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Review

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Divide et impera: boundaries shape the plant body and initiate new meristems

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Summary

Boundaries, established and maintained in different regions of the plant body, have diverse functions in development. One role is to separate different cell groups, for example the differentiating cells of a leaf primordium from the pluripotent cells of the apical meristem. Boundary zones are also established during compound leaf development, to separate young leaflets from each other, and in many other positions of the plant body. Recent studies have demonstrated that different boundary zones share similar properties. They are characterized by a low rate of cell divisions and specific patterns of gene expression. In addition, the levels of the plant hormones auxin and brassinosteroids are down-regulated in boundary zones, resulting in a low differentiation level of boundary cells. This feature seems to be crucial for a second important role of boundary zones, the formation of new meristems. The primary shoot meristem, as well as secondary and ectopic shoot meristems, initiate from boundary cells that exhibit competence for meristem formation.

I. Introduction

The architecture of flowering plants observed in nature shows an enormous heterogeneity. This variation is, to a large extent, caused by differences in the form of their leaves and in the branching patterns of their shoots. Different from animals, plants follow an open, indeterminate pattern of development. Although their basic body plan is determined by a species-specific genetic program, plants continue to form new organs after embryogenesis, which allows them to adapt their architecture to the prevailing conditions in the local environment. This ability can be traced back to the activity of meristems, groups of pluripotent cells, which are established and maintained at the tips of shoots and roots. The primary shoot apical meristem (SAM) is established during embryogenesis at the apical end of the embryonic axis. After germination, leaf primordia initiate in a regular pattern from cells at the flanks of the SAM. These leaf primordia, containing groups of differentiating cells, are separated from the pluripotent cells of the SAM by a boundary zone, to maintain functional integrity of the meristem and to enable maturation of leaves. Dependent on the species-specific genetic program and environmental conditions, leaf primordia develop either into simple leaves or into compound (complex) leaves. Simple leaves have an undivided leaf blade, whereas compound leaves, like tomato (*Solanum lycopersicum*) leaves, consist of individual small units, called leaflets. Such leaflets originate from a zone of transient organogenetic activity at the leaf

margins, the marginal blastozone (Hagemann & Gleissberg, 1996). Individual leaflets of a complex leaf are separated from each other and from their connecting structure, called the rachis, by boundary zones. Additional boundaries are established at many other positions of the plant body, separating for example different whorls of floral organs, the main axis of a plant from its side-shoots or a fruit from the rest of the plant. Recent experiments have shown that the boundary zones between the meristem and leaf primordia show similar properties to the boundaries between leaflets (Busch *et al.*, 2011), supporting the view that these are homologous structures as a result of descent from an ancestral shoot system (Floyd & Bowman, 2010). In general, boundaries are characterized by a low number of cell divisions (Hussey, 1971; Reddy *et al.*, 2004) and specific patterns of gene expression (Souer *et al.*, 1996; Aida *et al.*, 1997; Takada *et al.*, 2001; Vroemen *et al.*, 2003; Weir *et al.*, 2004; Berger *et al.*, 2009).

During post-embryonic development, plant architecture is modulated further by the formation of new shoot meristems. Secondary shoot meristems are formed in the axils of leaves. Furthermore, in many plant species, so-called ectopic meristems initiate at different positions of the plant body, for example on the compound tomato leaf (Rossmann *et al.*, 2015). Interestingly, meristem formation is preferentially associated with the boundaries that separate different parts of the plant body. Secondary shoot meristems function like the primary meristem, initiating the formation of a few leaf primordia. The newly established shoot (bud) may enter a resting period or it can grow out into a side-shoot. Modulating the pattern of secondary meristem formation as well as the timing and extent of growth of side-shoots allows a plant to adapt its architecture to the prevailing conditions.

To date, little is known about the different roles of boundary zones in the complex process of shaping plant architecture. Here, we describe the characteristics of boundary zones and the roles of different genetic regulators that are required for their establishment and maintenance. Furthermore, we review how boundaries structure different parts of the plant body, in particular how they shape the leaf. Finally, the initiation of meristems leading to new axes of growth will be discussed. In this review, we focus on flowering plants and the term 'plants' is used as a synonym for 'flowering plants'.

