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REVIEW PAPER

Control of patterning, growth, and differentiation by floral organ identity genes

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Abstract

In spite of the different morphologies of sepals, petals, stamens, and carpels, all these floral organs are believed to be modified versions of a ground-state organ similar to the leaf. Modifications of the ground-state developmental programme are orchestrated by different combinations of MADS-domain transcription factors encoded by floral organ identity genes. In recent years, much has been revealed about the gene regulatory networks controlled by the floral organ identity genes and about the genetic pathways that control leaf development. This review examines how floral organ identity is connected with the control of morphogenesis and differentiation of shoot organs, focusing on the model species *Arabidopsis thaliana*. Direct links have emerged between floral organ identity genes and genes involved in abaxial-adaxial patterning, organ boundary formation, tissue growth, and cell differentiation. In parallel, predictive models have been developed to explain how the activity of regulatory genes can be coordinated by intercellular signalling and constrained by tissue mechanics. When combined, these advances provide a unique opportunity for revealing exactly how leaf-like organs have been 'metamorphosed' into floral organs during evolution and showing crucial regulatory points in the generation of plant form.

Key words: *Arabidopsis thaliana*, floral homeotic genes, MADS domain, organ growth, target genes.

Introduction

Over two decades ago, research into the molecular genetics of floral development was in its heyday. Work in *Arabidopsis thaliana* and snapdragon (*Antirrhinum*) had converged on the well known ABC model, which explained how each type of floral organ is specified by a different combination of floral organ identity genes expressed in overlapping regions of the flower (Schwarz-Sommer *et al.*, 1990; Bowman *et al.*, 1991; Coen and Meyerowitz, 1991). Within a few years, all floral organ identity genes had been cloned, and all but one turned out to encode transcription factors containing the MADS DNA-binding domain (named after yeast MCM1, *Arabidopsis* AGAMOUS, snapdragon DEFICIENS, and mammalian Serum Response Factor) (Sommer *et al.*, 1990; Yanofsky *et al.*, 1990). Similar combinations of homologous genes encoding MADS-domain proteins were found to determine floral organ identity across distant species, including monocotyledons (Irish and Litt, 2005; Ito, 2011; Bowman *et al.*, 2012; Wellmer *et al.*, 2014).

Subsequently, the genetic interactions between *MADS*-organ identity genes were neatly mirrored by protein–protein interactions in what became known as the quartet model (Theißen and Saedler, 2001). The MADS-domain proteins required for each type of organ directly interact with each other to form different multimeric complexes (Melzer and Theissen, 2009), which are sufficient to convert any type of shoot organ into a specific floral organ (Pelaz *et al.*, 2000; Honma and Goto, 2001; Pelaz *et al.*, 2001). In *Arabidopsis*, the following combinations of MADS-domain proteins specify each floral organ type: APETALA1 (AP1) and SEPALLATA (SEP) proteins (SEP1, 2, 3, and 4) direct sepal development; petals are specified by AP1 and SEP1–3 together with APETALA3 (AP3) and PISTILLATA (PI);

AP3 and PI combined with SEP1–3 and AGAMOUS (AG) direct stamen development, and AG combined with SEP1–3 specifies carpels [Fig. 1; reviewed by Wellmer *et al.* (2014)].

The findings that any shoot organ can be converted into a floral organ, and that in the absence of organ identity genes, floral organs become leaf-like (Bowman *et al.*, 1991; Ditta *et al.*, 2004), matched the idea proposed by Goethe in the 18th century that floral organs are modified versions of a leaf-like archetypal organ (Goethe, 1790; Pelaz *et al.*, 2001). Therefore, the diverse morphology of each type of floral organ would be expected to arise from modifications of a basic, leaf-like developmental programme. It would also be expected that genes targeted by the floral homeotic genes would reveal key control points where morphological diversity can be generated. Until recent years, however, not enough was known about the genes that control leaf and floral organ growth to suggest what aspects of the basic, leaf-like programme would be modified by organ identity genes.

In the last few years, much has been learned about the gene regulatory network controlled by floral homeotic genes and about the mechanisms that control growth and morphogenesis of shoot organs. As reviewed below, links have emerged between floral homeotic genes and general regulators of lateral organ growth, including molecular links to the cellular activities that support tissue growth and shape organs (cell division, cell wall functions). More recently, molecular work and computer models have started to converge to explain how the control of organ growth unfolds from the molecular to cellular to organ scale. This creates new opportunities for revealing key regulatory points in the generation of morphological diversity between organs in the same plant and potentially between the same organs across plant species.

