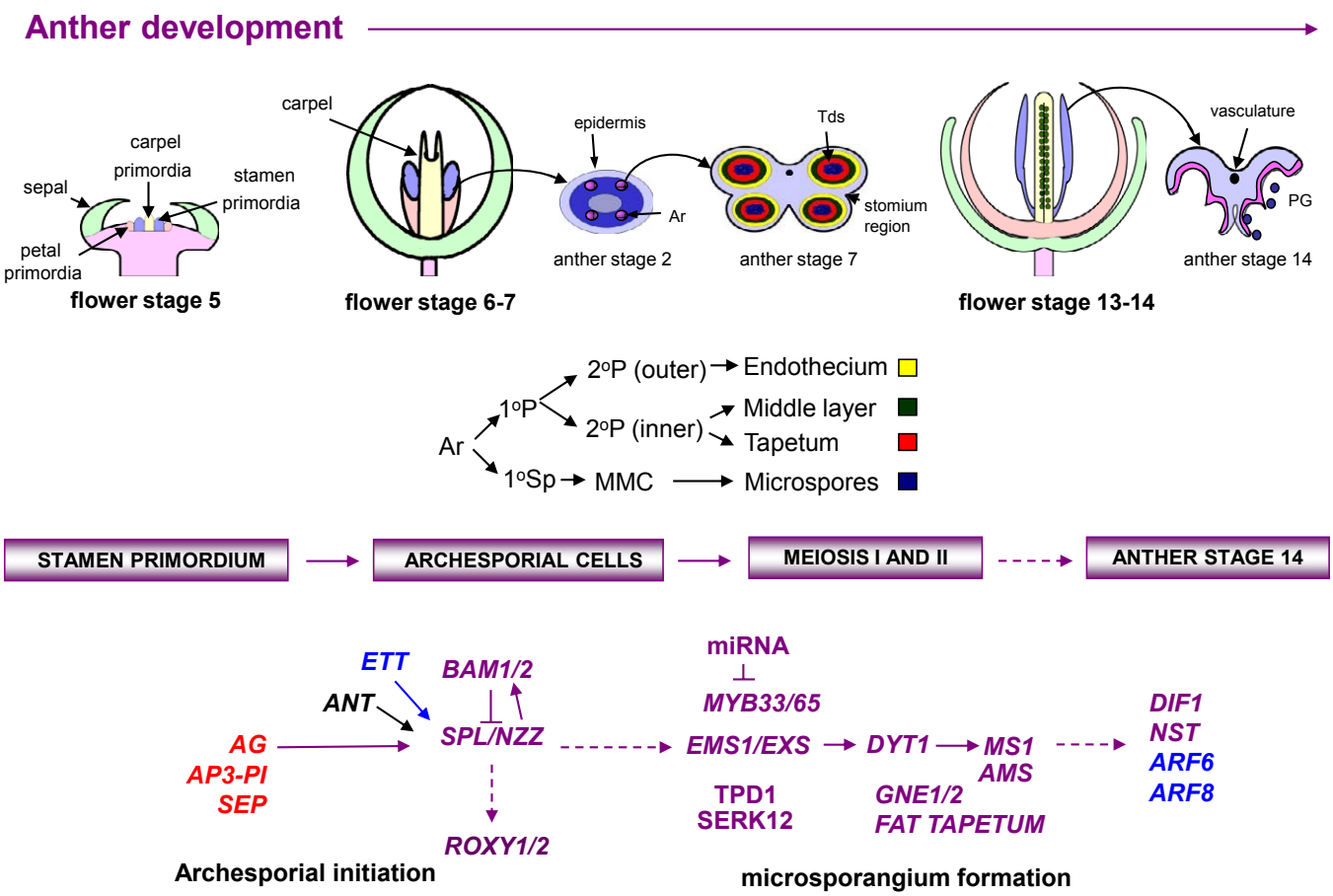


Figure 17. Stages of stamen development with emphasis on the genes implicated in anther formation.

Schemes of some stages of flower development showing representative stages of anther cell differentiation (Sanders et al., 1999) are shown at the top. At stage 1 of anther development and microspore formation, rounded stamen primordia emerge with three cell layers, L1, L2 and L3. During stage 2, the archesporial cells (Ar) arise in the four "corners" of the L2 layer and the epidermis in the L1. Before meiosis the Ar cells divide and generate the primary parietal layer (1°P) and the primary sporogenous layer (1°Sp). The 1°P then divides into two secondary parietal layers (outer and inner, 2°P). The outer layer gives rise to the endothecium, the inner cells to the middle layer and the tapetum. 1°Sp produces the microspore mother cell (MMC) that undergoes meiosis and gives rise to the microspores (Alves-Ferreira et al., 2007). At stage 7, meiosis is completed and the four locules carrying tetrads (Tds) of microspores are seen. At stage 14, cells shrink and the anther dehisces liberating the pollen grains (PG; Sanders et al., 1999). Some of the known genetic interactions important during anther development are shown in purple. AG (in red) induces the expression of *SPL* (the first gene known to be committed to anther development); later during microsporangium formation the action of the *EMS, DYT, MS1* and *AMS* genes is also indispensable (Feng and Dickinson, 2007). See section 3.4.6 for further explanation and Figure 15 for gene color code. Arrows and bars indicate positive and negative regulatory interactions, respectively, and dashed lines possible indirect interactions.

gradient with its maximum at the tip (Benková et al., 2003; Tanaka et al., 2006). As the primordium rapidly expands, auxin is depleted from the tip. Two hypotheses have been proposed to explain this observation: either auxin is transported through the primordium interior into the vascular network (Benková et al., 2003; Tanaka et al., 2006) or it is depleted from primordial regions as a result of specific reversals in PIN1 polarity (Heisler et al., 2005).

The ABCB/PGPs are also transmembrane proteins that belong to the ATP-binding cassette (ABC) transporter superfamily. In Arabidopsis, three of their members, ABCB1, ABCB4, and ABCB9, are able to transport auxin away from apical tissues and are important in maintaining long-distance auxin transport (Titapiwatanakun et al., 2009). One of the PGP proteins (PGP19) co-localizes and interacts with PIN1 and the ABCB protein is apparently important in stabilizing plasma membrane microdomains necessary for enhanced PIN1 activity (Bandyopadhyay et al., 2007; Titapiwatanakun et al., 2009).

Auxin enters the cell passively by simple diffusion and also by the import activity of AUX1 and related LAX proteins. The *AUX1* gene encodes a protein with 11 putative transmembrane domains (Hobbie, 2006) similar to plant amino acid permeases (Bennett et al., 1996). The mutant form (*aux1*) was identified in a screen

for auxin resistant and agravitropic mutants (Bennett et al., 1996; Vieten et al., 2007). The AUX1 protein also has polar subcellular localization in some cells and co-localizes with PIN1 in the shoot apical meristem. AUX1/LAX function could be essential for stabilizing the phyllotactic pattern. The proposed model for AUX1/LAX function is that these proteins concentrate auxin in the cytoplasm of cells of the L1 layer, preventing auxin diffusion in the apoplast (Bainbridge et al., 2008).

PINOID (*PID*) encodes a Ser/Thr protein kinase (Christensen et al., 2000) which has been implicated to function in redirecting subcellular PIN polarities, because the loss of its activity causes a shift in apical-basal PIN polarity (Friml et al., 2004; Berleth et al., 2007; Michniewicz et al., 2007). *pid* mutants have a defect in organ formation similar to that of *pin1*, but they do produce a few flowers (Reinhardt et al., 2003) with altered floral organ numbers (more petals but fewer stamens) (Bennett et al., 1995). Recently, Michniewicz et al., (2007) reported that *in vivo* PIN1 phosphorylation is directly dependent on the kinase PID and a phosphatase PP2A, which may act directly by dephosphorylating PIN1 or indirectly through PID. This phosphorylation status determines the intracellular apical-basal localization of PIN1 and therefore auxin transport-dependent development. PIN1 is targeted to the apex when it is phosphorylated and to the base when it is dephosphorylated (Michniewicz et al., 2007; Vieten et al., 2007).

Accumulation of auxin activates downstream processes through specific receptors and the combinatorial action of members of two large families of transcription factors, AUXIN RESPONSE FACTORS (ARF) and IAA/AUX (Kuhlemeier, 2007). The Aux/IAA proteins are degraded when the levels of free auxin rise, resulting in derepression of ARFs. *ETTIN* (*ETT*)/*ARF3* has a dynamic role in patterning by acting in specific cells within floral meristems and reproductive organs. At early stages, *ETT* functions in determining the number of organ primordia, whereas later it is involved in the outgrowth and patterning of tissues within organ primordia (Figure 15; Sessions et al., 1997). *ett* mutant plants show altered flower development; some flowers have missing petals and rudimentary radialized stamens, and others have normal fertile stamens, but radialized petals (Pekker et al., 2005). ETT is also involved in prepatterning apical and basal boundaries in the gynoecium primordium (see Table S1; Sessions and Zambryski, 1995; Sessions et al., 1997). MONOPTEROS (MP)/ARF5 mutants (*mp*) have inflorescences with smaller or absent flowers, similar to *pin1* mutants (Przemeck et al., 1996).

3.4.2. Floral organ primordia number, size, and boundaries

In Arabidopsis, which is a self-fertilizing (autogamous) and partially cleistogamous (before flower bud opens) plant, floral organ size might not be under strong evolutionary pressure compared to allogamous species. However, it has been an important model to study genes that control size and architectural traits of flowers (Weiss et al., 2005).

Several mutations that affect meristem size and maintenance lead to alterations in flower organ number or size. Mutations in the *CLV* genes (Clark et al., 1993 and 1995; Kayes and Clark, 1998) cause an increase in meristem size, thus yielding additional whorls and a change in floral organ number with altered phyllotaxis (Clark et al., 1993; Clark et al., 1997; Fletcher et al., 1999; Brand et al., 2000; Doerner, 2000). Mutations in genes that control cell proliferation in the SAM, such as the *CLV* genes, are similar to *ULT* in that they have larger SAM and primordia (Fletcher, 2001; Carles et al., 2004) and *WIGGUM* (*WIG*; Running et al., 1998).

When *WUS* is repressed and the number of cells for floral primordia formation is reduced, organ architecture is compromised suggesting that there is a threshold number of cells required to form a normal organ (Weiss et al., 2005). In fact, the loss of organs observed in A-function mutants, or any other AG repressor mutant could be explained as a result of premature repression of *WUS* by AG in these organs (Crone and Lord, 1994; Liu and Meyerowitz, 1995; Laux et al., 1996).

Other mutants that have altered floral organ numbers are *pan* (Running and Meyerowitz, 1996; Chuang et al., 1999), *ett* (Sessions et al., 1997) and *sup* (Jacobsen and Meyerowitz, 1997). Both *pan* and *ett* have more sepals and petals and fewer stamens, whereas *sup* produces more stamens at the expense of carpels (Weiss et al., 2005). Double *pan sup* mutants however have an attenuated *sup* phenotype in the fourth whorl, probably because in this mutant *AG* is downregulated and the domain of expression of *WUS* is expanded (Das et al., 2009).

The *PAN* gene mutation specifically alters floral organ number, yielding fertile plants with a pentamerous meristic pattern (Running and Meyerowitz, 1996). *PAN* encodes a member of the bZIP class of transcriptional regulators (Chuang et al., 1999) and is thought to act in the process by which cells assess their position within the developing floral meristem. This gene may affect the switch that commits floral organ primordia cells to enter an organ initiation program (Running and Meyerowitz, 1996). *PAN* and *WUS* expression overlaps and in *clv* mutants both genes are ectopically expressed (Chuang et al., 1999; Maier et al., 2009). *WUS* overexpression causes *PAN* overexpansion too suggesting that this gene is positively regulated by *WUS* (Maier et al., 2009).

Interestingly, pentameric symmetry is characteristic of flowers in early-diverging angiosperm lineages, thus suggesting that *PAN* may have been involved in changes to meristic patterns during angiosperm diversification; particularly the evolution from pentamerous to tetramerous flowers in the Brassicaceae lineage (Chuang et al., 1999).

Organ size is also regulated by the same components in all whorls. The *ANT* gene encodes a transcription factor of the AP2 family, which seems to be a general regulator of organ size during organogenesis (Elliott et al., 1996; Klucher et al., 1996; Krizek, 1999; Krizek et al., 2000; Mizukami and Fischer, 2000). The overexpression of *ANT* causes increased cell division in sepals and increased cell expansion in the inner three whorls, probably affecting both the rate and duration of cell divisions which are important determinants of the final size of lateral organs (Krizek, 1999; Mizukami and Fischer, 2000; Weiss et al., 2005). *ARGOS* participates in the same transduction pathway as *ANT* and acts downstream of *AUXIN RESISTANT 1* (*AXR1*). Interestingly, increased organ size observed in *ARGOS* overexpression lines is due to an extended period of cell division rather than to an increase in growth rate (Hu et al., 2003; Weiss et al., 2005). So, it is plausible to assume that these two genes (and probably others) affect organ size by transducing signals from plant growth regulators, such as auxin, which is a key player in establishing SAM

primordia and a general regulator of cell proliferation and expansion (Figure 15).

ANT also participates in defining abaxial-adaxial organ polarity in combination with *FILAMENTOUS FLOWER*/*YABBY1* (*FIL*/*YAB1*; Nole-Wilson and Krizek, 2006; see next section) and thus may be one of the links between the modules controlling primordia growth and the polarity establishment (Figure 15).

Ectopic expression of UFO (Levin and Meyerowitz, 1995) also causes increased floral organ size (Lee et al., 1997), due to increased cell division (Mizukami, 2001; Weiss et al., 2005). This pathway is regulated by UFO independently of its role in B gene expression, because ectopic expression of the B genes does not induce any increase in organ size, so missexpression of other unknown UFO-dependent factors may account for this phenotype (Ni et al., 2004). UFO and two gene enhancers of the *ufo*⁻ phenotype, *FUSED FLORAL ORGANS 1* and *3* (*FFO1* and *FFO3*), could also participate in establishing and maintaining organ boundaries probably by affecting cell proliferation (Levin et al., 1998).

Morphological boundaries are established in the early stages of the formation of a primordium separating it from surrounding tissues, and later from adjacent organ primordia (Figure 2C; Aida and Tasaka, 2006a). Cells in the boundary are distinctly narrow and elongated with low proliferation rates (Aida and Tasaka, 2006b). Genes expressed in the boundary may affect both meristem and organ development by upregulating cell differentiation genes and downregulating meristematic genes (Borghi et al., 2007). *CUC1, 2*, and *3* encode NAC-domain transcription factors that promote morphological separation of lateral organs through growth repression (Aida et al., 1997; Vroemen et al., 2003; Taoka et al., 2004). *cuc1 cuc2* double mutant seedlings have fused cotyledons with no shoots. However, when adventitious stems are induced in this genotype, flowers have fused sepals and stamens, fewer petals and stamens number, and reduced fertility (Aida et al., 1997). *CUC* genes are epigenetically regulated (Laufs et al., 2004; Kwon et al., 2006).