II. Development of boundary zones

1. Establishment and properties of boundary zones

Development of a distinct morphological structure, for example a leaf, is the result of a strictly controlled process that involves the formation of boundary zones, which separate the new entity from the rest of the plant body. Boundaries are established very early in organ development and, as growth proceeds, they become morphologically visible as concave grooves, a consequence of local growth repression (Fig. 1a,b). Boundary cells often acquire a characteristic elongated shape forming a saddle-shaped surface, as exemplified by epidermal cells in the meristem-to-leaf primordium boundary of *Anagallis arvensis* (Kwiatkowska & Dumais, 2003). Boundary zones are also characterized by specific patterns of cell division. In tomato, the number of cell divisions is reduced in the boundary region between the apical meristem and developing leaf primordia (Hussey, 1971; Fig. 1c). In Arabidopsis, boundary cells between inflorescence and floral meristems down-regulate DNA synthesis and expression of cell-cycle-related genes (Breuil-Broyer *et al.*, 2004). Similarly, cells in the sepal-to-meristem boundary show a reduced proliferation rate compared with those in the flower meristem (Laufs *et al.*, 2004). Cell form and the pattern of cell division in boundary zones are at least partially determined by mechanical stress that results from variations in growth rate and cell expansion in different regions of the shoot apex (Hamant *et al.*, 2008). In response to local mechanical stress, cells modulate the array of cortical microtubules, as predicted by mathematical modeling. Indeed, boundary cells between flower primordia and the SAM show a tangential orientation of cortical microtubules parallel to the direction of maximal stress (Hamant *et al.*, 2008).

Local variations in cell division activity and in cell size also play important roles during leaf development. In Arabidopsis, small cells accumulate at the sinuses of leaf teeth, whereas adjacent cells (i.e. along a tooth) adopt an elongated shape (Kawamura *et al.*, 2010). In complex-leaved species, boundary zones separate individual leaflets from each other (Blein *et al.*, 2008). The distal leaflet boundaries of tomato and *Cardamine pratensis* leaves were shown to harbor smaller cells in comparison to surrounding tissues (Rossmann *et al.*, 2015). At the same time, these cells are less differentiated, as indicated by the lack of differentiation markers, such as stomata or trichomes, and exhibit a down-regulation of *Histone H4* mRNA accumulation, indicating a low cell division rate (Rossmann *et al.*, 2015; Fig. 1d).

All these data clearly suggest that boundary cells adopt a unique behavior, encompassing infrequent cell divisions and limited cell expansion, leading to the establishment of a zone of reduced growth (Reddy *et al.*, 2004). These observations already indicate that boundary zones are more than a physical separation layer between neighboring parts of the plant body; rather they provide a versatile module that is utilized in different developmental processes.

2. Genes that make boundaries

Some of the best studied regulators of boundary development belong to the NAC (*NO APICAL MERISTEM* (*NAM*); *ARABIDOPSIS TRANSCRIPTION ACTIVATION FACTOR1/2* (*ATAF1/2*); *CUP-SHAPED COTYLEDON2* (*CUC2*)) family, including the petunia gene *NAM* (Souer *et al.*, 1996), the snapdragon (*Antirrhinum majus*) gene *CUPULIFORMIS* (*CUP*) (Weir *et al.*, 2004), the Arabidopsis genes *CUC1*, *CUC2* and *CUC3* (Aida *et al.*, 1997; Takada *et al.*, 2001; Vroemen *et al.*, 2003) and their tomato ortholog *Goblet* (*Gob*) (Brand *et al.*, 2007; Berger *et al.*, 2009). These genes are consistently expressed in boundary zones and their loss of function causes fusion of adjacent organs (e.g. cotyledons, floral organs, and ovules) that would normally be separated (Souer *et al.*, 1996; Aida *et al.*, 1997; Takada *et al.*, 2001; Vroemen *et al.*, 2003; Weir *et al.*, 2004; Fig. 1e,f). *CUC1* and *CUC2* transcript accumulation is modulated by the action of microRNA *miR164* (Laufs *et al.*, 2004). The role of *NAM*/*CUC* genes is well conserved among a large range of species. Recently, it