MADS-domain organ identity proteins orchestrate gene expression throughout floral organ development

The finding that all floral homeotic genes encode transcription factors prompted numerous studies into the changes in gene expression downstream of the floral organ identity genes [reviewed in Wellmer *et al.* (2014)]. Initial comparisons of organ identity mutants and the wild type revealed large numbers of changes in gene expression, most of which are likely to be indirectly caused by the organ identity genes. Subsequent studies using inducible versions of the MADS-domain proteins revealed immediate target genes and were extended more recently by chromatin immunoprecipitation – high-throughput sequencing (ChIP-Seq) to identify genome-wide binding sites of the MADS-domain protein complexes. Comprehensive stage-specific expression and ChIP-Seq data are now available for all classes of organ identity genes in

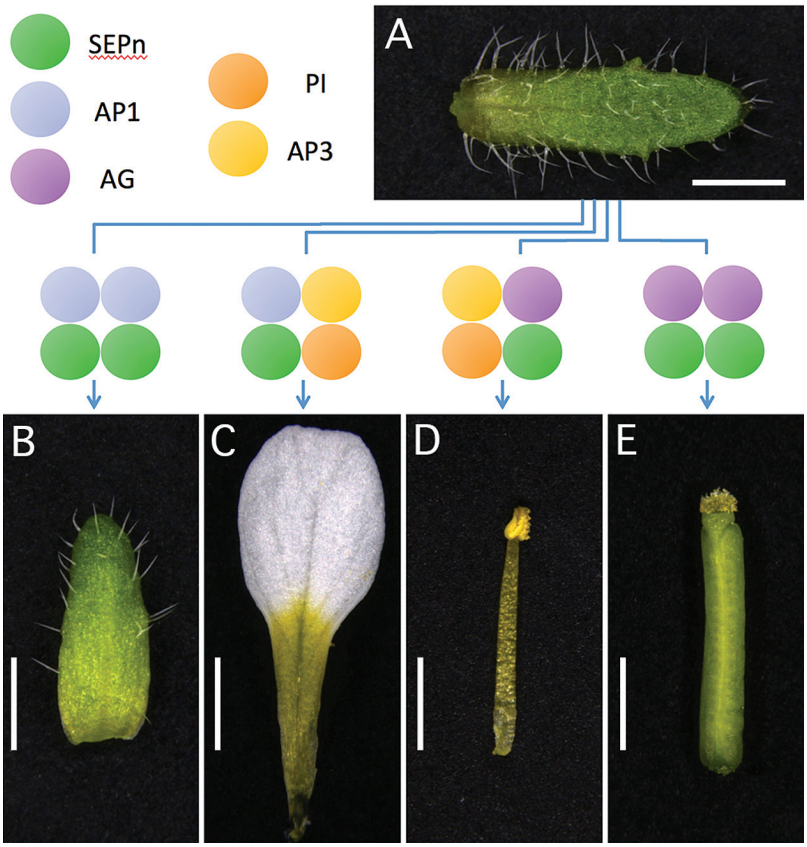

Fig. 1. MADS-domain proteins function in combination to modify organ identity. Coloured circles represent the organ identity MADS-domain proteins from *Arabidopsis*; for simplicity, SEPn represents multiple, partially redundant SEP proteins. (A) The ground state of floral organs is similar to leaves (here, an *Arabidopsis* cauline leaf is shown); in different floral whorls, different combinations of organ identity modify the ground-state organ to sepals (B), petals (C), stamens (D), or carpels (E). Scale bar: 1 mm.

Arabidopsis: AP1 (Kaufmann *et al.*, 2010; Pajoro *et al.*, 2014), AP3/PI (Wuest *et al.*, 2012), AG (Ó'Maoiléidigh *et al.*, 2013), and SEP3 (Kaufmann *et al.*, 2009; Pajoro *et al.*, 2014).

These studies revealed that MADS-domain organ identity proteins directly interact with thousands of loci (between 1500 high-confidence target sites for AP3/PI and more than 4000 for SEP3). In part, the large number of downstream targets reflects the fact that, as indicated by early work using temperature-sensitive alleles, organ identity genes are required at all stages of organ development (Bowman *et al.*, 1989), and as shown by time-course transcriptome analysis, control distinct sets of genes at different stages of floral development (Gómez-Mena *et al.*, 2005; Wellmer *et al.*, 2006). In contrast to the detailed analysis of temporal changes in gene expression, much less is known about cell type-specific target genes. It is possible that even in a single developmental stage, organ identity genes will control different genes in specific tissues and regions of the organ. Thus the gene expression programmes directed by organ identity genes in individual cells might be less complex than our current picture based on whole developing buds.