Other genes, such as *LATERAL ORGAN BOUNDARY* (*LOB*) and *JAGGED LATERAL ORGANS* (*JLO*), members of the *LATERAL ORGAN BOUNDARY DOMAIN* (*LBD*) gene family, encode putative transcription factors with a leucine-zipper motif that are also expressed in boundary cells (Shuai et al., 2002; Borghi et al., 2007). *JLO* along with the *CUC* genes probably coordinate auxin accumulation and loss of meristem-specific gene expression in organ anlagen (Takada et al., 2001; Borghi et al., 2007).

3.4.3. Floral organ polarity

Establishing organ polarity is an important aspect of morphogenesis and it is sometimes clearly associated with specific functions of plant organs. Both, adaxial-abaxial and proximal-distal polarities are regulated by genetic circuits that are similar for all lateral organs (Figure 2C; Feng and Dickinson, 2007), although each organ type has distinct cell types and morphogenesis in the abaxial versus adaxial surfaces, and in the proximal versus distal regions (Figures 2C and 8). Organ polarity is also linked to the establishment of hormone gradients.

Briefly, abaxial fate is conferred by members of the *YABBY* family (Sawa et al., 1999; Siegfried et al., 1999) and by some of the *KANADI* genes (Eshed et al., 2001; Kerstetter et al., 2001), whereas adaxial cell fate is determined by members of the *PHAB* family: *REVOLUTA* (*REV*), *PHABULOSA* (*PHB*), and *PHAVOLUTA* (*PHV*) (McConnell et al., 2001; Emery et al., 2003; reviewed in Bowman et al., 2002; Zik and Irish, 2003b; Golz, 2006) together with *JAGGED (JAG)* and *NUBBIN (NUB)* (Figure 15; Dinneny et al., 2004; Dinneny et al., 2006).

YABBY proteins (YAB) are transcription factors with a Zn-finger and a helix-loop-helix (YABBY) domain that are promoters of abaxial cell fate in all lateral organs, among other functions (Bowman 1999; Sawa et al., 1999; Siegfried et al., 1999). During flower development they participate in establishing the primordium domain and meristem patterns, and later in maintaining abaxial polarity (Siegfried et al., 1999; Goldshmidt et al., 2008). *FIL/YAB1, YAB2,* and *YAB3* are expressed in a polar manner in all lateral organs of the flower meristem, while *CRABS CLAW* (*CRC*/*YAB6*) is only expressed in carpels and nectaries, and *INNER NO OUTER* (*INO/YAB4*) is restricted to outer integuments (see section 3.4.6 and 3.4.7; Alvarez and Smyth, 1999; Bowman and Smyth, 1999; Villanueva et al., 1999).

KANADI (KAN) genes encode transcription factors of the GARP family. *KAN1, KAN2*, and *KAN3* have been implicated in promoting abaxial cell fates (Eshed et al., 1999; Eshed et al., 2001; Kerstetter et al., 2001). The *kan1* mutant was selected as a genetic enhancer of *crc* gynoecium phenotype, producing a mirror-image of adaxial tissues in the *kan1 crc* double mutant, indicating that both genes participate in a redundant manner to promote abaxial identity (Eshed et al., 1999). In *kan1 kan2* double mutants, all floral organs are also extremely adaxialized (Eshed et al., 2001; Kerstetter et al 2001). Although these *KAN* genes are not necessary for the activation of *YAB* genes, they are important in controling their proper abaxial localization (Eshed et al., 2001). Even though *KAN* and *YAB* genes may have common targets, they also have different ones, since the phenotype of the *fil yab3* double mutant is not quite the same as the extreme phenotype of *kan1 kan2* (Bowman et al., 2002).

It has been hypothesized that the "default" state of cells is the abaxial fate (Sussex 1954, 1955). Genes that belong to the *PHAB* family (class III homeodomain-leucine zipper, HD-ZIP III; Sessa et al., 1998; McConnell et al., 2001; Golz, 2006) of transcription factors, like *PHB* and *PHV*, might be activated by a proximal signal coming from the apical meristem. These cells that are programmed to yield the adaxial portion of the lateral organ, are predicted to in turn have *YAB* and *KAN* genes repressed (Bowman et al., 2002). In this respect, semidominant gain-of-function mutants of *PHB* and *PHV* genes cause adaxialization of lateral organs (McConnell and Barton, 1998; McConnell et al., 2001). *PHB*, *PHV*, and *REV* have similar expression patterns. They are expressed in the SAM initiating lateral organs and later become adaxially restricted as the primordium emerges (McConnell et al., 2001; Otsuga et al., 2001; Prigge et al., 2005). Finally, phenotypes of the loss-of-function *rev* mutants could be interpreted as having a partial loss of adaxial identity (Talbert et al., 1995; Otsuga et al., 2001).

Besides the PHAB function in polarity, it is also interesting to note that a *phb phv cna* (*corona*, another member of the HD-ZIP III gene family) triple mutant has a very similar phenotype to those of *clv* mutants with a distinct increase in organ number in each whorl. This would suggest that HD-ZIP III genes and the

CLV pathway regulate meristem function in a similar manner. The possible interrelation of these modules could contribute to homeostasis between stem cell maintenance and organ formation (Prigge et al., 2005).

NUB and *JAG* are similar genes which encode C2H2-zinc finger transcription factors that are proposed to play redundant functions in proliferation and differentiation of adaxial cells, particularly during anther and carpel development (Dinneny et al., 2004; Ohno et al., 2004; Dinneny et al., 2006; Xu et al., 2008). They specifically work together in determining the number of cell layers formed in floral organs, and like the *PHAB* family, they are not cell-fate genes. Hypothetically, *JAG* suppresses the premature differentiation of tissues by slowing down the cessation of cell division in distal regions of organs until it finally arrests after normal morphogenesis has occurred (Dinneny et al., 2004).

AS1 and *AS2* have redundant functions in the establishment of adaxial identity (Ori et al., 2000; Sun et al., 2000; Semiarti et al., 2001). *AS1* encodes a MYB-domain transcription factor, and *AS2* is a member of the LBD gene family (Serrano-Cartagena et al., 1999; Byrne et al., 2000; Semiarti et al., 2001; Sun et al., 2002). AS1 protein is expressed in organ initials and physically interacts with AS2 to inhibit KNOX gene expression, thus guiding primordia toward differentiation (Figure 15; Ori et al., 2000; Byrne et al., 2002; Xu et al., 2003; Guo et al., 2008).

Other reviews on polarity determination in embryos and in leaves are found in other chapters in this series: “Embryogenesis: pattern formation from a single cell” (Berleth and Chatfield, 2002) and “Leaf development” (Tsukaya, 2002).

3.4.4 Sepals and petals

Sepals and petals constitute the sterile perianth in the first and second flower whorls, respectively. The sepal whorl or calyx protects the developing floral bud and in some plants, but not in Arabidopsis, it may be involved in fruit development (He et al., 2004). The petal whorl or corolla is generally thought to be important for attracting pollinators (Krizek and Fletcher, 2005), but in an autogamous plant such as Arabidopsis, the corolla is generally not showy.

According to the ABC model, sepal identity specification depends on the activity of both A and *SEP* genes (see section 3.3; Coen and Meyerowitz, 1991; Pelaz et al., 2000), and petal identity specification depends on the overlapping activities of A, B and *SEP* genes (see section 3.3; Coen and Meyerowitz, 1991; Pelaz et al., 2000). Also, it has been shown that sepal and petal identity specification depends, at least in part, on the correct downregulation of *AG* expression in the second whorl (see below).

Several molecular components known to influence development of sepals, influence petals too. But knowledge is still limited especially of sepal developmental gene networks. However, a basic GRN for petal development can be constructed based on available data (Figure 16). As stated earlier, organ identity determination, boundary establishment, and expression of polarity determinants are common features needed for the correct development of all the flower organs (Figure 15). There are several pieces of evidence that suggest that genes involved in these processes might be acting at the same time (for example, expression profiles and *in situ* hybridization assays), at least momentarily during flower development. However, we still do not understand fully how such functional modules interact with each other.

As it was said before sepal and petal boundary and organ number establishment are controlled by the *CUC* and *FFO2* genes (see Figure 16 and section 3.4.2; Aida et al., 1997; Levin et al., 1998). *CUC* gene expression is regulated by the miR164c (encoded by *EEP1*) in an organ specific manner (Laufs et al., 2004; Baker et al., 2005).

Several genes are involved in establishing and maintaining the sepal and/or petal domain and, in a way, determining the boundaries between the organs. One of the main activities of these genes is to exclude *AG* expression from the first and second whorl. As stated in section 3.3, *AG* is repressed by *RBE*, *LUG*, *SEU, ROXY1, AP2*, *BLR*, *ANT* and *SAP* (for more information about each gene, see Table S1; Figures 15 and 16).

Briefly, *RBE* is mainly involved in boundary and organ number determination of both sepals (non-autonomously) and petals, and in AG exclusion from the second whorl at early stages of flower organ development. But it is also important during late petal development as mutants may form filamentous organs in the second whorl. *RBE* expression is controlled by both PTL and UFO (Takeda et al., 2004; Krizek et al., 2006). PTL is a trihelix transcription factor that is expressed at early stages in four zones between the initiating sepal primordia and in lateral regions of stamen primordial. Later on, *PTL* expression can be detected at the margins of expanding sepals, petals, and stamens (Brewer et al., 2004). Thus PTL may delimit the *AG* expression region indirectly by activating *RBE* expression (Irish, 2008), and it may also be controlling lateral outgrowth of mature sepals, petals and stamens defining their final shape and orientation (Griffith et al, 1999; Brewer et al., 2004).

UFO is also an important regulator of petal development. Its action toward *RBE* may be indirect, as it may be degrading (as part of an SCF E3 ubiquitin ligase complex) an unknown repressor of *RBE* (Irish, 2008). But *UFO* is an important network link between the *AG* inactivation pathway and the B gene identity determination pathway, because UFO interacts with LFY to activate *AP3* expression (See section 3.3; Lee et al., 1997; Samach et al., 1999; Chae et al., 2008). Importantly, *UFO* expression is also required for normal petal blade outgrowth after petal identity has been established (Laufs et al., 2003), as well as for determination of sepal shape and number in the first whorl (Levin and Meyerowitz, 1995; Samach et al., 1999).

SEU and *LUG* also repress *AG* expression in the first and second whorls by forming a protein complex with AP1 and SEP3 (see section 3.3; Sridhar et al., 2004; Sridhar et al., 2006). But these genes are also part of the adaxial/abaxial polarity establishment pathway in the petal GRN, as they are required for normal *PHB* and *FIL* expression (Figure 15). SEU and LUG participate in petal shape regulation by controlling blade cell number and petal vasculature development in an AG independent manner (Franks et al, 2006). Finally, SEU is also involved in auxin response pathways by directly interacting with ETT, and influencing the final shape, number and phyllotaxy of petals (Pfluger and Zambryski, 2004).

As part of the regulatory network that represses *AG* expression, AP2 is itself negatively regulated by miR172 when second whorl boundaries are determined (Chen, 2004; Zhao et al., 2007). Besides being a negative regulator of *AG*, ANT also affects organ number and morphology in the first three whorls (Elliott et al., 1996; Klucher et al., 1996). *SAP*, another regulator of the mor-

phology of all organs, but mostly of petals, is unexpectedly more important in later flowers (Byzova et al., 1999*).*

Another important indirect repressor of *AG* is ROXY1. As a glutaredoxin, ROXY1 seems to be a postranslational modifier of AP2, LUG, UFO and RBE giving them the specificity to repress AG in the second whorl (Xing et al., 2005; Irish, 2008). ROXY1 is also important for repressing *PAN* expression and for activating other *TGA* factors at different stages of petal development (Li et al., 2009).

Genes that usually work in the establishment of lateral organ polarity (see section 3.4.3) are also important in determining the polarity of sepals and petals, e.g. *PHB*, *JAG*, *FIL*, *YAB3*, *KAN*, *AS1* and *AS2* (Figure 16). Experimental data suggest that AS1, AS2 and JAG are negative regulators of *CUC1/2* and *PLT* (Xu et al., 2008). This links the expression of these genes with those important for boundary determination in the GRN of both sepals and petals. *PHB* and *FIL* expression are also part of the network and are regulated by SEU and LUG (Franks et al., 2006). Lateral-axis dependent development is determined by the *PRESSED FLOWER* (*PRS*) homeobox gene (Matsumoto and Okada, 2001). As with some other genes involved, its position in the GRN is unknown, but by analyzing the mutant phenotypes, it becomes clear that the same regulatory modules that underlie polarity determination are involved in organ shape regulation.

In Arabidopsis, as in other plants, several mutants featuring a foliose-sepal-syndrome (FSS) (leaf-like sepals) have been isolated. Ectopic expression of the MADS-box genes *AGL24, SVP*, and *ZMM19* (from *Zea mays)*, belonging to the *STMADS11*-clade (according to Theissen et al., 2000), result in FSS (He et al., 2004). The main feature of these leaf-like sepals is that they are large and have leaf-like stellate trichomes on their outer surface. One of the characteristics of *ap1* mutant plants is that they also have large or foliose sepals. Thus, it has been proposed that, in addition to their roles in floral transition and/or organ determination, *AP1*, *SVP*, and *AGL24* may also have a role regulating sepal size (He et al., 2004). But how they interact among themselves or with other sepal specific genes is still unknown.

Final sepal and/or petal morphology is also determined by *FRL1* (Hase et al., 2000; Hase et al., 2005), *TSO1* (Hauser et al., 1998), the AP3/PI regulated genes *GNC*, *GNL*, *At4g30270*, *HXK1* (Mara and Irish, 2008), and *NAP* (Sablowski and Meyerowitz, 1998). Except for FRL1, which is involved in endoreduplication control, and TSO1, which is likely involved in chromatin remodelling, the position of these genes in the petal GRN has already been established (see Figure 16).

Using microarray approaches Wellmer et al. (2004), compared gene expression levels within different floral homeotic mutants (see section 3.3.1). Their first study of stage 2 flowers identified only 13 genes as being sepal-specific and only 18 genes expressed exclusively (or predominantly) in petals. However, a more recent study of flowers at stage 3, when sepal primordia have just formed, revealed that 199 genes are upregulated and 161 genes downregulated (Figures 3-4; Wellmer et al., 2006). One speculation is that sepals are relatively simple organs and not many specific genes are involved in their development. But more detailed studies are still required. Results also suggest that genes regulating sepal and petal development may have been recruited from leaf developmental pathways, and, hence, are not specific for the development of these organs.

Petals have been proposed as an excellent model system in which to study development because they have a simple organization and are not essential for survival or reproduction (Irish, 2008). Although much progress has been made, much work is still needed for an integrated and dynamical understanding of petal development.

3.4.5 Stamens

Six stamens occupy the third whorl in the Arabidopsis flower. Stamen specification depends on the overlapping activities of B, C and *SEP* MADS-box genes (Coen and Meyerowitz, 1991; Pelaz et al., 2000). A complex network of gene regulatory modules is simultaneously activated in young stamen primordia, and these are also important for organ morphogenesis (Figure 15). These modules include those that regulate adaxial-abaxial primordium polarity (also affecting other vegetative and reproductive lateral organs) including genes from the *PHAB* (*PHB, PHV*, and *REV*), *KANADI* (*KAN1-4*), and *YABBY* (*FIL/YAB1, YAB2*, *and YAB3*) families. At later stages of stamen development, genes involved in sporogenesis such as *SPL and BAM1/2*, and in anther development, such as *JAG* and *NUB*, are activated (see Figures 15 and 17 for regulatory modules and genes; Scott et al., 2004; Ma, 2005; Feng and Dickinson, 2007).