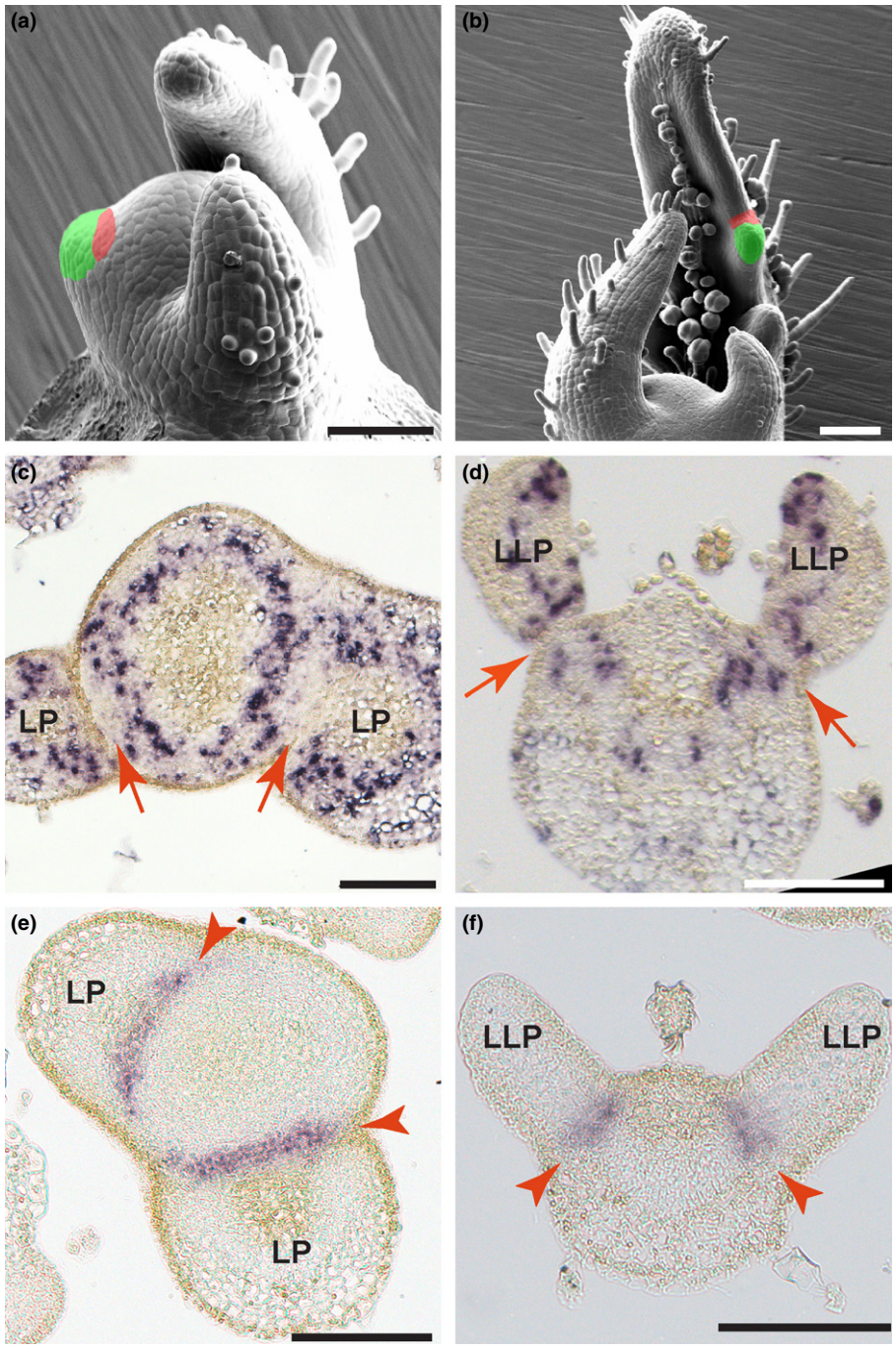

Fig. 1 Leaf and leaflet boundaries share similar properties. (a) In tomato, leaf primordium (green) formation at the flank of the shoot apical meristem (SAM) creates an adaxial boundary (red) between the leaf primordium and the SAM. (b) Formation of a leaflet primordium (green) at the leaf margin is accompanied by the development of similar boundaries. The distal leaflet boundary between leaflet and rachis, marked in red, is different from the proximal boundary. (c–f) Cross-sections through shoot apices (c, d) and leaf primordia (e, f). (c, d) Expression of the cell division marker *Histone H4* is absent in leaf axils (c, arrows) and in distal leaflet boundaries (d, arrows). (e, f) *Goblet* is expressed in the adaxial boundary of leaf primordia (e, arrowheads) and in the distal boundary of leaflets (f, arrowheads). Bars, 100 μm. LP, leaf primordium; LLP, leaflet primordium. Images (except a) are from Rossmann *et al.* (2015), with kind permission of *The Plant Journal*.

has been reported that the *NAM/CUC* ortholog from *Medicago truncatula* is necessary for organ separation including compound leaf development, and is also required for floral organ identity and development (Cheng *et al.