Context-specific interactions with target genes are also suggested by comparing ChIP-Seq and expression data. In the case of SEP3, 72% of the bound genes were differentially expressed at some point in flower development or in at least one of the floral homeotic mutants, suggesting that the majority of SEP3 binding sites are functionally relevant (Kaufmann *et al.*, 2009). However, this does not imply that every binding event causes a transcriptional response. This has been shown clearly for AP1 and AG, for which only about 10% of genes bound during early floral development also showed differential expression in the same experimental conditions (Kaufmann *et al.*, 2009; Ó'Maoiléidigh *et al.*, 2013). The discrepancy between binding and expression differences suggests that many of the binding sites identified by ChIP-Seq may be functionally irrelevant. Alternatively, MADS-domain proteins could 'tag' genes that are due to be regulated at some point or in some cell type during floral organ development, but only be able to change their activity when co-factors become available. This has been confirmed in the case of AG: a significant number of loci bound by AG early in development only showed AG-dependent transcriptional changes at later developmental stages (O'Maoileidigh, 2013).

One mechanism by which MADS-domain proteins could prime target genes for subsequent regulation by other factors could be by inducing changes in chromatin accessibility. This idea has been supported by careful comparison between binding of AP1 and SEP3 and genome-wide changes in DNase I-sensitive sites (Pajoro *et al.*, 2014), and by the direct interaction between MADS-domain proteins and chromatin-modifying enzymes (Sridhar *et al.*, 2006; Smaczniak *et al.*, 2012). In addition to chromatin regulators, MADS-domain proteins interact with several other transcription factors, such as BELL-like homeodomain and AUXIN RESPONSE FACTOR (ARF) proteins (Smaczniak *et al.*, 2012). These transcription factors control patterning and growth of both leaves and floral organs (see below), so direct interaction with general regulators of organ development appears to be one of the mechanisms by which MADS-domain proteins modify the basal leaf-like developmental programme. The ubiquitous, but context-specific, function of organ identity proteins, combined with their direct interaction with core regulators of shoot organ development, support the idea that MADS-domain proteins function as organ identity co-factors that modify the function of a variety of transcription factors with more specialized functions (Sablowski, 2010), as proposed for Hox proteins in *Drosophila* (Akam, 1998).

Interaction with genetic pathways for organ patterning

The sets of target genes in early organ development are especially enriched in genes that encode additional transcription factors (Gómez-Mena *et al.*, 2005; Kaufmann *et al.*, 2010; Wuest *et al.*, 2012; Ó'Maoiléidigh *et al.*, 2013). Relevant to morphogenesis, these transcription factors provide multiple links between the organ identity genes and regulatory networks that control adaxial-abaxial patterning, formation of organ boundaries, and development of the organ margins.

One of the earliest acting patterning networks establishes the differences between the adaxial (facing the meristem) and the abaxial (facing away from the meristem) sides of the organ. The initial clue that distinguishes the adaxial and abaxial sides of the primordium is probably derived from the radial axis of the shoot apex (meristem in the centre, initiating organs in the periphery) (McConnell *et al.*, 2001; Emery *et al.*, 2003). The different identities of the two sides are consolidated and maintained by the antagonistic activity of adaxial (*AS1, AS2,* and *HD-ZIPIII*) and abaxial (*YABBY*, *KANADI*, and *ETT*/*ARF4*) identity genes [reviewed in Khan *et al.* (2014) and Rodriguez *et al.* (2014)]. These genes perform comparable functions during leaf and floral organ development, but there is some specialization of family members. For example, mutation of the *YABBY* gene *FILAMENTOUS FLOWER* (*FIL*) is sufficient to cause severe abaxial-adaxial polarity defects in floral organs (Sawa *et al.*, 1999), whereas in leaves there is a higher level of redundancy between *YABBY* genes, and comparable defects are only seen in the triple mutant *fil yab3 yab5* (Stahle *et al.*, 2009). Other *YABBY* genes function in abaxial-adaxial patterning specifically in flowers: *CRABS CLAW* (*CRC*) in carpels (Bowman and Smyth, 1999) and *INNER NO OUTER* (*INO*) in ovules (Villanueva *et al.*, 1999). Organ identity genes interact directly with abaxial-adaxial polarity genes, for example, AP1/SEP3 bind to *FIL*, *AS1*, and *AS2* (Pajoro *et al.*, 2014) and *CRC* is directly activated by AG (Gómez-Mena *et al.*, 2005). These interactions may have a role in floral-specific variations in adaxial-abaxial patterning, but it is not clear yet what role this may play in morphological differences between leaves and floral organs.