Among the most striking stamen development mutants is *fil* (also called *antherless* and *undeveloped anther*) which bears normal filaments with neither anthers nor pollen. The *FIL* gene is *YABBY*-like and the *fil* phenotype suggests that the developmental programs of the filament and anther are controlled by independent regulatory modules (Sanders et al., 1999).

As mentioned in section 3.3.1, *SPL/NZZ* is essential for male and female reproductive development and is probably the first reproductive gene to be activated in the anther or, at least, it is the only gene that remains active during most of early anther development. This transcription factor gene is expressed during micro- and megasporogenesis. *AG* directly induces *SPL* but *AG* is not necessary for maintaining its expression (Ito et al., 2004). *spl* mutants are not able to produce microsporogenous cells or tapetal tissue, and show several alterations in anther wall and nucellus development (Schiefthaler et al., 1999; Yang et al., 1999). Interestingly, BAM1 and BAM2, which participate in the first cell division of the archesporial cells and the subsequent periclinal divisions to produce the somatic cell layers, are proposed to form a regulatory loop with *SPL* (Figure 17; Hord et al*.,* 2006; Feng and Dickinson, 2007). Since *SPL* maintains the sporogenous activity in the microsporogenous cells, and *BAM1/2* maintain somatic differentiation, *bam1 bam2* anthers have cells interior to the epidermis with characteristics of pollen mother cells (Hord et al*.,* 2006).

Although *SPL* is one of the genes expressed the earliest in stamen development, it is not the only one. Ectopic expression of *SPL* in all the whorls of an *ag* mutant, results in the formation of microsporangia only in the lateral parts of the staminoid 'petals', suggesting that microsporangial localization is established independently of *AG*, and that there is at least one other *SPL* inducer that is expressed in the second whorl, and not in other whorls (Ito et al., 2004; Feng and Dickinson, 2007). Two other genes, *JAG* and *NUB* play a crucial role in the formation of the four-locular anther architecture, independent of *SPL* induction. *jag nub* double mutants do not have a proper microsporangium. Instead, they

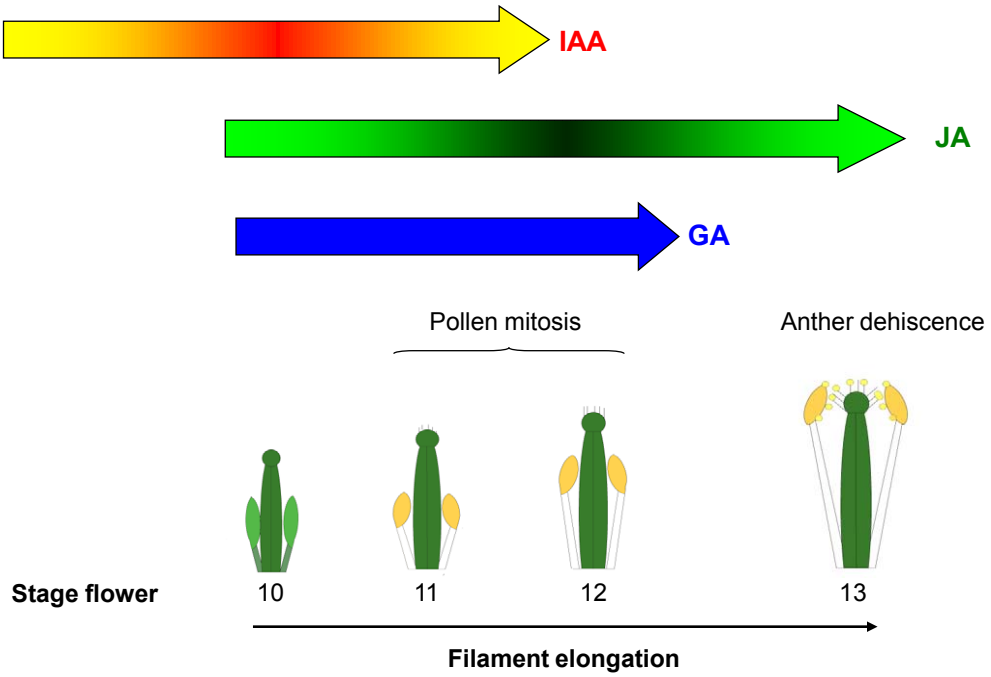


Figure 18. Hormones in late stages of stamen development.

At stage 10 of flower development, the auxin (IAA) concentration (yellow arrow) peaks (red gradient) in the stamens. During this period filaments start to elongate and auxin prevents premature dehiscence. Auxin also participates in later anther dehiscence, probably by inducing JA production (green arrow) that peaks (dark green gradient) at stages 11 and 12 (Nagpal et al., 2005). JA coordinates filament elongation, pollen maturation, anther dehiscence and flower opening (Ishiguro et al., 2001). Although it has not been quantified, GA (blue arrow) is involved in filament elongation and participates in microsporogenesis. Pollen development in anthers of GA-biosynthetic mutants is arrested before microspore mitosis (for details see section 3.4.6; Cheng et al., 2004; Iuchi et al., 2007).

form a finger-like structure that expresses *SPL* in its tips (Dinneny et al., 2006; Feng and Dickinson, 2007).

The correct number of microsporangial initials and the subsequent production of the tapetal cell and middle cell layer identities are properties specified by a putative LRR receptor kinase, EXCESS MICROSPOROCYTES1 (EMS1)/EXTRA SPOROGENOUS CELLS (EXS) (Canales et al., 2002). Until recently, the ligand for EMS1 was unknown, though it was hypothesized that it could be involved in the same signaling pathway as the *TAPETAL DETERMINANT1* (*TPD1*) gene. Both *tpd1* and *tpd1 ems1* mutants are similar to the single *ems1* mutant with arrested meiotic cytokinesis and degenerated microsporocytes (Yang et al., 2003). TPD1, is a small putatively secreted protein that interacts with EMS1 and induces its phosphorylation suggesting that TPD1 is the ligand of the EMS1 receptor that signals cell fate determination during sexual cell morphogenesis (Jia et al., 2008).

ROXY1 and *ROXY2* redundantly regulate anther development in Arabidopsis (Xing and Zachgo, 2008). Lateral and medial stamens of *roxy1* mutants might be fused and the former are sometimes missing (Xing et al., 2005). In these mutants, the adaxial anther lobes are affected in sporogenous cell formation during early differentiation steps, abaxial lobes develop normally but pollen mother cells degenerate, while the tapetum overgrows and occupies most of the locule space. Eventually, the tapetum degenerates too. *ROXY1* and *ROXY2* function downstream of *SPL* and upstream of *DYSFUNCTIONAL TAPETUM1* (*DYT1*). As with other glutaredoxins, they may need an interaction with glutathione to catalyze biosynthetic reactions, suggesting that they may have a role in redox regulation and/or plant stress defense mechanisms involved in the control of male gametogenesis (Xing and Zachgo, 2008).

After tapetal cells are specified, a range of genes are essential for subsequent development. *DYT1* encodes a putative bHLH transcription factor which functions downstream of *SPL* and *EMS1*. However *DYT1* is not able to complement the *spl* or *ems1* mutant phenotypes when it is overexpressed, indicating that it is required but not sufficient for normal tapetum development. *dyt1* exhibits abnormal anther morphology with largely vacuolated tapetal cells that eventually collapse. Several tapetum-expressed genes, such as *MALE STERILE 1* (*MS1*) and *ABORTED MICROSPORES* (*AMS*) are upregulated by *DYT1* (Zhang et al., 2006). In *ms1* mutants for example, tapetal cell abnormalities can be seen and pollen development is arrested just after microspores are released from the tetrads (Bowman, 1994; Wilson et al., 2001; Yang et al., 2007a). Other genes that participate in tapetum development include *RECEPTOR-LIKE PROTEIN KINASE2* (*RPK2*), *FAT TAPETUM* and *GUS-NEGATIVE1 and 2* (*GNE1*, *GNE2*). *RPK2* regulates tapetal function and middle layer differentiation (Mizuno et al., 2007). *FAT TAPETUM,* when mutated, has a middle layer that fails to collapse

after meiosis and shows tapetal-like behavior (Sanders et al., 1999; Ma, 2005). In *gne1* and *gne2* mutants the sporogenous cells enter meiosis, but cytokinesis is frequently arrested. The few highly aberrant tetrads formed degenerate early and microsporangia of mature anthers end up empty (Sorensen et al., 2002).

Several mutants affecting pollen development have been described: *pollenless3; three division mutant (tdm1)*; *ms5*, *ms3* and *ms15*; *determinate infertile1* (*dif1*); *switch1* (*swi1*); *defective-pollen 1*; and *6492* among others (Bhatt et al., 1999; Sanders et al., 1999; Sorensen 2002). Meiotic cells in *pollenless3* anthers undergo a third division without DNA replication generating some cells with unbalanced chromosome numbers (Sanders et al., 1999) or "tetrads" with more than four microspores. *dif1* and *swi1* mutants have micro- and megaspores of uneven sizes because the encoded proteins are essential for sister chromatid cohesion in male and female meiosis and so mutants are totally infertile (Bhatt et al., 1999; Parisi et al., 1999; Mercier et al., 2001; Ma, 2005). Finally, other pollen mutants exhibit abnormal callose deposition (*ms32*, *ms31*, *ms37*, *7219*, and *7593*).

There are late-developmental anther mutants that affect anther dehiscence. In *non-dehiscence1* mutant plants, anthers contain apparently wild-type pollen but do not dehisce. It has been hypothesized that a cell death suppression program, which is normally responsible for dehiscence, might be inactive in this mutant (Sanders et al., 1999). *ms35* is also affected in anther dehiscence, because endothecial cells fail to develop the lignified secondary walls that after desiccation shrink differentially leading to the retraction of the anther wall and full opening of the stomium (Dawson et al., 1999; Scott et al., 2004). *MS35*, now *MYB26* (Steiner-Lange, 2003), is expressed during early anther development and may be a regulator of *NAC SECONDARY WALL-PROMOTING FACTOR 1* and 2 (*NST1*, *NST2*), which have also been linked to secondary thickening in the anther endothecium (Yang et al., 2007b). In *delayed-dehiscence* mutants (*dd1*, *dd2*, *dd3*, *dd4*, *dd5*) anther dehiscence and pollen release occurs after the stigma is no longer receptive preventing successful pollination, but stamens look wild-type and pollen is viable (Goldberg et al., 1993). On the contrary, in *defective-pollen1*, *2*, and *3*, anthers are able to dehisce, but the pollen is aberrant and unviable.

Recent publications have established that gibberellic acid (GA), jasmonic acid (JA), and auxins are involved during stamen development (Figure 18; Fleet and Sun, 2005; Nagpal et al., 2005; Wu et al., 2006; Cecchetti et al., 2008). The GA-deficient mutant, *ga1-3*, produces an abortive anther where microsporogenesis is arrested prior to pollen mitosis (Cheng et al., 2004). Mutations in two GA receptors, GA-INSENSITIVE DWARF1a and b (AtGID1a, b), affect the elongation of stamens, suggesting that these receptors have specific roles during stamen development (Iuchi et al., 2007). GA induces the degradation of the DELLA protein REPRESSOR OF GA1-3 (RGA) upon ubiquitination. Microarray analysis shows that 38% of the RGA downregulated genes are expressed in the male gametophyte at various stages of microsporogenesis (Hou et al., 2008).

Temporal coordination of the elongation of filaments, pollen maturation, and dehiscence of anthers is important for efficient fertilization. The expression overlap of RGA-regulated genes and jasmonate-responsive genes during stamen development suggest a crosstalk between GA and JA signaling pathways in these processes (Hou et al., 2008).

JA has been shown to be involved in at least three androecial developmental pathways: filament elongation, anther dehiscence and pollen production (Mandaokar et al., 2006). Different male sterile mutants have been found to be JA biosynthetic mutants (McConn and Browse, 1996; Sanders et al., 2000) including: the triple *fad* mutant (*fad3–2 fad7–2 fad8*), which lacks the fatty acid precursors of JA; *defective in anther dehiscence 1* (*dad1*), which encodes a phospholipase A1 that catalyzes the initial step of JA biosynthesis; and *dd1*, a member of the *12-OXOPHYTODIENOATE REDUCTASE* (*OPR3*) gene family (Stintzi and Browse, 2000; Ishiguro et al., 2001). *OPR3/DD1* is expressed in the stomium and in the septum cells of the anther that are involved in pollen release. All these mutant phenotypes can be rescued by exogenous application of JA, suggesting that this hormone plays an important role in controlling the timing of anther dehiscence. Interestingly, *DAD1* is a direct target of AG (Ito et al., 2007).

Similarly, the *coronatine insensitive 1* (*coi1*; JA receptor) mutant is defective in both pollen development and anther dehiscence. Stamens of *coi1* flowers have shorter filaments than those of wild-type flowers and anthers are indehiscent containing pollen grains with conspicuous vacuoles (Feys et al., 1994; Xie et al., 1998).

Three related polygalacturonases, enzymes involved in pectin degradation that promotes cell separation, are also involved in JA-regulated anther dehiscence. ARABIDOPSIS *DEHISCENCE ZONE POLYGALACTURONASE 1 (ADPG1) and 2 (ADPG2)*, *and QUARTET2 (QRT 2)* gene expression are distinctly regulated by JA (Ogawa et al., 2009).

To determine the jasmonate-regulated stamen-specific transcriptome the expression profiles of JA-treated and untreated *opr3* mutants were compared (Mandaokar et al., 2006). It was found that 821 genes were induced (70% of them expressed in the sporophytic tissue) and 480 genes were repressed by JA and 13 transcription factors were identified that could be important for stamen maturation pathway(s). Of these, *MYB21*, *MYB24*, and *MYB28* are JA-responsive genes (Mandaokar et al., 2006). *myb21* mutants have short filaments, are late to dehisce and have reduced fertility. Though *myb24* mutants look like wild type, *myb21 myb24* double mutants have a more severe phenotype than *myb21*, suggesting that these two genes might be redundantly involved in important aspects of JA-dependent stamen development. *MYB28* is involved in amino acid metabolism and it is downregulated by both JA and RGA. This study also uncovered several other biochemical pathways that could be important during stamen and pollen maturation. Other results indicate that JA coordinates pollen maturation, anther dehiscence, and flower opening (Ishiguro et al., 2001). Auxins have also been proved to participate in these processes *arf6 arf8* double mutants are defective in anther dehiscence probably because they produce too little JA. Accordingly, this phenotype can be rescued by application of JA (Nagpal et al., 2005). However, auxins trigger filament elongation and prevent premature anther dehiscence and pollen maturation at earlier stages of stamen development. While JA production peaks at stages 11-12 of flower development (see Figure 6 and 18; Nagpal et al., 2005) auxin receptors (*TIR1* and *AFBs*) are already expressed at the end of meiosis. Mutants in these genes cause the release of mature pollen grains before filaments elongate. At later stages, the amount of JA decreases allowing these processes to continue (Figure 18; Cecchetti et al., 2008).