*, 2012). Beside fused cotyledons, the loss-of-function *gob-3* mutant of tomato produces simpler leaves with smooth leaflet margins and lacks secondary leaflets (Fig. 2d), suggesting that *Gob* is also required for the formation of boundaries between leaflets in the compound tomato leaf (Berger *et al.*, 2009). Similarly, a reduction in the expression of three orthologous *CUC* genes from *Cardamine hirsuta* leads to the formation of fused leaflets, resulting in a simpler leaf (Blein *et al.*, 2008). In Arabidopsis, *CUC2* and *CUC3* genes act redundantly with the myeloblastosis oncoprotein (MYB)-domain transcription factor *LATERAL ORGAN FUSION1 (LOF1)* (Lee *et al.*, 2009). *LOF1* and *LOF2* loss-of-function mutants develop fused lateral organs, caused by abnormal cell divisions and cell expansions during early boundary establishment. In addition, *LOF1* and *CUC* genes show complex interactions, regulating each other's expression (Lee *et al.*, 2009; Gendron *et al.*, 2012; Fig. 3). It has been proposed that NAM/CUC proteins exert their function by repressing growth in the boundary regions, because mutations in these genes or down-regulation of their expression leads to ectopic growth in the boundaries between adjacent morphological structures (Souer *et al.*, 1996; Aida *et al.*, 1997; Breuil-Broyer *et al.*, 2004; Blein *et al.*,