The boundary between adaxial and abaxial regions of the organ primordium is important for establishing domains at the organ margins, which promote lateral growth (Waites and Hudson, 1995; Eshed *et al.*, 2004) to produce the planar structures of leaves, petals, sepals, and carpel walls. In leaf development, these marginal regions can retain the activity of

a subset of genes that control meristem function: the homeodomain-encoding *KNOX* family, which includes the meristem maintenance genes *SHOOT MERISTEMLESS* (*STM*) and *BREVIPEDICELLUS* (*BP*) (Hay and Tsiantis, 2010), and *CUP-SHAPED COTYLEDON* (*CUC*) *1* and *2*, which are initially required for the establishment of *KNOX* expression but subsequently repress *KNOX* genes to establish the lateral boundaries of shoot organs (Aida and Tasaka, 2006). This meristematic 'module' also functions in the leaf margins to control the formation of leaf lobes and leaflets, and has been repeatedly involved in the independent evolution of compound leaves in different clades (Blein *et al.*, 2008; Townsley and Sinha, 2012). In extreme cases, such as in *Kalanchoe*, *KNOX* gene expression in the sinuses of serrations maintain meristematic regions that generate new plants (Garcês *et al.*, 2007).

In the gynoecium, organ margins also have an organogenic role. The gynoecium is probably derived from leaf-like organs that fused at their margins (Hawkins and Liu, 2014). The similarity between each of the fused units (carpels) and leaves is readily apparent in homeotic mutations such as *ap2-2*, which replace sepals by single carpels with ovules on their margins (Bowman *et al.*, 1989). The region of the gynoecium corresponding to the fused carpel margins is called the carpel margin meristem (CMM), which produces the inner structures of the gynoecium, including the placenta, ovules, septum, and transmitting tract (Hawkins and Liu, 2014). Numerous mutations affect carpel fusion and development of the CMM, many of which affect flower-specific regulatory genes such as *CRC*, *SPATULA* (*SPL*), *ALCATRAZ* (*ALC*), and *INDEHISCENT* (*IND*) (Reyes-Olalde *et al.*, 2013). Thus CMM development appears to be a particularly specialized aspect of the gene expression programme downstream of the organ identity genes. However, there are also aspects shared with leaf margin development, in particular the central role of the meristematic module including *CUC* and *KNOX* genes (Hasson *et al.*, 2011; Kamiuchi *et al.*, 2014). Organ identity proteins directly interact with *CUC* genes (Kaufmann *et al.*, 2009; Wuest *et al.*, 2012; Ó'Maoiléidigh *et al.*, 2013), but it is not known whether this interaction is involved in elaborating the function of *CUC* genes in marginal tissues, such as the carpel CMM.

CUC genes are not the only organ boundary genes that modify organ shape. Development of the basal region of shoot organs is controlled by a different set of organ boundary genes, notably *BLADE-ON-PETIOLE* (*BOP*) *1* and *2* (Ha *et al.*, 2003; Hepworth *et al.*, 2005; Norberg *et al.*, 2005; Khan *et al.*, 2014), and *ARABIDOPSIS THALIANA HOMEOBOX 1* (*ATH1*) (Gómez-Mena and Sablowski, 2008). *BOP1/2* are required for proper development of the leaf petiole, preventing outgrowth of the leaf lamina at least in part by regulating adaxial-abaxial polarity genes and antagonizing *KNOX* genes (Ha *et al.*, 2007; Jun *et al.*, 2010). Both *BOP1/2* and *ATH1* are also required for the development of basal organ structures, such as the abscission zone. The direct interaction of organ identity proteins with *BOP1*, *BOP2*, and *ATH1* (Wuest *et al.*, 2012; Ó'Maoiléidigh *et al.*, 2013) may play a role in generating the diverse basal structures of floral organs: sepals lack recognizable petioles, carpels normally have very short petiole-like structures (the gynophores), petals have petioles comparable to those of leaves, and the petiole-like structures of stamens (the filaments) are very enlarged compared to the other floral organs. It must be noted, however, that *BOP* genes also interact genetically with *AP1* at the transition from inflorescence meristem to floral meristem (Xu *et al.*, 2010), so the interaction with *AP1/SEP3* may reflect functions that precede floral organ development.