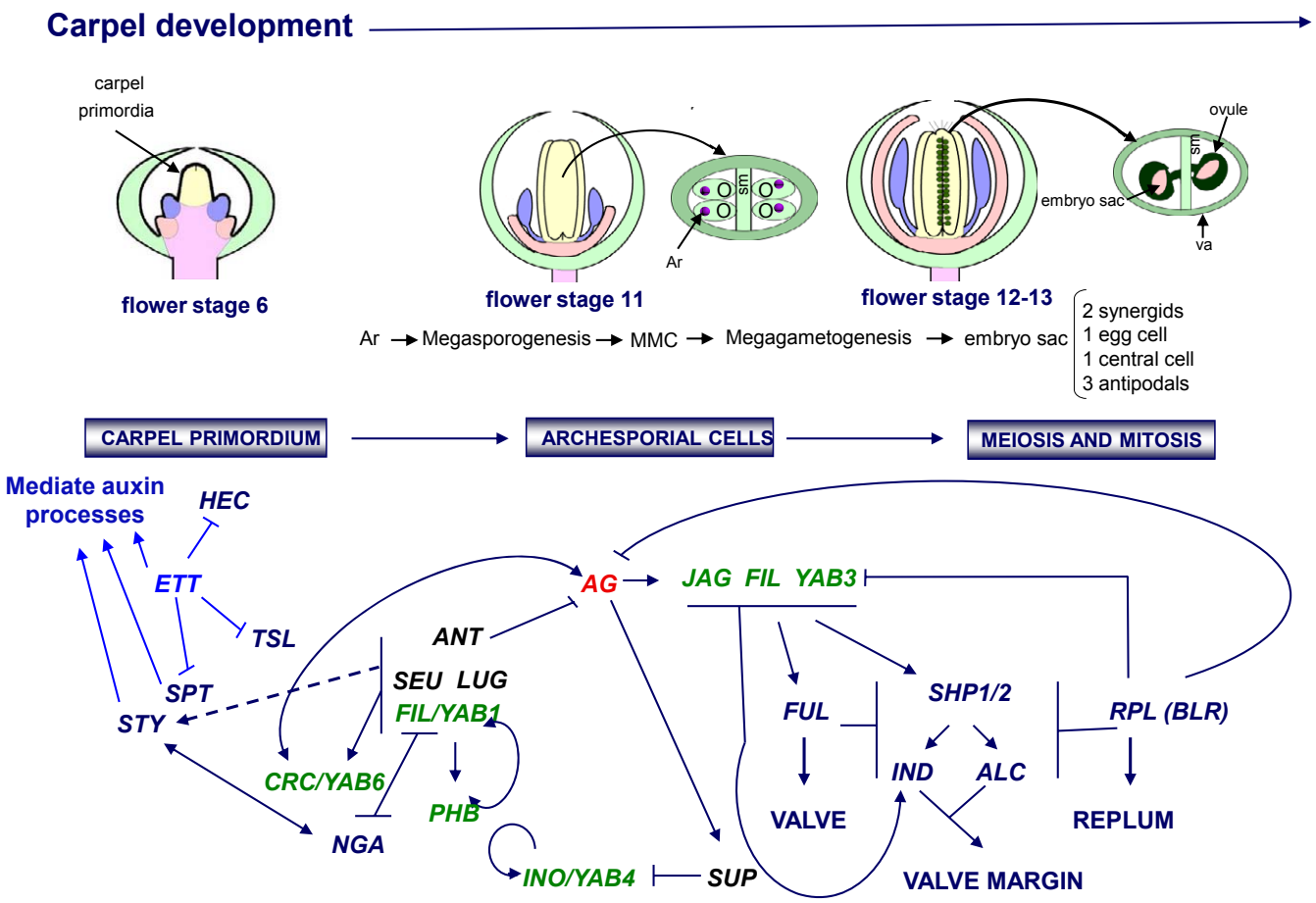


Figure 19. Main stages of carpel development and some genes involved.

Three different stages of carpel development are represented by the schemes in the upper part of the figure. Briefly, at stage 6, the central zone of the FM begins to grow upward and eventually will form the gynoecium. From stages 11 to 13, the ovule primordia (O) arise from the placenta flanking the medial ridges, and the Archesporial cell (Ar) develops from a single hypodermal cell at the ovule. The Ar then forms the megaspore mother cell (MMC) through megasporogenesis, and the MMC forms the embryo sac through megagametogenesis. The embryo sac consists of 2 synergids, 1 egg cell, 1 central cell and 3 antipodal cells. The medial ridges meet in the center of the fruit to form the septum (sm) which divides the gynoecium in two internal compartments. The mature gynoecium is externally formed by the fusion of two valves (va); internally, it also carries totally differentiated ovules each one containing its own embryo sac.

Carpel-specific gene networks are shown in blue. For genes and references not in the main text, see Table S1. Part of the network shown here was taken from Roeder and Yanofsky (2006) and Balanzá et al. (2006). Color codes of interactions and gene/floral organs are according to those of functional modules identified in Figure 15. Arrows and bars indicate positive and negative regulatory interactions, respectively.

Additional stamen or pollen microarray analyses have been performed recently. For example, a clear difference was found between the genes that are expressed in the sporophyte and in pollen with 39% of the expressed genes being pollen specific (Honys and Twell, 2003; Pina et al., 2005). The global gene expression profiles of wild-type reproductive axes have been compared to those of the floral mutants *ap3*, *spl/nzz*, and *ms1* in order to study gene expression during stamen development and microspore formation (Alves-Ferreira et al., 2007). The data suggest that different interconnected regulatory modules may control specific stages of anther and microspore development (for further details see: Amagai et al., 2003; Cnudde et al., 2003; Honys and Twell, 2003; Zik and Irish, 2003a; Wellmer et al., 2004; Pina et al., 2005; Alves-Ferreira et al., 2007; Verelst et al., 2007).

3.4.6 Carpels and ovules

Carpels are specified by the C gene *AG*, and the *SHP1, SHP2, and STK* genes (in an *AG* independent manner) together with the *SEP* genes (Bowman et al., 1989; Coen and Meyerowitz, 1991; Favaro et al., 2003; Pelaz et al., 2000; Pinyopich et al., 2003). They arise in the center of the flower meristem and when carpels are fully developed the floral meristematic cells are completely consumed. Carpels are the most complex structures within flowers and a GRN underlies their development (Figure 19; Table S1). Comprehensive reviews on carpel and fruit development can be found in Bowman et al., (1999), Ferrándiz et al., (1999), Balanzá et al., (2006) and in Roeder and Yanofsky (2006) in this book.

3.4.7 Nectaries

Little is known about the molecular genetics of nectary development. It is clear that nectaries are ABC-independent because *ap2-2 pi-1 ag-1* triple mutant flowers develop nectaries, although in these mutants nectaries are clearly smaller. However, ABC genes may play a role in nectary patterning as *pi-1 ag-1* and *ap3-3 ag-3* double mutants lack them (Baum *et al*., 2001). Also, single mutant *lfy* and *ufo* plants show reduced nectary formation (Lee *et al*., 2005a).

Several genes have been found to be expressed in the nectaries (e.g., *SHP1*, *ALC, SPL, MS35* and *XAL1*), but no detectable defect is observed in their respective mutants (Figure 5G; Schiefthaler et al., 1999; Roeder and Yanofsky, 2006; Yang et al., 2007b; Tapia-López et al., 2008). The only gene that has been clearly related to nectary development is *CRC,* which is also important for gynoecial development (Alvarez and Smyth, 1999; Bowman and Smyth, 1999). The regulation of *CRC* in the nectaries seems to be independent of its expression in the gynoecium. Expression of this gene is already detectable at stage 6 of flower development in the area where the nectaries will be formed. Thus, *CRC* may be important for the early specification of nectary cells (Bowman and Smyth, 1999). *CRC* may also be necessary for maturation or maintenance of the nectaries, because it is expressed at high levels when they develop (Bowman and Smyth, 1999) and *crc* mutants have defects in nectary development. But *CRC* is not sufficient for nectary development, because its ectopic expression does not yield ectopic nectaries (Lee et al., 2005b). Bioinformatic and functional molecular genetic approaches have been taken to discover components of the regulatory network in which *CRC* participates during nectary and carpel development. A combination of floral homeotic gene activities acting redundantly with each other, involving *AP3*, *PI* and, *AG* and in combination with SEP proteins, activate *CRC* in both organs (Lee et al., 2005a). Interestingly in another study, *CRC* was also found to be a direct target gene of AG (Gómez-Mena et al., 2005) and to be indirectly regulated by *LFY* and *UFO* (Lee et al., 2005a). A model has been proposed in which *LFY* and *UFO* activate downstream MADS-box genes which could be working in conjunction with region-specific factors to activate *CRC* during nectary and carpel development (Lee et al., 2005a).

Evolutionary studies have indicated that the *CRC* gene may have been recruited as a regulator of nectary development in the core eudicot plant lineage, but its ancestral function could have been related to carpel development (Lee et al., 2005b).

4. THEORETICAL MODELS: INTEGRATIVE AND DYNAMIC TOOLS FOR UNDERSTANDING FLOWER DEVELOPMENT

As shown throughout this chapter, morphogenetic patterns underlying flower development arise from complex, often non-additive, interactions among several molecular and other kinds of components (e.g., cells) and factors (e.g., morphogen gradients, physical and geometrical constraints) at different levels of organization. Dynamical models can be used to study the concerted action of many elements at different spatio-temporal scales and levels of organization; an approach which is becoming both necessary and possible for understanding how morphogenetic patterns emerge and are maintained during development in general, and in particular, in flower development (for reviews Alvarez-Buylla et al., 2007; Grieneisen and Scheres, 2009). At the level of GRN, mathematical and computational models provide useful tools for integrating and interpreting molecular genetic information, or for detecting gaps and contradictions in the evidence for particular functional regulatory modules. At other levels, two or three-dimensional morphogenetic models of coupled GRNs within cells or among cells are useful for understanding spatiotemporal cell patterning in individual organs and overall plant architecture; and this enables novel insights into the mechanisms underlying developmental processes to be made. Such morphogenetic models are also a way of posing informed non-trivial predictions, testing hypotheses, uncovering potentially generic mechanisms underlying conserved features, and performing *in silico* investigations that guide novel experiments in biological development.

As the amount of experimental evidence increases and novel theoretical approaches and techniques develop, there continue to arise experimentally-grounded models of development and theoretical tools useful in posing predictions amenable to further experimental testing. These advances contribute to discussions of central issues in developmental and evolutionary biology (e.g., Kauffman, 1969; Berg et al., 2004; Milo et al., 2004; Wagner, 2005; Alvarez-Buylla et al., 2007; Balleza et al., 2008). In Arabidopsis, early flower development has been studied using dynamic gene regulatory network (GRN) models. Such models have helped capture the logic of gene regulation, mostly at the transcriptional level, during cell-type specification in various systems (e.g. von Dassow et al., 2000; Espinosa-Soto et al., 2004; Huang and Ingber, 2006; Li et al., 2006; Benítez et al., 2008). In this section we focus on this modeling approach and present some of the main results derived from network modeling in flower development.

4.1 Gene Regulatory Network Models

In this section we review some central notions in GRN theory and the main assumptions that are made and present some of the main results derived from network modeling in flower development. GRN models are composed of nodes, which stand for genes or proteins, and edges or connections, which represent the interactions among nodes (arrows for upregulation and bars for downregulation; for an example see Figure 20). Genes in the GRN model may take different activation states, depending on the activation states of their inputs. Given the architecture of the network and the sign of the interactions, it is possible to define a set of rules or kinetic functions that govern the GRN dynamics, that is, the way activation states of the genes change over time. These rules or kinetic functions may be defined and studied in the context of different mathematical frameworks, some of which have been thoroughly reviewed elsewhere (Gibson and Mjolsness, 2004; Alvarez-Buylla et al., 2007). In experimentally-based GRNs, the dynamic rules may be obtained from reported molecular genetics data as well as from functional genomics datasets.

The kinetic functions of gene activation depend on the states of the input nodes and are multivariate. These may be modeled with discrete or continuous functions. In the former, Boolean functions that allow only “0” (OFF; not expressed) or “1” (ON; expressed) values for the nodes have been successfully used

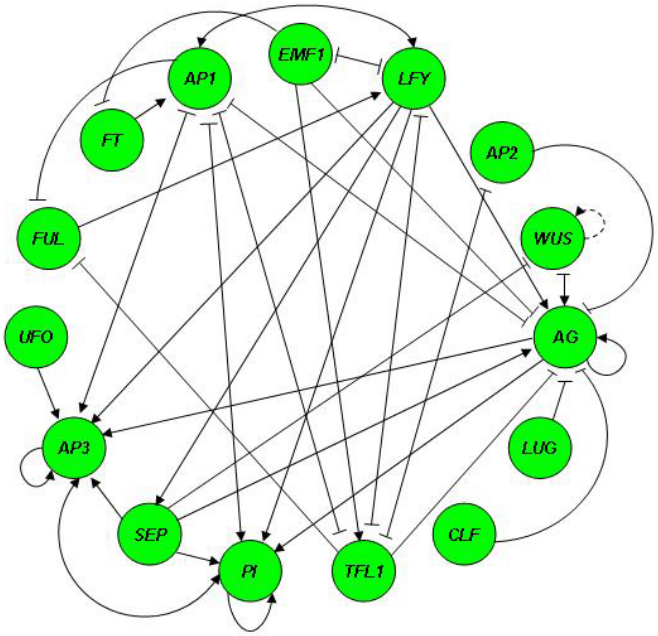


Figure 20. Floral organ specification gene regulatory network (FOS-GRN) model.

The diagram shows GRN topology where circles or nodes correspond to genes or proteins, and arrows and bars correspond to positive and negative regulatory interactions, respectively. The *SEP* node represents the *SEP1*, *2*, and *3* genes together. The interactions are updated with respect to previous publications (Espinosa-Soto et al., 2004; Chaos et al., 2006). The GRN attractors or steady states match the gene expression profiles that characterize inflorescence meristem regions and flower organ primordia. See text and Table 1 for details and experimental data supporting this model (and Table S2 for the dynamics truth tables). Dotted lines represent interactions predicted by the model.

to recover the key qualitative aspects of GRNs (e.g., Albert and Othmer, 2003; Espinosa-Soto et al., 2004). In Boolean networks, parameters of specific kinetic functions are not required. It is appropriate to assume that the GRN nodes are Boolean variables given that: (1) transcriptional regulation may be discrete and take place in the form of pulses, rather being continuous (Ross et al. 1994, Fiering et al., 2000, Ozbudak et al., 2002); (2) the experimental data at hand can be readily formalized as logical rules (see detailed discussions in Albert and Othmer, 2003; Espinosa-Soto et al., 2004; Chaos et al., 2006), while there are no or very few available data on parameters required to postulate continuous functions; and (3) in complex GRNs with many components interacting in non-linear manners, the overall topology of the GRN and the form of the logical rules of gene interaction, rather than the details of the kinetic functions, are what determine the qualitative network dynamics.