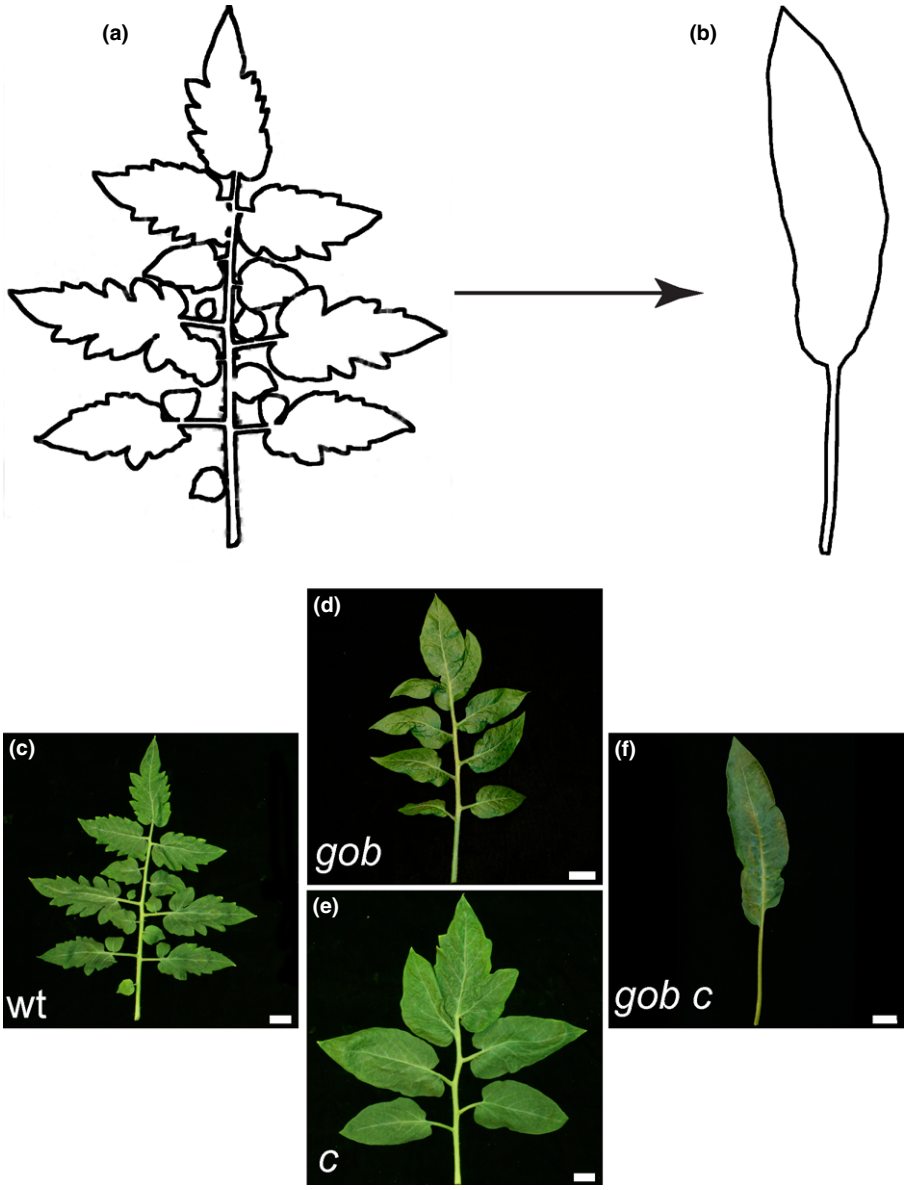

Fig. 2 Boundaries shape the leaf. (a) Silhouette of a typical compound leaf with leaflets and serrations of the leaf margin. (b) Silhouette of a simple leaf without any leaflets. (c) A wild-type (wt) tomato leaf develops leaflets and serrations of the leaf margin. (d) A leaf of the *goblet-3* (*gob*) mutant showing a reduced number of leaflets and serrations, compared with wt. (e) The *potato leaf* (*c*) mutant also develops leaves with strongly reduced complexity and a smooth leaf margin. (f) Leaflets and serrations are missing in the *c gob* double mutant. Genotypes in (c–f) are indicated in the lower left corner. Bars: (c–f) 2 cm. Images (c) and (e) are from Busch *et al.* (2011), with kind permission of *Plant Cell* (www.plantcell.org), Copyright American Society of Plant Biologists.

2008). However, the molecular mechanism by which these regulators control cell proliferation and differentiation in the boundaries is still not understood.