The development of distinct tissues along the apical-basal axis patterning has also been linked to auxin function, particularly in carpel development. It was initially proposed that an auxin gradient patterns the gynoecium, but more recent evidence supports a model in which the apical-basal defects seen in auxin-related mutants result from growth defects very early in primordium development (Hawkins and Liu, 2014). There is evidence that input from organ identity genes is important for this role of auxin in carpel development: SEP3 binds to genes involved in auxin transport and auxin responses (*PIN-LIKE 4*, *PINOID*, *ARF3*, *ARF8*, and *IAA4*), and the *sep1 sep2 sep3* triple mutant has elongated gynophores similar to those seen in the *pid* mutant (Kaufmann *et al.*, 2009). Furthermore, plants in which SEP3 was converted from a transcriptional activator to a repressor by fusion to the EAR (ERF-associated Amphiphilic Repression) domain showed severe defects in floral organ development, including defects in apical-basal development of carpels, similar to those of the auxin-related mutants *pin1* and *arf3* or of plants treated with the auxin transport inhibitor NPA (Kaufmann *et al.*, 2009). Thus organ identity complexes are likely to influence apical-basal patterning through direct regulation of genes involved in auxin transport and signalling.

Given the extensive use of hormone signalling in all aspects of plant development, it is not surprising that in addition to the auxin-related genes mentioned above, organ identity genes have many direct links to hormone synthesis and signalling. These include regulation by AG of jasmonic acid synthesis, which is essential for anther development (Ito *et al.*, 2007), and direct regulation of genes involved in GA biosynthesis (*GA2ox1*) and response (*RGL2*) by multiple organ identity genes (Gómez-Mena *et al.*, 2005; Kaufmann *et al.*, 2010), although in the latter case the specific consequences for floral organ development are not clear.

Interaction with growth regulatory genes

Ultimately, organ identity genes alter organ shape by controlling rates and directions of tissue growth (Coen *et al.*, 2004). This role probably involves interactions with intermediate regulatory genes that control the growth of both vegetative and floral organs.

One of the best-characterized genetic pathways that control organ growth is centred on the *GROWTH REGULATING FACTOR* (*GRF*) genes (Rodriguez *et al.*, 2014). GRFs are a family of transcription factors that promote cell proliferation during lateral organ development (Kim *et al.*, 2003; Rodriguez *et al.*, 2010), in association with the co-activator GRF-INTERACTING FACTOR

(GIF), also called ANGUSTIFOLIA 3 (AN3) (Kim and Kende, 2004; Horiguchi *et al.*, 2005). In *Arabidopsis*, *GRF*s are antagonized by the micro RNA miR396, which targets seven of the nine family members for degradation, and is in turn activated by transcription factors of the TCP family (TEOSINTE-BRANCHED 1, CYCLOIDEA, and PROLIFERATING CELL FACTORS 1 and 2) (Rodriguez *et al.*, 2010). Both *TCP*s and *GRF*s are overrepresented among the targets of organ identity genes (Kaufmann *et al.*, 2009), and SEP3 binds to all nine *GRF* genes (Pajoro *et al.*, 2014). DNA sequences bound by TCP proteins are enriched in the vicinity of genomic binding sites for SEP3, suggesting that organ identity proteins could influence the regulation of at least a subset of TCP target genes (Kaufmann *et al.*, 2009). The combined data make the TCP/GRF pathway an obvious candidate in mediating the effects of organ identity genes on organ growth.