Independently of the mathematical formalism used, dynamical analyses of GRNs mostly focus on finding the steady gene activation profiles, that is, the configurations of the network that, once reached, remain in that configuration. These configurations are called *attractors*. The GRN model may be initialized on a particular gene-activation configuration known as an *initial condition* and then the elements of the GRN change their activation state according to the dynamic rules until they reach an attractor. Kauffman (1969) proposed that Boolean GRN attractors correspond to the activation profiles typical of different cell types and therefore that exploring the GRN architecture and dynamics is fundamental to understanding cell-type determination processes. This idea has now been verified experimentally and explored further (e.g. Albert and Othmer, 2003; Huang and Ingber, 2006; Alvarez-Buylla et al., 2007).

Another helpful notion in GRN dynamical studies is that of *basins of attraction*. Given the dynamic rules of the network, the set of initial conditions that lead to each of the attractors is known as its basin of attraction. As we discuss below, the concepts related to a GRN - attractor, initial condition and basin of attraction - may be useful in addressing some pertinent aspects of flower development.

4.1.2 Functional Modules in Flower Development

The functional data on genes involved in flower development reviewed in this Chapter suggest that several regulatory modules act at different stages and in different structures (Figures 9, 15-17 and 19). We define a regulatory module as a set of interacting genes that have more interactions among themselves than with other genes. These modules are semi-autonomous, meaning that their dynamic outcomes are fairly independent of other modules. In Figure 15 we have presented the best-studied modules associated with flower development. The approach described here for the functional module that includes the ABC genes could in principle be used for all of these modules as sufficient nodes have been identified and their regulatory interactions characterized. Eventually, models of coupled GRN that consider several such models together, both within and among cells, will be possible. For now, we have focused in just one such regulatory module.

In previous studies, we have proposed and analyzed the regulatory module, which includes the ABC genes as well as other components, that is sufficient to regulate the partitioning of the inflorescence and floral meristems into subregions of primordial cells. In the case of the flower meristem, each one of the four subregions is composed of the primordial cells that eventually give rise to each of the four types of floral organs: sepals, petals, stamens and carpels.

We use this functional module as a working example of the protocol that has been used in order to assemble an experimentally grounded gene regulatory network (GRN) model corresponding to a functional module. Then we demonstrate how once such a GRN model is postulated, it is possible to follow its dynamics, and explore how the concerted action of multiple interconnected molecular components eventually lead to stable gene expression profiles that may be compared to those characterizing different cell types. Then we delineate some theoretical approaches put forward to model coupled GRN dynamics that may underlie pattern formation and morphogenesis during the early stages of flower development, when the floral meristem is partitioned into four concentric rings of primordial cells. Finally, we review other modeling approaches that are useful to study signal transduction pathways.

4.2 Arabidopsis Flower Organ Specification GRN (FOS-GRN)

Soon after flowering is induced, the flower meristem is partioned into four concentric regions of primordial cells from which floral organs will later form. During the last decade, an experimentally-grounded GRN model for flower organ specification (FOS-GRN) has been built and investigated (Figure 20; Mendoza and Alvarez-Buylla 1998; Espinosa-Soto et al., 2004; Chaos et al., 2006; Alvarez-Buylla et al., 2008). This model incorporates the intricate regulatory interactions among ABC genes themselves and among ABC and non-ABC genes that are key to this process. This functional module includes: some key regulators underlying the transition from IM to FM (*FT, TFL, EMF*, *LFY, AP1*, *FUL*); the ABCs and some of their interacting genes (*AP1*, *AP3*, *PI*, *AP2*, *AG*, *SEP*); some genes that link floral organ specification to other modules regulating primordia formation and homeostasis (*AG*, *CLF* and *WUS)*; and some regulators of organ boundaries (*UFO* and *LUG*; Figures 9, 15 and 20).

The main result obtained from analyzing this GRN is that the postulated network converges to only ten attractors—even though it can be initialized in more than 130,000 different configurations of gene activation. Furthermore, the attractors—the stable configurations recovered—match gene activation profiles typical of the four inflorescence meristem regions (i.e., a region lacking *WUS* and *UFO*, two regions with either one of these two genes turned on, and a fourth region with both genes turned on; see Espinosa-Soto et al., 2004), as well as those of primordial sepal, petal, stamen and carpel cells (Figure 21). This shows that the FOS-GRN is suf-

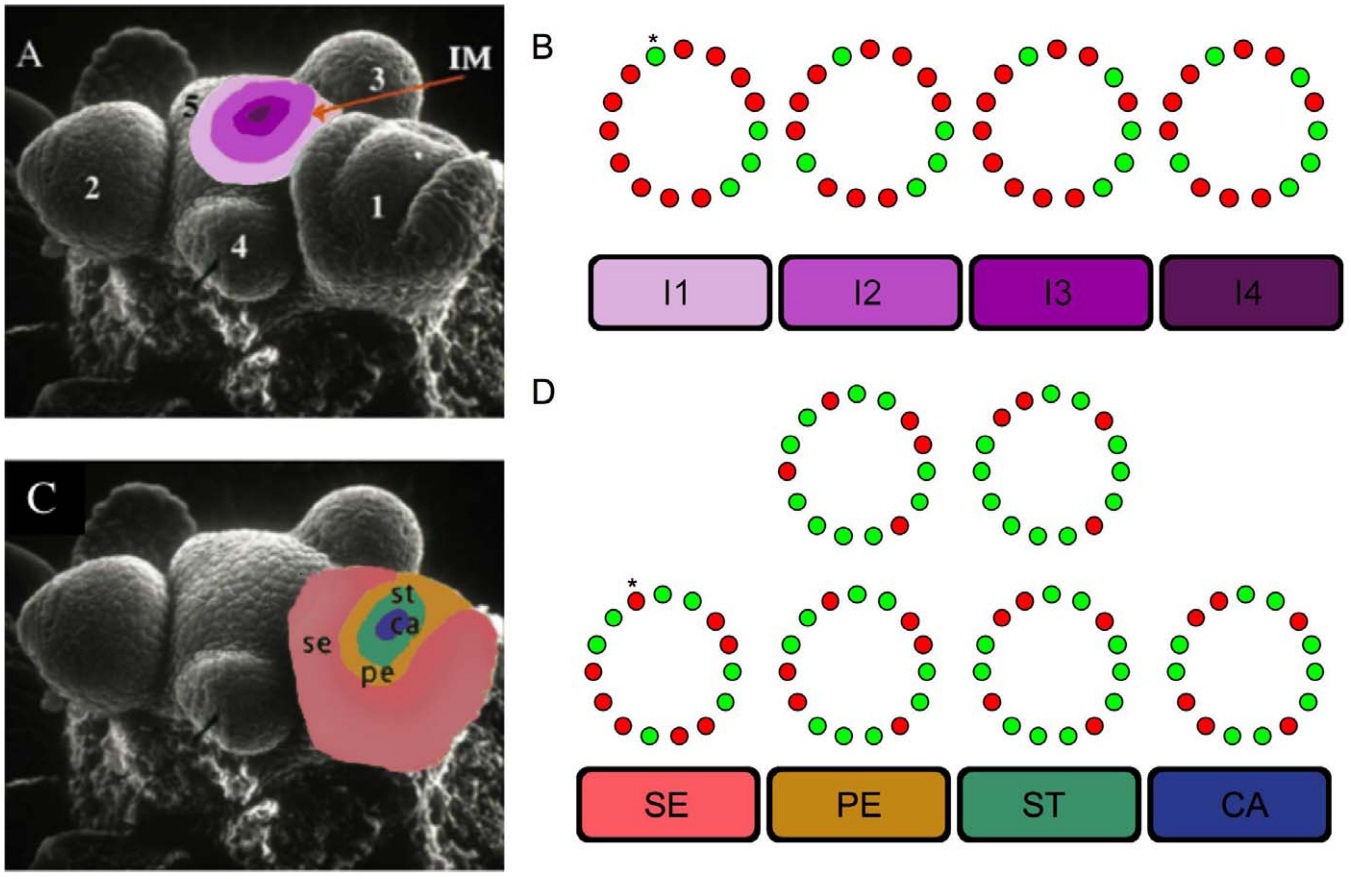


Figure 21. *Arabidopsis* inflorescence and flower development and FOS-GRN.

(A) SEM colored where four regions I1, I2, I3 and I4 are distinguished within the IM. FMs are also seen arising from the flanks of the IM,1 the oldest and 5 the youngest.

(B) I1, I2, I3 and I4 regions of the IM correspond to four of the FOS-GRN attractors. Expressed genes for each attractor are represented as green circles, while non-expressed genes correspond to red circles (nodes are in the same relative position as in Figure 20. * marks the position of the *EMF1* node for further reference). Note that this model recovers the respective regions in the IM with both *WUS* and *UFO*, with either one of these two genes, or with neither expressed.

(C) SEM colored to distinguish four types of primordial cells in young flower meristems. Each will eventually develop into the different flower organs, from the flower periphery to the center, sepals (se), petals (pe), stamens (st) and carpels (ca).

(D) The six attractors of the FOS-GRN model match gene expression profiles characteristic of sepal, petal (p1 and p2), stamen (st1 and st2) and carpel primordial cells. The gene activation profiles of the attractors are congruent with the combinatorial activities of A, B, and C genes described in the ABC model of floral organ determination (adapted from Alvarez-Buylla et al., 2008).

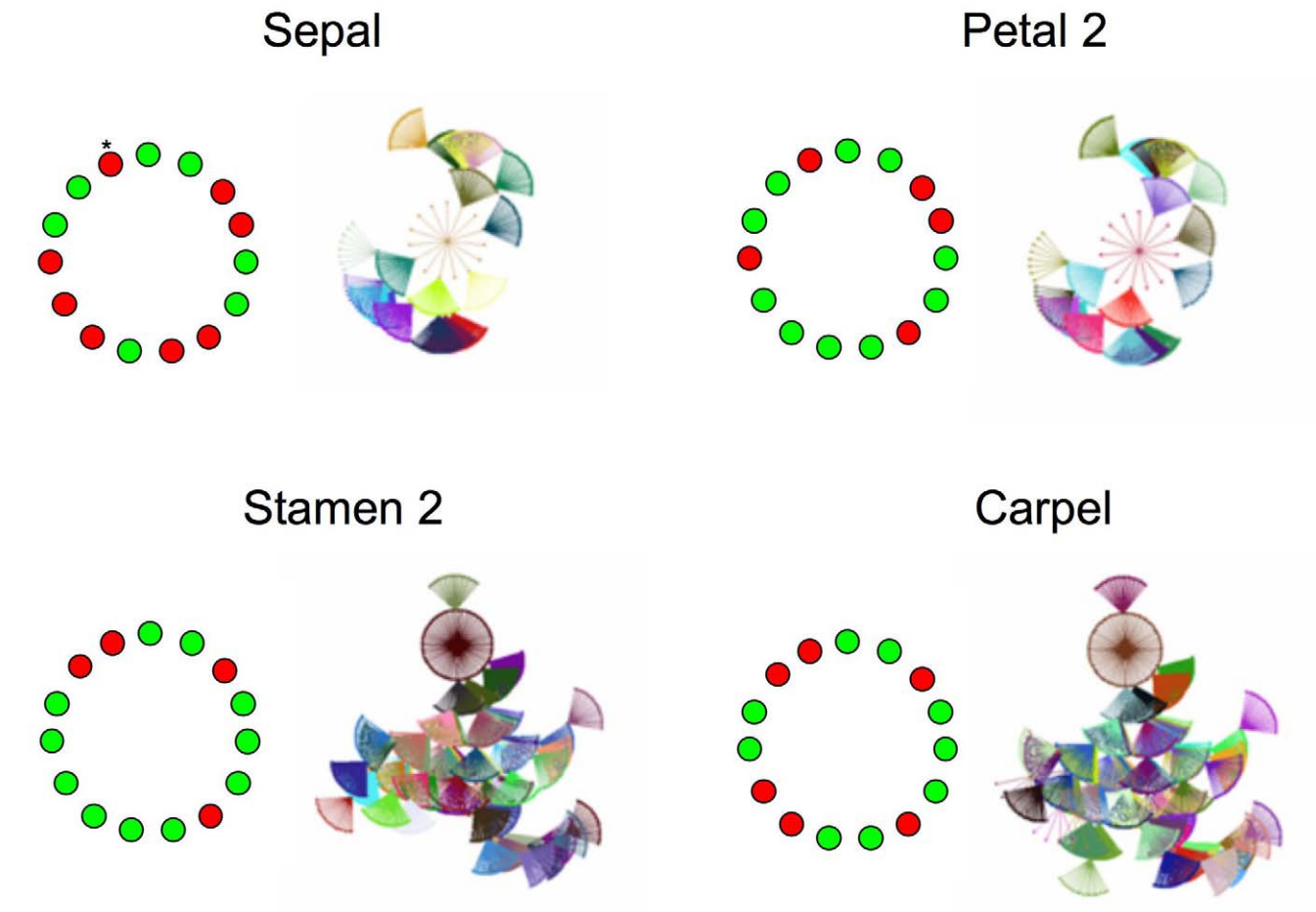


Figure 22. Basins of attraction for the four flower organ FOS-GRN attractors.

Attractors of FOS-GRN match the gene expression profiles of the four types of floral organ primordia of young floral buds (sepal, petal, stamen and carpel). The fan diagrams depict the GRN configurations (combinations of 0s and 1s corresponding to gene activation profiles) that lead to each of the attractors. Points in the outermost layers of these fan diagrams correspond to initial configurations of the network and they are linked to the transitory configurations. Petal2 and Stamen2 stand for one of the two possible attractors for each one of these organs. Relative position of nodes and their colors as in Figure 21. * marks the position of the *EMF1* node for further reference.

ficient to recover the gene activation profiles required to specify primordial cells during the first stages of flower organ development. Therefore the GRN itself constitutes a functional module that robustly leads to the gene configurations that characterize different regions of inflorescence and flower meristems during early flower development; and this independently of the activation states of additional genes that are connected to this elucidated regulatory module. Furthermore, various robustness analyses have been performed showing that the recovered attractors are also robust in response to permanent alterations in the logical functions of gene interactions and the inclusion of gene duplications. Therefore, these results (Espinosa-Soto, et al., 2004; Chaos et al., 2006) suggest that FOS dynamically and robustly emerges from complex networks of molecular components, rather than from a series of linear or hierarchical gene interactions or from the action of particular genes. The FOS-GRN model not only recovers the ABC gene combinations that are necessary for FOS, but it also provides a dynamic explanation for the formation of such gene combinations, and postulates a set of gene interactions with the ABC genes, that are also sufficient for FOS. The functions and interactions of the genes included are reviewed earlier in this chapter.

The FOS-GRN was validated by using this model to simulate the effect of loss-of-function mutations or overexpression, and comparing the results recovered from the model with the gene activation profiles determined experimentally in mutant or overexpressor lines. The mutants were simulated by fixing the state of the gene to 0 for loss of function, and to 1 for gain of function or overexpression (Figure 20; Table 1 and Table S2). In all cases tested, the simulated and empirically-reported profiles matched (Espinosa-Soto et al., 2004).