JAGGED LATERAL ORGANS (*JLO*) has been identified as a modulator of boundary establishment in Arabidopsis (Borghi *et al.*, 2007). The initially characterized *JLO* allele conditions a gain-of-function phenotype with an SAM arrest. *JLO* is expressed at the boundary between the SAM and organ primordia (Borghi *et al.*, 2007; Bureau *et al.*, 2010; Rast & Simon, 2012) and *jlo* loss-of-function mutants show fusions of cotyledons and of floral organs. *JLO* encodes a transcription factor of the *LATERAL ORGAN BOUNDARIES DOMAIN* (*LBD*) family. The founding member of the *LBD* family, *LATERAL ORGAN BOUNDARIES* (*LOB*), was identified as an enhancer trap line showing expression in the boundaries between meristems and lateral organs (Shuai *et al.*, 2002). *LOB* inactivation leads to the fusion of axillary branches and their subtending cauline leaves, suggesting an organ separation defect. *LOB* is activated by *BLADE-ON-PETIOLE1* (*BOP1*) and *BOP2* genes, which are transiently expressed in leaf primordia and restricted to the boundary when primordia first appear as morphologically distinct from the meristem (Ha *et al.*, 2004, 2007; Norberg *et al.*, 2005). Both *bop1* and *bop2* mutants display defects in inflorescence development, as they frequently form fused inflorescences and generate multiple flowers from the same node (Hepworth *et al.*, 2005; Ha *et al.*, 2007). *bop1 bop2* double mutants show ectopic tissue growth from the lamina, and lack a distinct petiole (Norberg *et al.*, 2005; Ha *et al.*, 2007). In the boundary, *BOP1/2* genes modulate differentiation and growth through the repression of *class I KNOTTED1-like homeobox* (*KNOX*) genes (Ha *et al.*, 2003, 2007). There is no evidence that *CUC* gene expression is altered in the *bop1 bop2* double mutant, suggesting that *BOP1/2* are not playing a role in the initial establishment of boundary domains (Khan *et al.*, 2014). Nevertheless, it has been recently shown that BOP activities are well conserved in a variety of species