Another transcription factor with well studied roles in organ growth is JAGGED (JAG). Mutations in *JAG* and in its paralogue, *NUBBIN* (*NUB*), enhance leaf serration and impair growth of the apical region of floral organs (Dinneny *et al.*, 2004; Ohno *et al.*, 2004; Dinneny *et al.*, 2006). The preferential role of *JAG* in the distal region of floral organs led to the suggestion that it functions as a mediator between organ patterning and growth processes (Breuninger and Lenhard, 2010). This idea has been corroborated by the finding that JAG directly binds to genes involved in boundary formation (e.g. *BOP1* and *BOP2*) and organ growth (*TCP4*, *GRF5*, *AN3*, and *miRNA396*), in addition to directly regulating genes involved in the cellular activities required for tissue growth, such as cell cycle control and cell wall functions (Schiessl *et al.*, 2014). Quantitative analysis of the effects of *JAG* at the cellular level revealed roles in both the rate of cell growth and proliferation, and in promoting oriented cell expansion (Schiessl *et al.*, 2012), and genetic analysis confirmed that *JAG* stimulates organ growth to a large extent by repressing the expression of cell cycle inhibitors (Schiessl *et al.*, 2014). *JAG* and *NUB* are direct target genes of AG, SEP3, and AP3/PI (Gómez-Mena *et al.*, 2005; Kaufmann *et al.*, 2009; Wuest *et al.*, 2012; Ó'Maoiléidigh *et al.*, 2013), so *JAG*/*NUB* are also good candidates to mediate between organ identity and growth.

As with most of the examples discussed above, direct targets of organ identity proteins reveal molecular links to processes such as organ growth. However, exactly how the temporal or spatial expression pattern of these genes is altered by organ identity genes, and how these changes are translated into the patterns of cell proliferation and expansion that shape organs, remains virtually unknown. Some insight into how *JAG* may shape different organ types came from recent computational models of organ growth (Sauret-Güeto *et al.*, 2013). The model had three main components: (i) a polarity factor whose distribution and orientation in the tissues depends on the location of proximal and distal organizers; (ii) growth factors that determine rates of growth perpendicular and parallel to local polarity; (iii) mechanical connectedness leading to accumulation of stresses during growth, which are resolved in part by tissue deformation. Using this modelling framework, the different shapes of petals and leaves were simulated by assuming different patterns of tissue polarity (convergent at the distal end in leaves, divergent in petals). Considering its expression pattern and mutant phenotype, *JAG* was proposed as a candidate for the distal growth factor, which would preferentially promote growth perpendicular to local polarity. Based on the effect of the *jag* mutation on reporters for auxin transport and response (which are connected to local tissue polarity), *JAG* was also proposed to be required for establishing a continuous distal organizer along the edge of the petal. Changes in the corresponding parameters resulted in models that correctly captured the main features of *jag* petals (narrow organs with serrated edges; Fig. 2). Therefore this type