In addition, this GRN model has enabled investigations to be made into the sufficiency and necessity of particular gene regulatory interactions, which have led to novel predictions. For example, these analyses predicted that *AG* upregulated itself (Espinosa-Soto el al., 2004), which seemed somewhat counterintuitive at the time, but which was then verified by independent experiments

(Gómez-Mena et al., 2005). Also, computer simulations of the FOS-GRN that show that its attractors are robust to different types of perturbation and to duplications (Espinosa-Soto et al., 2004; Chaos et al., 2006) can account for the overall conservation of the flower structure throughout angiosperm (particularly eudicot) evolution (Rudall, 2007; Whipple et al., 2004; Adam et al., 2007).

Since the FOS-GRN model was based on thorough molecular data and is one of the few well-characterized regulatory modules, it has been used as a "model GRN" for further methodological, theoretical and conceptual developments in GRN and systems biology research (Table 2). The main conclusions obtained from the first versions of this GRN have been confirmed. New data regarding FOS are continuously being generated (novel data are also summarized in Table 1) and the FOS-GRN constitutes a basic theoretical framework in which to integrate it alongside previous data. Here, we have updated the FOS-GRN taking these novel data into account and conclude that the basic module originally put forward (Espinosa-Soto et al., 2004; Chaos et al., 2006) is robust to the addition of these newly discovered interactions. We consider, for instance, that *EMF1* downregulates *AG* (Calonje et al., 2008), and *AP3*/*PI* downregulate *AP1* (Sundström et al., 2006), so the postulated module seems to be robust to the addition of intermediary components or previously missing interactions.

Simulations of the updated FOS-GRN have been performed with the new software, ATALIA (http://www.ecologia.unam.mx/~achaos/Atalia/atalia.htm) developed in the Alvarez-Buylla laboratory by A. Chaos-Cador. This tool can be used to readily update this and other GRN models and explore their dynamics. We illustrate the use of this software with a visualization of the attractors' basins (Figure 22) and a simulation of the updated wild-type and certain mutant FOS-GRN dynamics (Figure 23).

In the simulated FOS-GRN, genes can take only two activation states: 0 for no expression and 1 for expression. Hence, by using combinations of 0s and 1s, we can describe all the possible initial conditions of the GRN. Figure 22 presents the so-called *fan diagrams* that show all the GRN configurations leading to each of the attractors. Knowing the relative sizes of the basins of attraction of each steady state is the key to exploring the robustness of each attractor in the face of perturbations.

ATALIA can also calculate the attractor that every possible initial condition will eventually reach and show this information in a tapestry of destinies. In such tapestries, each possible configuration of the GRN is represented by a square in a lattice and is colored according to the attractor it reaches. Moreover, ATALIA can draw a tapestry that represents the difference between the original wild-type tapestry and a mutant one (Figure 23). For example, if we want to know whether an *ap2* mutation has a more or less drastic effect in terms of the GRN dynamics than a *pi* mutation, we can analyze the tapestries of *ap2* and *pi* shown in Figure 23 and conclude that *ap2* mutation has stronger dynamic effects than *pi* given the GRN postulated up to now. Given the complexity of the network involved, such predictions are impossible to make without a tool like ATALIA. As the regulatory interactions in other modules that participate in flower development are gradually uncovered, for each one the experimental data can be exhaustively mined and formalized in the form of a GRN topology and logical rules governing its components' interactions. ATALIA can then be used to explore their dynamics, validate the proposed GRN models by simulating experimental reports of mutants or overexpressing lines, and to postulate novel interactions. Eventually, two or more functional modules may be interconnected via common components to postulate GRN aggregates. Such an approach will be useful in beginning to uncover the types of microtopological trait that characterize the nodes connecting different functional modules, for example.

We have illustrated the potential of using dynamic GRN models to understand cell differentiation using a relatively small and well-characterized module. Approaches used for small regulatory modules that are well-characterized in terms of molecular genetics, should feedback from functional genomic efforts that span the dynamics of a larger number of genes or proteins under diverse conditions and developmental stages or tissues.

4.2. Temporal and Spatial Patterns of Cell-fate Attainment During Early Flower Development

In real biological systems, populations of meristematic cells differentiate into different cell types in stereotyped temporal sequences and spatial patterns. The first primordial cells to be determined in the flower meristem are those of sepals, then those of petals, stamens and carpels going from the periphery to the center of the floral meristem. This suggests that in the population of meristematic cells the most probable temporal order in which each attractor is visited follows the same sequence (Alvarez-Buylla et al., 2008). Recent results from another theoretical approach show that the sequence of floral organ determination can be recovered by introducing some level of stochasticity (random noise) in the GRN dynamics, namely, a degree of error in the updating dynamical rules of the GRN (Alvarez-Buylla et al., 2008). These results are consistent with a handful of other recent studies showing that stochasticity at the molecular scale may contribute to the formation of spatiotemporal patterns in development (see review in Raj and van Oudenaarden, 2008). Studies with the stochastic version of the FOS-GRN also concluded that the relative position of the basins is important in determining the most probable temporal sequence of cell-fate attainment referred to above (Alvarez-Buylla et al., 2008). This fascinating result certainly suggests that the stereotypical temporal pattern of cell fate specification at early stages of flower development may be an emergent and robust consequence of the complex GRN underlying cell-fate determination and that, in principle, it could take place in the absence of inducing signals, emerging only as a result of the stochastic fluctuations that occur during transcriptional regulation (Alvarez-Buylla et al., 2008). Ongoing modeling efforts are explicitly focusing on spatial domains, and exploring the need and sufficiency of different cell-cell communication mechanisms or physical fields (e.g., created by curvature or tension forces) that could provide positional information for spatio-temporal cell patterning during early stages of flower development.

It is important to mention that the FOS-GRN modeled up to now is an abstraction of the qualitative regulatory logic underlying the IM and FM subregionalization during early stages of flower development when the ABC patterns are established. However, other regulatory modules for meristem positioning, growth and polarity, among others, still need to be considered in order to fully understand spatiotemporal cell patterning and morphogenesis of IM and FM. Some genes interacting with FOS-GRN components

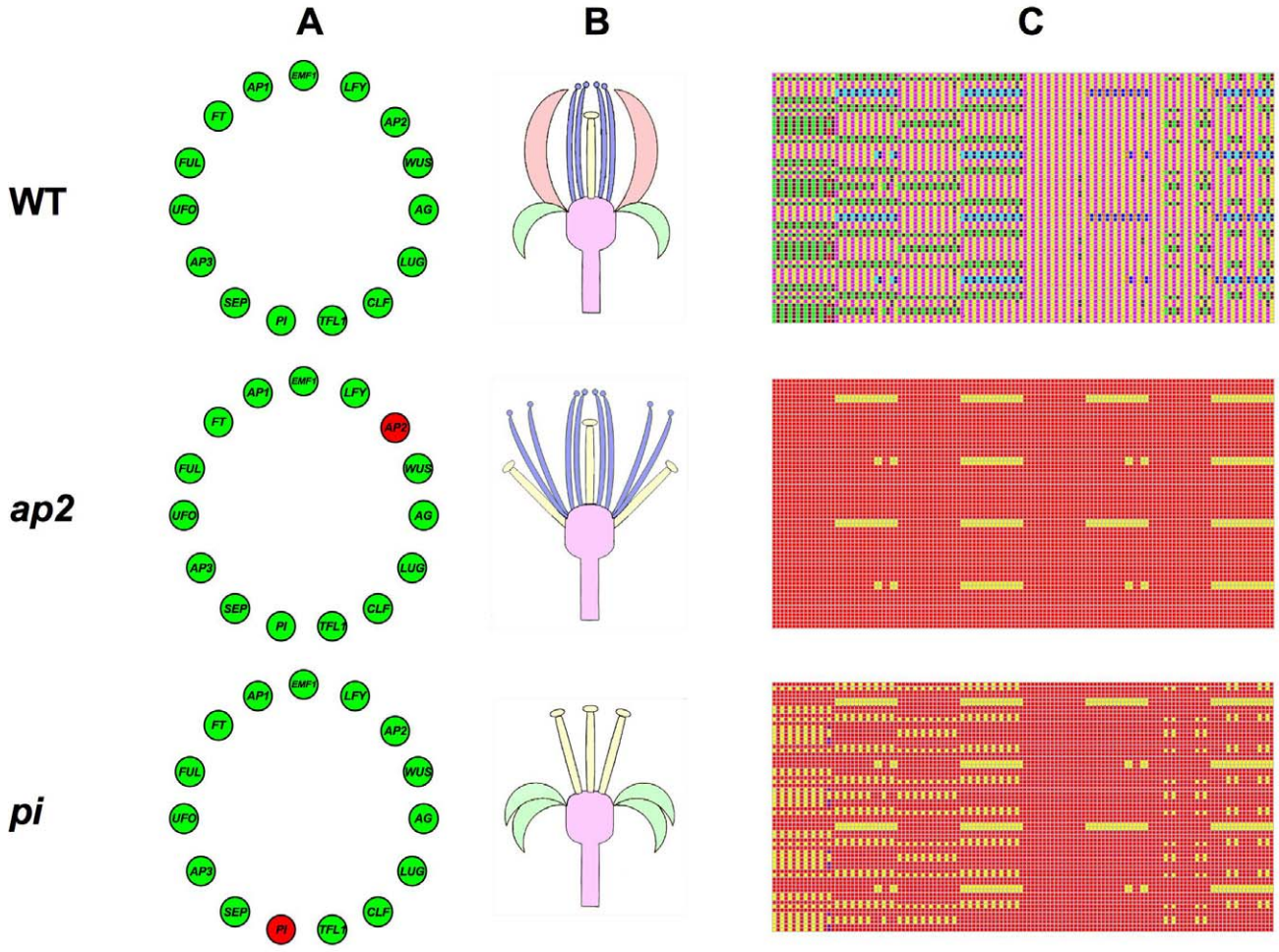


Figure 23. Simulation results for wild type (WT) and two mutants.

(A) Simplified representation of the FOS-GRN. The mutated genes are in red (nodes are in the same relative position as in Figure 20). Mutations were simulated by constitutively turning "off" (loss-of-function) mutated genes regardless of the dynamical rules.
(B) Floral diagrams showing floral organs of the simulated WT and mutant plants. These correspond to the steady-state gene expression arrays (attractors) attained in the simulation.
(C) Tapestries of gene configuration destinies corresponding to the simulated WT and mutant lines. In the WT simulation each square in the tapestry represents an initial condition and they are colored according to the attractor they eventually reach. In the mutant simulation for *ap2* and *pi*, the tapestries illustrate the difference between the WT tapestry of destinies and that obtained for the mutant simulations. Yellow squares, configuration attained is the same attractor as in the WT; red squares, configurations that reached a new attractor; purple squares, configurations that attained a pre-existing attractor but not the same one reached in the WT simulations. Images generated with ATALIA (http://www.ecologia.unam.mx/~achaos/Atalia/atalia.htm).

(e.g. *AGL24*, *BEL*, *RBE* and those described in the last section of Table 1) that do not seem to directly affect cell-type determination in the floral meristem, could link the FOS-GRN with: a) signaling pathways (e.g. Díaz and Alvarez-Buylla, 2006); b) genes involved in cell growth and proliferation both before and after the partitioning of the floral meristem into the four concentric regions; and c) other types of downstream genes or modules that are important during cell sub-differentiation and organogenesis at later stages of flower development.

A complete understanding of flower morphogenesis will continue to require multidisciplinary approaches and modeling tools that help unravel how such single-cell GRNs are coupled in explicit cellularized spatial domains and physicochemical fields (e.g. Jönsson et al., 2005, Savage et al., 2008; Benítez et al., 2008), including metabolism, signaling, and emergent gradients of morphogens (e.g., auxin), cell growth and proliferation, mechanical forces and cell-cell communication mechanisms. All of these are likely to feedback in non-linear ways from and to the GRNs underlying cell differentiation or proliferation (for example see Hamant et al., 2008).

It is important to keep in mind, for example, that plant cell growth in meristems is symplastic. This implies that the contacts

Table 1. Summary of evidence for the FOS-GRN gene interactions shown in Figures 20-23 (ChIP, chromosome immunoprecipitation; EMSA, electrophoretic mobility shift assays; arrows indicate gene induction and bars repression; Espinosa-Soto et al., 2004; Chaos et al., 2006).

INTERACTIONS	EXPERIMENTAL EVIDENCE	REFERENCE
AG (AT4G18960) → *AG*	ChIP shows that AG interacts *in vivo* with predicted regulatory sequences of *AG* .	Gómez-Mena et al., 2005.
AP1 (AT1G69120) --| *AG*	Sepals are replaced by carpels, and petals by stamens in *ap1* mutants. *AG* mRNA found in all flower primordia of *ap1-1* plants. First whorl organs are sometimes carpelloid, and second whorl organs are staminoid in *ap1* mutants.	Bowman et al., 1993; Weigel and Meyerowitz, 1993; Liu and Meyerowitz, 1995.
CLF (AT2G23380) --| *AG*	In *clf* mutants, first whorl sepals are frequently carpelloid, second whorl organs are staminoid petals and *AG* mRNA is detected in sepals. It is likely that CLF is part of a complex with EMF2, MSI1, and FIE that epigenetically regulate *AG*.	Goodrich et al., 1997; Calonje et al., 2008.
LFY (AT5G61850) → *AG*	Expression of *AG* is reduced in *lfy-6* flowers. The expression of *LFY* fused to a strong activation domain produces increased and ectopic AG expression. LFY binds to the first intron of *AG*, and cooperates with the WUS homeodomain to activate it.	Weigel and Meyerowitz, 1993; Parcy et al., 1998; Busch et al., 1999; Lohmann et al., 2001.
LUG (AT4G32551)--| *AG*	*AG* is ectopically expressed in *lug-1* mutants. LUG functions as a repressor of *AG* via its the second regulatory intron.	Liu and Meyerowitz, 1995; Sieburth and Meyerowitz, 1997; Deyholos and Sieburth, 2000; Gregis et al., 2006.
SEP3 (AT1G24260) → *AG*	There is *AG* expression in rosette leaves of *35S:SEP3* plants. In addition, *35S:AG 35S:SEP3* plants have more pronounced carpelloid features.	Castillejo et al., 2005.
TFL1 (AT5G03840) --| *AG*	Stigmas and styles of terminal flowers in *lfy ap1* double mutants are normal if the *tfl1* mutation is added.	Shannon and Meeks-Wagner, 1993.
WUS (AT2G17950) → *AG*	*wus* mutants lack carpels and most stamens. In *AP3:WUS* transgenic plants, second whorl organs are carpelloid stamens instead of petals, whereas in *AP3:WUS* ag plants, second and third whorl organs do not differentiate into carpelloid stamens.	Laux et al., 1996; Lenhard et al., 2001; Lohmann et al., 2001.
AG --| *AP1*	*AP1* mRNA accumulates uniformly in *ag-1* mutant flowers.	Gustafson-Brown et al., 1994.
FT (AT1G65480) → *AP1*	In *ft lfy* double mutants, there is no *AP1 mRNA* unlike in the respective single mutants, suggesting that at least one of these two genes needs to be active for *AP1* activation	Ruiz-García et al., 1997.
LFY → *AP1*	*AP1* expression is delayed in *lfy-6* null mutants, ectopic in *35S:LFY* plants and increased when *LFY-VP16* is induced. LFY directly binds the *AP1* promoter and activates this gene.	Parcy et al., 1998; Liljegren et al., 1999. Weigel and Nilsson, 1995; Wagner et al., 1999.
TFL1 --| *AP1*	In *tfl1* mutants, *AP1* is ectopically expressed in the basal lateral meristems and in terminal flowers. *AP1* expression is also retarded in *35S:TFL1*	Gustafson-Brown et al., 1994; Ratcliffe et al., 1998.
TFL1 --| *AP2* (AT4G36920)	The absence of petals in *tfl1 ap2* flowers and the presence of petals in *tfl1* single mutants suggest there is ectopic *AP2* activity in the terminal flowers of *tfl1* single mutants.	Shannon and Meeks-Wagner, 1993.
AG → *AP3* (AT3G54340)	There is weaker *GUS* expression in the third whorl of *ag-1 AP3:GUS* flowers than in the transgenic control. *AG* may maintain *AP3* expression because cauline leaves of *35S:PI 35S:AP3 35S:SEP3 35S:AG* are converted into stamen-like organs. ChIP shows that AG interacts *in vivo* with predicted regulatory sequences of *AP3.* Also, *AP3 RNA* is absent from the center of the *ag-1* meristem.	Hill et al., 1998; Honma and Goto, 2001; Gómez-Mena et al., 2005; Zhao et al., 2007.
AP1 → *AP3*	*AP3* expression is quite normal in *ap1* mutants but is almost undetectable in *lfy ap1* double mutants, indicating that *AP1* can act with *LFY* to regulate *AP3* expression. Futhermore, AP1 seems to bind *AP3 cis*-regulatory elements.	Weigel and Meyerowitz, 1993; Hill et al., 1998; Ng and Yanofsky, 2001; Lamb et al., 2002.
AP3 → *AP3*	Endogenous *AP3* is upregulated in *35S:AP3-GR* plants induced with dexamethasone, supporting the notion that *AP3* self-activates.	Hill et al., 1998; Honma and Goto, 2000.