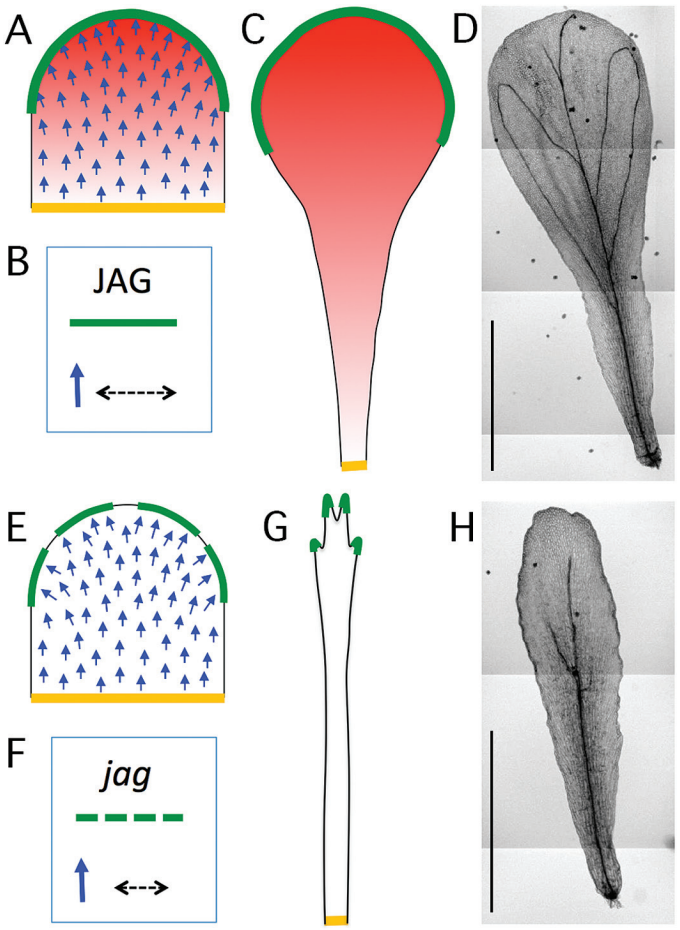

Fig. 2. Example of computational modelling of the effect of *JAG* (one of the targets of organ identity proteins) on organ growth (based on Sauret-Güeto *et al*, 2013). (A) Schematic wild-type organ primordium with key model assumptions represented: proximal and distal organizers (orange and green lines, respectively) orient local tissue polarity (blue arrows); and a growth factor expressed more highly in the distal region of the organ (red gradient) preferentially promotes growth perpendicular to local polarity. (B) *JAG* function is assumed to correspond to the growth factor (red gradient) in (A), and in addition is required to establish a continuous distal organizer (green line); the broken black arrow represents growth perpendicular to local polarity (blue arrow). (C) Running the model to a state corresponding to a mature petal results in a morphology similar to that of a wild-type petal (D). (E, F) Initial state and assumptions of the model corresponding to the *jag* mutant: growth perpendicular to local polarity is reduced, and the distal organizer (green line) is discontinuous. (G, H) Running the simulation to a state corresponding to a mature petal results in a narrow organ with serrated edges, which are features of *jag* petals (G). Scale bar: 1 mm.

of modelling approach has the potential to reveal how organ identity genes generate different organ morphologies through changes in tissue polarity and the localized activity of growth regulators.

Interaction with cellular differentiation pathways

As the organ grows and takes shape, cell differentiation is initiated. Floral organs differ from leaves not only in morphology, but also in their repertoire of cell types. Accordingly, organ identity genes directly interact with genes that control cell identity, both to repress leaf-specific cell types and to promote floral-specific differentiation.

Photosynthetic capacity is a prominent feature of leaves that is lost in petals and stamens. Presumably B-function genes suppress the differentiation of photosynthetic tissues, but the molecular basis for this is only partially understood. One of the few direct targets of organ identity genes specifically regulated in petals is the *BANQUO3* (*BNQ3*) gene, which encodes an atypical bHLH protein that does not have a DNA- binding domain but is believed to interact with other bHLH transcription factors to modify their function (Mara *et al.*, 2010). *BNQ3* is widely expressed in the shoot but is directly repressed by AP3/PI in developing petals. Loss of *BNQ3* function caused reduced chlorophyll levels in cauline leaves, stems, sepal,s and carpels, while *BNQ3* overexpression interfered with light-induced hypocotyl elongation. Thus one way in which organ identity genes turn green leaves into pale petals is by interfering with light signalling and chloroplast development through repression of *BNQ3*.

An example of a leaf cell type in which development is suppressed during floral development is branched trichomes; their development is promoted by *GLABROUS1* (*GL1*) (Larkin *et al.*, 1994) and inhibited by *CAPRICE* (*CPC*) (Schellmann *et al.*, 2002). AP1, AP3, PI, and AG all directly bind to the *GL1* and *CPC* loci, and consistent with the absence of trichomes on stamens and carpels, AG repressed *GL1* and activated *CPC* (Ó'Maoiléidigh *et al.*, 2013). Further support for the role of AG in repressing this aspect of the leaf development programme came from experiments in which loss of AG function was caused during development by artificial miRNAs. Loss of AG function during mid-stages of organ development, when cell differentiation is under way, caused ectopic trichome development on carpels (Ó'Maoiléidigh *et al.*, 2013).

There are also examples of differentiation pathways that are unique to floral organs and are directly activated by the organ identity genes. *SPOROCYTELESS* (*SPL*), also known as *NOZZLE* (*NZZ*), is required for development of sporogenic tissues, which produce the male and female gametophytes (Schiefthaler *et al.*, 1999; Yang *et al.*, 1999). AG directly binds to and activates *SPL* (Ito *et al.*, 2004; Ó'Maoiléidigh *et al.*, 2013), and ectopic activation of SPL is sufficient to activate pollen development in petals, revealing that male sporogenesis is a developmental module invoked by *AG* through *SPL* (Ito *et al.*, 2004). However, competence to respond to ectopic *SPL* was only seen in the inner organs of the *ag-1* mutant and was limited to the distal petal margins, showing that the exact timing and location of *SPL* function depend on additional, unidentified floral factors.

Another differentiation pathway that is specific to flowers leads to the formation of conical cells on the petal epidermis, which have characteristic cuticular wax ridges. These conical cells are a conserved feature of petals that has been implicated in the interaction with pollinators (Glover and Martin, 1998). *SHINE1*, which is directly bound by AP1 during petal development (Pajoro *et al.*, 2014), coordinates the expression of biosynthetic genes required for the production of the cuticular ridges (Shi *et al.*, 2011). The latter example illustrates how organ identity genes direct the gene expression programme up to the 'finishing touches' in floral organ development.

Conclusions and perspectives

We now have a much better understanding of the genetic networks that control leaf development, and numerous direct molecular links between floral organ identity genes and key nodes in these networks. Rather than functioning as master genes at the top of a regulatory hierarchy that overrides the leaf developmental programme, MADS-domain proteins directly modify every step of organ development, from early patterning to growth to final differentiation (Fig. 3). The current picture suggests that the interaction between organ identity genes and general regulators of organ development may produce the overall structure of floral organs on which organ-specific cell types and structures are added or suppressed by interaction with more specialized gene expression programmes.

One important next step will be to test how floral organ identity genes modify organ morphology through changes in the temporal or spatial expression patterns of general regulators of shoot development. To achieve this, at least three challenges lie ahead. First, we will need higher resolution, quantitative measurements of gene expression during organ development. An example of how this type of data can be integrated into 3D models of floral buds has been produced for early sepal development (La Rota *et al.*, 2011). Second, it will be necessary to reveal the links between the relevant regulatory genes and the cellular activities that constrain tissue growth (e.g. oriented cell expansion and cell cycle progression) (Schiessl *et al.*, 2014). Third, spatial modelling will be required to simulate and predict the feedbacks between gene expression, growth, and tissue mechanics. Progress has been made in establishing predictive models of leaf and floral organ growth (Robinson *et al.*, 2011; Sauret-Güeto *et al.*, 2013), and in understanding the feedbacks between tissue mechanics and growth (Kierzkowski *et al.*, 2012; Routier-Kierzkowska and Smith, 2013). In the years to come, these approaches may finally give a full understanding of exactly how shoot organs can be 'metamorphosed' as described by Goethe.

Another interesting point is the question of how variation on developmental programmes between organs in the same organisms relates to variation across organisms. In particular,

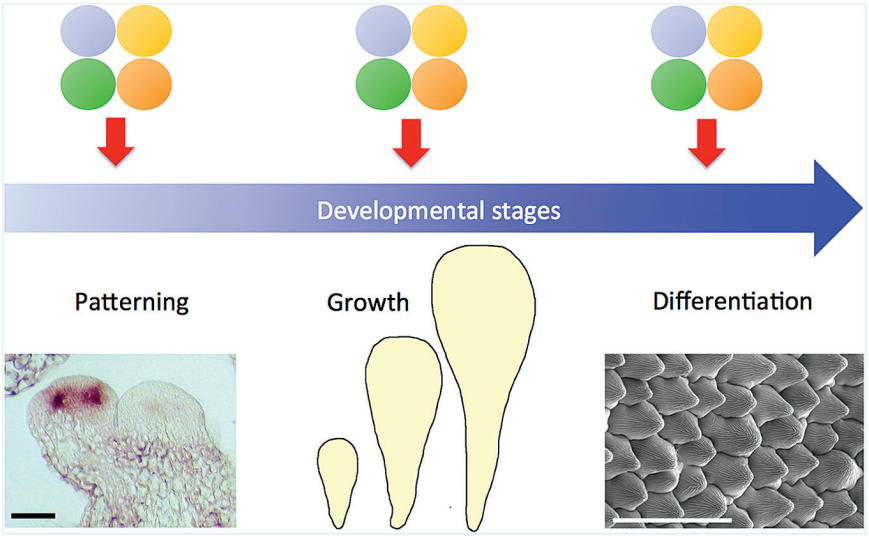

Fig. 3. Complexes of organ identity MADS-domain proteins (represented by the coloured circles; see Fig. 1) directly regulate processes required at all stages of organ development, including early organ patterning, subsequent organ growth, and final cellular differentiation. Lower panels: (left) expression of *CUC1* (as an example of a patterning gene) revealed by *in situ* RNA hybridization on a section through an early floral bud; (middle) outlines of a growing petal (based on Sauret-Güeto *et al.* 2013); (right) scanning electron micrograph of conical cells of the petal epidermis (as an example of differentiated cells in a mature organ). Scale bar: 50 μm.

it would be interesting to know to what extent the regulatory pathways that produced evolutionary variation in leaf development (Townsley and Sinha, 2012; Tsukaya, 2014) also played a role in establishing the differences between leaves and floral organs. Parallels between morphological diversity between segments of the same organisms and across species may give insight not only into organ development in individual species, but also into the evolutionary diversity of plant form.

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