(Continued)

Table 1. (continued)

INTERACTIONS	EXPERIMENTAL EVIDENCE	REFERENCE
LFY+*UFO* (AT1G30950) → *AP3*	Both the amount and the domain of *AP3* expression are reduced in *lfy-6* mutants. *ufo-2* plants have less *AP3* protein and less AP3 mRNA. Both *LFY* and *UFO* have to be overexpressed to induce ectopic expression of *AP3*. EMSA show that LFY binds directly to sequences in the *AP3* promoter. ChIP shows that UFO associates with the *AP3* promoter. This association was abolished when ChIP was performed using extracts from *lfy-26* plants harboring the *35S:UFO-Myc* transgene.	Weigel and Meyerowitz, 1993; Meyerowitz, 1995; Parcy et al., 1998; Lamb et al., 2002; Levin and Chae, 2008.
SEP (AT5G15800, AT3G02310, AT1G24260, AT2G03710) → *AP3*	In *AP3:GUS 35S:PI 35S:AP3 35S:AP1* mutants, AP3-GUS is expressed throughout the plant supporting the idea that full activation of the B-function genes requires tetramer formation to include SEP. The ectopic expression of *SEP3* resulted in the induction of ectopic *AP3* expression. Stronger *35S:SEP3* lines are also capable of activating *AP3:GUS* ectopically	Honma and Goto, 2001; Castillejo et al., 2005.
LFY --| *EMF1* (AT5G11530)	Ectopic *LFY* expression in *emfl-1* mutants increases the severity of the *emf* phenotype.	Chen et al., 1999.
EMF1 --| *FT*	*FT RNA* levels are higher in the *emf1-1* mutant and are detected earlier than in the wild type.	Moon et al., 2003.
AP1 --| *FUL* (AT5G60910)	*FRUITFULL* is ectopically expressed in *ap1* mutants.	Mandel and Yanofski, 1995b; Ferrándiz et al., 2000a.
TFL1 --| *FUL*	TFL1 has been postulated to be an inhibitor but it also is possible that other factors have this posttranscriptional inhibitory role. This interaction is necessary as when the negative posttranscriptional regulation of FUL by TFL1 is not considered, the nonfloral gene steady states disappear. No experimental evidence.	Espinosa-Soto et al., 2004.
AP1 → *LFY*	In *ap1* and *ap1 cal* double mutants, *LFY* expression is reduced. Additionally, *LFY* is activated earlier in *35S:AP1* plants than in the wild type.	Bowman et al., 1993; Kempin et al., 1995; Weigel and Nilson, 1995; Piñeiro and Coupland, 1998; Liljegren et al., 1999.
EMF1 --| *LFY*	Double mutants of the weak *emf1-1* allele and *lfy-1* bear *lfy*-like flowers suggesting that, for this trait, *lfy* is epistatic. These genes have antagonistic activities.	Yang et al., 1995.
FUL → *LFY*	Even though *LFY* expression is similar in wild type and *LFY:GUS ful-2* plants, there is less expression in *ful ap1 cal* triple mutants than in *ap1 cal* double mutants, suggesting that the role of *FUL* in *LFY* upregulation is only important when *AP1* is inactive.	Ferrándiz et al., 2000a.
TFL1 --| *LFY*	In *tfl1* mutant plants *LFY* is ectopically expressed in the shoot apex.	Weigel et al., 1992; Ratcliffe et al., 1999.
LFY→ *PI* (AT5G20240)	Amount and domain of *PI* expression are reduced in *lfy-6* mutants. There is no *GUS* expression in early *lfy PI:GUS* flowers.	Weigel and Meyerowitz, 1993; Honma and Goto, 2000.
PI → *PI*	AP3 and PI co-immunoprecipitate. *AP3* and *PI* mRNA levels are not maintained in *ap3-3 pi-1* double mutants. In *AP3:GUS 35S:PI 35S:AP3 35S:AP1* mutants, *AP3:GUS* is expressed throughout the plant supporting the idea that full activation of the B-function genes requires PI	Jack et al., 1992; Goto and Meyerowitz, 1994; Honma and Goto, 2001.
LFY → *SEP*	Microarray experiments show that the group of *LFY* dependent genes includes the homeotic cofactors *SEP1-3*.	Schmid et al., 2003.
AP1 --| *TFL1*	In *35S:AP1, TFL1* expression is greatly diminished. *TFL1* is ectopically expressed in *ap1 cal* double mutants.	Liljegren et al., 1999.
AP2 --| *TFL1*	The *tfl1-1* mutation partially suppresses the *ap2-1 ap1-1* inflorescence phenotype.	Schultz and Haughn, 1993; Shannon and Meeks-Wagner, 1993.
EMF1 → *TFL1*	In *emf1-2 tfl1* double mutants, the *emf1-2* mutation is epistatic with respect to flower initiation. These genes do not have antagonistic activities. This suggests that *EMF1* upregulates *TFL1*.	Chen et al., 1997.
LFY --| *TFL1*	The *35S:LFY* plants resemble the *tfl1* mutant and have no *TFL1* expression. *LFY* can inhibit *TFL1* at the transcriptional level. *TFL1* is also ectopically expressed in *lfy* mutants.	Weigel and Nilsson, 1995; Liljegren et al., 1999; Ratcliffe et al., 1999.
AG --| *WUS*	There is strong *WUS* expression in the center of *ag* floral meristem.	Lenhard et al., 2001; Lohmann et al., 2001.

Table 1. (continued)

INTERACTIONS	EXPERIMENTAL EVIDENCE	REFERENCE
SEP --| *WUS*	*SEP* activity is required for *WUS* downregulation by *AG* because *sep1 sep2 sep3* triple mutant plants bear indeterminate flowers.	Pelaz et al., 2000.
WUS → *WUS*	No experimental evidence. Assumption of model.	Espinosa-Soto et al., 2004; Chaos et al., 2006
UPDATES (Chaos et al., 2006 and this chapter)		
EMF1 --| *AG*	In ChIP experiments, *EMF1* is associated with sites in the promoter and second intron of AG. *EMF1* interferes with transcription by RNA polymerase II and T7 RNA polymerase *in vitro*.	Calonje et al., 2008.
AP3 --| *AP1*	*AP1* transcript levels are significantly higher in *ap3-3* mutant plants than in both *WT* and *35S:AP3*.	Sundström et al., 2006.
PI --| *AP1*	ChIP shows that PI binds to target sequences in the *AP1* promoter	Sundström et al., 2006
MiR172 (AT2G28056, AT5G04275, AT3G11435) + *HEN1* (AT4G20910) --| *AP2*	Elevated *miR172* accumulation results in floral organ identity defects similar to those in loss-of-function *ap2* mutants. On the other hand, the *miR172* abundance depends on the activity of DICER-like protein HUA ENHANCER 1 (HEN1), which is expressed through the plant. This observation suggests that a cofactor expressed in the inner floral whorls is required to give specificity to the HEN1-dependent repression of *AP2*. The need for *AG* inactivity for *AP2* function is added to the *AP2* logical rules	Chen et al., 2002; Park et al., 2002; Chen et al., 2004; Zhao et al., 2007.
LFY → *SEP1-3*	Microarray experiments show that the group of *LFY* dependent genes includes the homeotic cofactors *SEP1-3*.	Schmid et al., 2003.
INTERACTIONS NOT INCLUDED IN THE MODEL		
AGL24 (AT4G24540) + *SVP* (AT2G22540) --| *AG*	In the *agl24 svp* double mutant, *AG* mRNAs are detected in the inflorescence and floral meristems as early as stage 1, indicative of early *AG* expression. In later stages, *AG* is still expressed in all floral organs. Probably, this interaction is part of a different GRN that ocurs before the cell fate determination	Gregis et al., 2006.
BLR (AT5G02030) --| *AG*	*AG* is expressed ectopically in *blr* mutants. *BLR* directly binds to *AG cis* elements (identified by EMSA). This interaction is probably important in organogenesis.	Bao et al., 2004.
RBE (AT5G06070) --| *AG*	In *rbe* mutants, there is ectopic expression of *AG* in second-whorl cells. This interaction may be important in organogenesis.	Krizek et al., 2006.
SEU (AT1G43850) --| *AG*	The direct *in vivo* association of SEUSS (SEU) with the *AG cis*-regulatory element was shown by ChIP. SEU interacts with LUG in a repressor complex to regulate *AG*, and LUG is already considered in the GRN model.	Sridhar et al., 2006.
AGL24+*SVP* --| *AP3*	An *in situ* analysis shows that in the *agl24 svp* double mutant, *AP3* is expressed in all parts of the floral meristem and later in all floral organs. Probably, this interaction is part of a different GRN occurring before the cell fate determination.	Gregis et al., 2006.
LFY → *CAL*(AT1G26310)	Using posttranslational activation of LFY-GR, it is demonstrated that CAL is a direct LFY target. cis-regulatory elements in the putative CAL promoter are bound by LFY. AP1 forms heterodimers with CAL and AP1 is already included.	William et al., 2004.
AP3 --| *FUL*	The domain of *FUL* expression is expanded to the third whorl in stage-3 *ap3* mutants, but no direct interaction is detected by ChIP analysis.	Mandel and Yanofsky, 1995b; Sundström et al., 2006.
FT --| *FUL*	*FUL* is expressed at higher levels in 35S:FT-VP16. It is not considered because this interaction could be mediated by TFL1 and LFY.	Teper-Bamnolker and Samach, 2005.
PNY (AT5G02030) → *LFY* *PNF* (AT2G27990) → *LFY*	The transcripts of *LFY* are substantially reduced in shoot apices of *pny pnf* double mutants after floral induction. *pny pnf* double mutants do not produce flowers but, *35S:LFY pny pnf* plants do produce flowers. This interaction is part of a different GRN.	Anrar et al., 2008.
AP2 → *PI*	*In situ* hybridization shows there is less *PI RNA* occupying a smaller area in *ap2-2* flowers than in wild type. Probably an indirect effect.	Zhao et al., 2007.
AG --- *SEP3*	ChIP shows that AG interacts *in vivo* with predicted regulatory sequences of *SEP3*. Insufficient experimental data.	Gómez-Mena et al., 2005.
FT → *SEP3*	Overexpression of *FT* causes ectopic expression of *SEP3* in leaves. No further experimental evidence.	Teper-Bamnolker and Samach, 2005.

Table 2. Some of the contributions that have used the flower organ specification GRN model in order to test, advance or discuss novel conceptual or methodological approaches.

Contribution	Reference
Logical analysis of the flower organ specification (FOS) GRN.	Mendoza et al., 1999
Introduction of the transsys formalism to represent GRN and implementation of FOS-GRN in this framework.	Kim, 2001
Method for gene network inference based on nonlinear differential equations and logical approaches. Predictions were tested using FOS-GRN.	Perkins et al., 2004
New method for automatically inferring gene regulation functions modeled as logical functions. The method is applied to FOS-GRN.	Bozek et al., 2006
Automatic Petri-net-based method, applied to FOS-GRN, for finding stationary states.	Gambin et al., 2006
Analysis of the dynamic role of feedback loops in networks including FOS-GRN.	Kwon and Cho, 2007
Application of the GenYsis software to model the discrete and multiple valued FOS-GRN.	Garg et al., 2007
Analysis of the effect of feedback loops on the robustness of Boolean networks, such as that of flower organ specification.	Kwon and Cho, 2008
Dynamic study of FOS-GRN and other GRNs with the finding that these exhibit a property known as criticality.	Balleza et al., 2008
Formal analysis of the main sources of perturbation and their effects in biological regulatory networks, with the FOS-GRN as example.	Demongeot et al., 2008

between cells are preserved because there is no displacement or sliding at middle lamellas that join neighboring cells (Priestley, 1930 and Erickson, 1986; cited in Kwiatkowska, 2008). Therefore, overall plant growth could be modeled using the principles of solid body mechanics (see review in Kwiatkowska, 2008). However plant cells also grow anisotropically which implies a variation in the directional growth rates at a given point (Baskin, 2005). Hence, meristem growth has rather been modeled using the principles of continuum mechanics, computing variables that characterize plastic strain (Goodall and Green, 1986; for review see Green, 1999).

Some quantitative mesoscopic models for flower development and growth in Arabidopsis and other angiosperms have been put forward (e.g., Rolland-Lagan et al., 2003; Lee et al., 2004; Skryabin et al., 2004; Mündermann et al., 2005). A finite element model of the SAM has also shown, for example, that lateral bulging of the meristem surface leading to the formation of a primordium results in a gradient of shear stresses with high shear stress at the point where the future primordium emerges (Selker et al., 1992; reviewed in Kwiatkowska 2008). More recently, it was shown that cells in the Arabidopsis SAM orient their cortical microtubules along lines of mechanical stress generated during tissue formation, and this then affects the mechanical properties of the cell, thus establishing a feedback loop (Hamant et al., 2008). This seems to be particularly relevant during the formation of the groove between the apical meristem and the primordium of lateral organs, but less so during growth and differentiation, because the lateral organ primordia are not affected when the microtubular network is disintegrated by a drug (Hamant et al., 2008). This implies that the mechanical feedback loop described is likely to act in parallel with the previously described auxin-mediated patterning mechanism (Laufs et al., 2009). Similar morphogenetic mechanisms are likely to be at work in flower meristem and floral organ development, and both morphogenetic mechanisms connected to the functional regulatory modules, including FOS-GRN and others that have been partly elucidated and reviewed in this Chapter.

5. CONCLUSIONS AND PERSPECTIVES

Arabidopsis has been indispensable in unraveling the molecular genetic bases of the stereotypical and most conserved aspects of flower development. It has also been used to resolve some basic mechanisms of floral meristem determination, as well as floral organ cell differentiation and morphogenesis. The challenge ahead will be to understand how modules regulating each aspect of flower development are interconnected among themselves and with signal transduction pathways that respond to environmental and internal cues to yield coupled GRN spatiotemporal dynamics during flower development. Such dynamics likely involve feedback from physical or mechanical forces, structural and geometric characteristics of domains of activity and from cell dynamics (cell growth and division) in complex ways still requiring multiple theoretical multilevel models and coordinated experimental research. Different functional modules are now being characterized (Figure 24 and Table S1) and shown to regulate some of the main processes involved in flower development. Some of these modules or their components may participate in one or more flower developmental process and data on the functions and interactions of genes are becoming available to enable new dynamic computational models of GRN and signaling pathways during flower development (Figure 24 and Table S1).

Computational models for the gene regulatory module that underlies patterning of the inflorescence meristem and determination of the primordial cell types during early stages of flower organ specification, have demonstrated the potential and need of theoretical dynamic approaches in understanding complex GRN underlying flower development. But information on each regulatory module and the interconnections between modules and with signal transduction pathways is still scanty.

It would be fascinating to unravel which molecular components, circuits, or sub-networks underlie the development and evolution of the diversity of flower forms and the variations

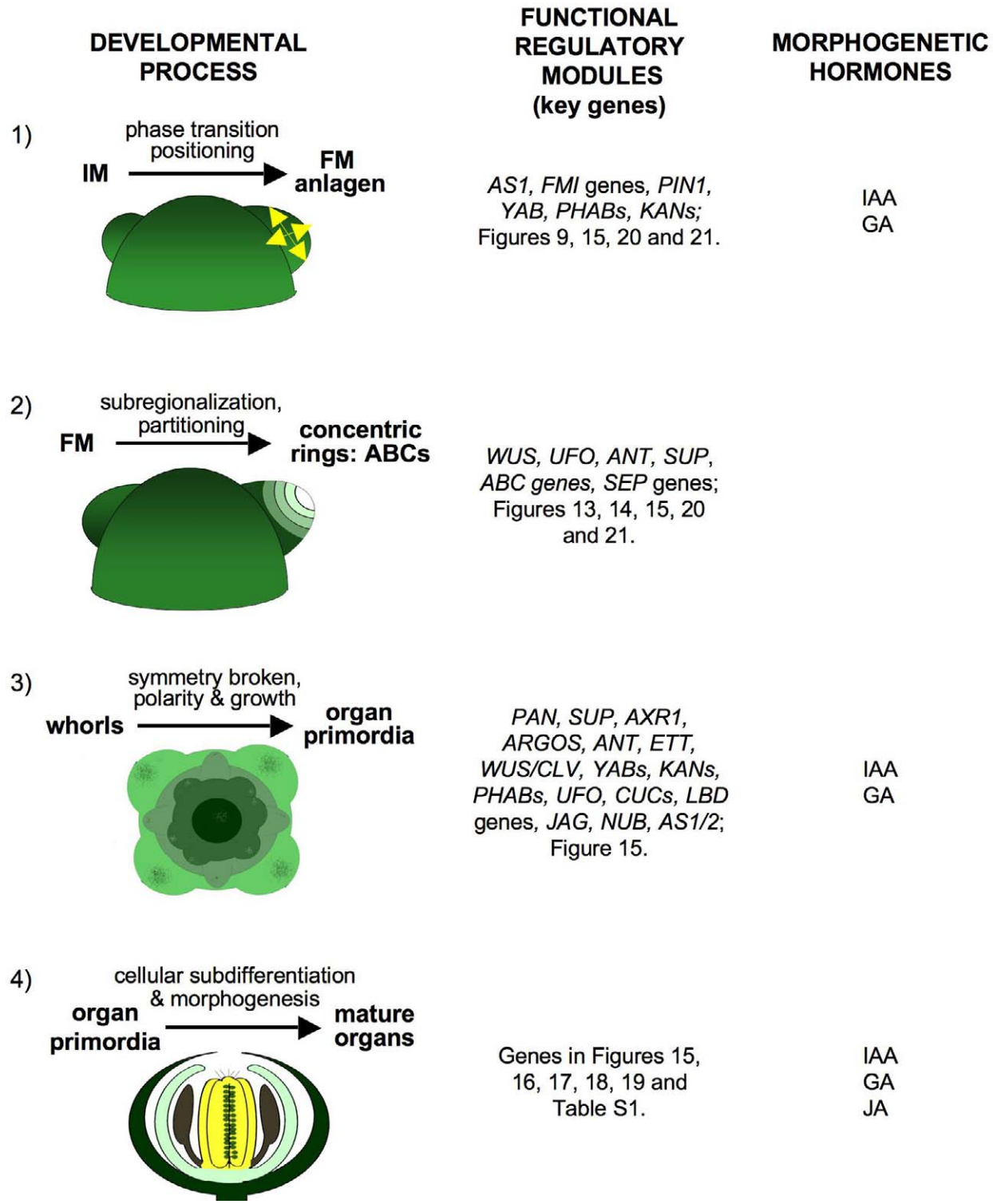
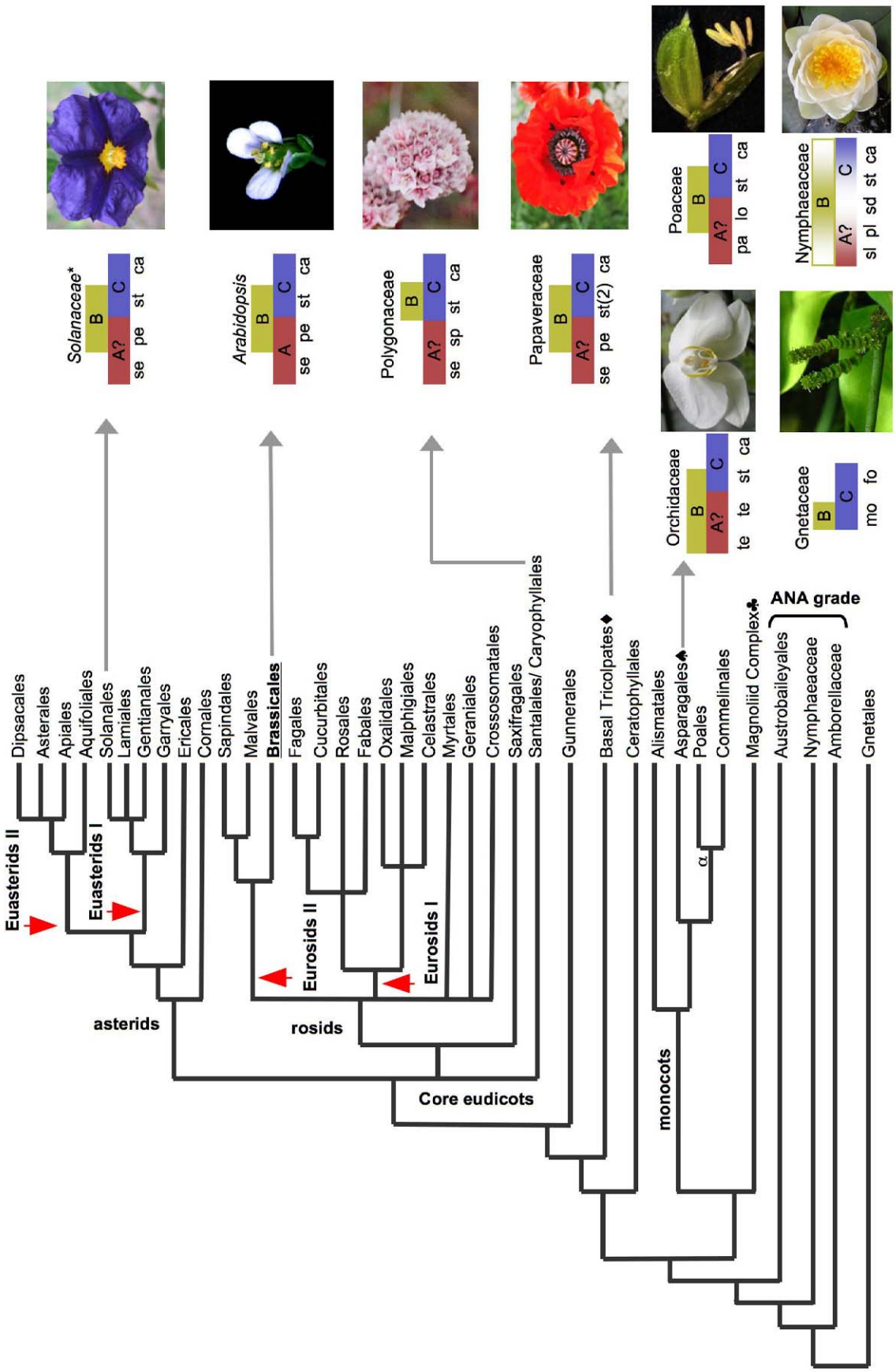


Figure 24. The main regulatory gene modules and hormone signaling pathways during flower developmental processes.

Four main developmental processes in flowers shown schematically from FM formation to mature flower formation. 1) Specification of the floral meristem anlagen. To initiate this process, FMI genes like *LFY* and *AP1* are upregulated. However the position and polarity of these meristems are determined by other gene families and hormones like auxin (IAA) and gibberellins (GA). 2) Specification of whorls of organ primordia. The ABC identity genes and *SEP* are necessary and, together with other genes, sufficient to specify floral organ primordial cells (FOS-GRN module). 3) Organ primordia cell proliferation, boundary establishment and organ polarity are regulated by additional modules that are presumably coordinated during floral organ primordia formation. 4) Cellular differentiation and organ morphogenesis yield the final shape, size and tissue composition of functional sepals, petals, stamens and carpels.



around the overall conserved "theme" of floral structure among angiosperms. This will be possible with integrated multidisciplinary approaches addressing pending questions. For example, in order to understand how a flower meristem forms will require knowledge of the regulatory mechanisms underlying mechanoreception and cell wall, microfibril and microtubule behaviour. How are such mechanisms interconnected or coordinated with the cell differentiation GRNs as well as with the morphogen-mediated patterning mechanisms? The challenge ahead consists in integrating mesoscopic mechanical and morphogen-gradient models with experimentally grounded models of the GRNs underlying cell behaviour, dynamics and differentiation. The aim is to build multilevel computational modeling frameworks that can be used to test the sufficiency and necessity of contrasting mechanisms, which scale from the biochemical and GRN level to the physical factors constraining plant growth (Hogeweg, 2002). Ideally, joint efforts in modeling, bioinformatics and experimentation continually feeding back on each other should give a better understanding of flower, and more generally, plant development and evolution.

Notwithstanding the usefulness of Arabidopsis, such a grand challenge will surely benefit or require comparative experimental and evolutionary studies of other angiosperms with divergent floral structures such as the monocots, other eudicots and basal angiosperms. Such an approach has been successful in understanding and interpreting morphological traits of plants (Kaplan, 2001). Recently, studies in non-model monocots such as orchids (Tsai et al., 2004; Xu et al., 2006) and commelinids, (Ochiai et al., 2004), in maize and rice (Whipple et al., 2004; Xu and Kong, 2007), in members of the Solanaceae, such as tomato (Hileman et al., 2006; de Martino et al., 2006), and in basal angiosperms (Soltis et al., 2007) among others, have started to demonstrate the power of coupling functional and evolutionary questions of a comparative approach with detailed molecular experimentation in several species.

Findings from diverse groups of angiosperms, mostly comparative analyses of ABC gene expression data among diverse angiosperm groups (especially basal angiosperm taxa), with emphasis on the A and B class genes, have already been used to account for the underlying genetic differences in the diversity of petal and stamen morphology among extant flowering plants (Kim et al., 2005; Rudall, 2007). Figure 25 shows a diagrammatic and very simplified angiosperm phylogeny and the variations observed in the domains of expression of the ABC class genes in selected species, representative of the morphological diversity present in their respective angiosperm lineages. Overall, these approaches are helping refine our knowledge of flower development, and will be instrumental in understanding the canonical GRN modules involved in flower formation and discovering variations.

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Figure 25. Angiosperm phylogeny and schematic representation of ABC gene expression patterns of selected taxa.

Schematic phylogeny based on APGII (2003) conventions with variations in the ABC model among angiosperm groups shown (see section 3.3). We present all rosids and asterids, but taxa comprising basal angiosperms, the magnoliid complex, monocots and core eudicots have been compacted and simplified. *Arabidopsis thaliana* belongs to the order Brassicales (bold and underlined). In the ABC model, the A function for sepal specification is maintained for all groups, although the class A genes involved in Arabidopsis are not functionally conserved for other taxa and may not be separable from floral meristem determination. The A function for all lineages was kept to enable comparison with Arabidopsis although a question mark was added to underline its dubious role. For B function, it should be noted that B class genes have undergone extensive duplications within different angiosperm lineages; while these duplications do not affect overall B function, on occasion they implicate subfunctionalization of the resulting paralogs (Irish and Litt, 2005; Soltis et al., 2007). For example, in species of Solanaceae such as tomato (de Martino et al., 2006) and petunia (Vandenbussche et al., 2004), and in the majority of eudicot taxa in which B function expression has been analyzed, two copies of the *AP3* gene are found that have undergone subfunctionalization, *AP3* and *TM6*. Specified floral organs are indicated underneath each ABC model (Theissen and Melzer, 2007). Abbreviations: male organs (mo); female organs (fo); sepal-like tepals (sl); petal-like tepals (pl); staminodes (sd); stamens (st); carpels (ca); petaloid tepals (te); petals (pe); palea/lemma (pa); lodicules (lo); sepals (se); sepaloid petals (sp). Symbols used to refer to compacted plant lineages are: Basal tricolpates (♦), including orders Ranunculales and Proteales and families Buxaceae, Sabiaceae and Trochodendraceae; Asparagales (♠) including Dioscorales, Liliales and Pandanales; (a) the Commelinid grade that, in addition to Poales and Commelinales, includes Dasipogonaeae, Arecales and Zingiberales; the Magnoliid complex (♣) including Canellales, Piperales, Laurales and Magnoliales. Images of rice spikelet, *Nymphaea alba* and the male *Gnetum gnemon* reproductive structure were taken from Yale Virtual Centre for Cellular Expression Profiling of Rice http://bioinformatics.med.yale.edu/riceatlas/anatomy.jspx; http://commons.wikimedia.org/wiki/Image:Nymphaea_alba.jpg and http://commons.wikimedia.org/wiki/Image:Gnetum_gnemon_male.jpg respectively.

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