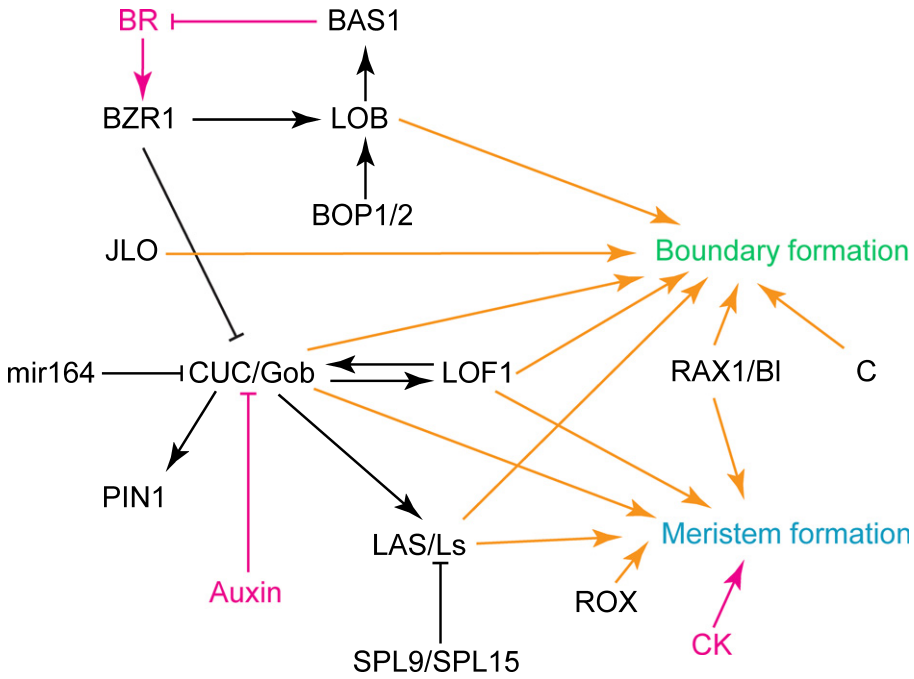

Fig. 3 Network of genetic and hormonal interactions modulating boundary establishment and meristem formation. Boundary establishment and meristem formation require the interplay of several transcription factors and plant hormones, including auxin, brassinosteroids (BRs) and cytokinin (CK). In Arabidopsis, boundary formation is promoted by *CUP-SHAPED COTYLEDON* (*CUC*) genes, *LATERAL ORGAN FUSION1* (*LOF1*), *LATERAL SUPPRESSOR* (*LAS*), *JAGGED LATERAL ORGANS* (*JLO*), *LATERAL ORGAN BOUNDARIES* (*LOB*) and *REGULATOR OF AXILLARY MERISTEMS1* (*RAX1*). *CUC* genes positively regulate *LOF1* expression, which in return activates *CUC* transcript accumulation. *CUC2* is needed to promote the formation of auxin convergence points through its action on *PIN-FORMED1* (*PIN1*). *CUC1* and *CUC2* expression is repressed by *miR164*, and that of *CUC2* also by auxin. *LOB* expression is activated by BLADE-ON-PETIOLE1/2 (BOP1/2) and by BRASSINAZOLE-RESISTANT1 (BZR1), a key transcriptional regulator in brassinosteroid (BR) signaling. LOB represses BR signaling by activating the BR-inactivating enzyme PHYB ACTIVATION TAGGED SUPPRESSOR1 (BAS1). Low BR concentrations release *CUC* genes and *LOF1* from BZR1 repression. Goblet (Gob), Lateral suppressor (Ls), Blind (Bl) and Potato leaf (C) modulate boundary formation in tomato. *LAS/Ls* expression is promoted by CUC/Gob and down-regulated by SQUAMOSA PROMOTER-BINDING PROTEIN-LIKE9 (SPL9)/SPL15. Apart from boundary establishment, *CUC/Gob*, *LOF*, *LAS/Ls* and *RAX1/Bl* genes are also required for meristem formation. In Arabidopsis, the basic helix-loop-helix (bHLH) transcription factor REGULATOR OF AXILLARY MERISTEM FORMATION (ROX) controls axillary meristem formation during vegetative development. Cytokinin promotes meristem formation. Positive interactions between genes are depicted by black arrows, promotion of developmental processes by orange arrows. Plant hormones and their interactions are indicated in magenta. mir164, microRNA miR164.

and developmental contexts. Indeed, *BOP1/2* orthologous genes *NODULE ROOT* (*NOOT*) and *COCHLEATA* (*COCH*), from *M. truncatula* and *Pisum sativum* (pea), respectively, have the same functions as *BOP* genes in Arabidopsis (Couzigou *et al.*, 2012). The interesting point here is that, even though functions of *NOOT* and *COCH* are needed in *M. truncatula* and pea leaf and flower development, their respective mutants were first characterized by the abnormal development of roots from nodules (Couzigou *et al.*, 2012). The authors proposed that *NOOT* and *COCH* define the boundaries between meristem and vascular tissue territories, leading to the conclusion that legume plants have recruited pre-existing developmental modules to create an apparently unrelated organ.

Another group of regulatory proteins that is specifically expressed in boundary zones was primarily identified because of its role in axillary meristem (AM) formation. The GRAS (GA INSENSITIVE, REPRESSOR OF GA1-3, SCARECROW) protein LATERAL SUPPRESSOR and its orthologs (Lateral Suppressor (Ls)/LATERAL SUPPRESSOR (LAS)/MONOCLUM1 (MOC1)) regulate the formation of axillary buds in tomato (Schumacher *et al.*, 1999), Arabidopsis (Greb *et al.*, 2003), *Oryza sativa* (rice; Li *et al.*, 2003), and other species. Furthermore, a small subgroup of *MYB* genes, related to the tomato *Blind* gene, were shown to modulate axillary meristem formation along the shoot axis in tomato (Schmitz *et al.*, 2002) and Arabidopsis (Keller *et al.*, 2006; Müller *et al.*, 2006). These genes are specifically expressed in the leaf axil and plants homozygous for loss-of-function alleles show fusions of axillary shoots to their subtending leaves or to the main axis (Greb *et al.*, 2003; Busch *et al.*, 2011), indicating that the respective gene product is required not only for axillary meristem formation, but also for organ separation.

To date, a number of boundary-specific genes have been described. An open question is: How do these regulators exert their function? Until recently, the *ORGAN BOUNDARY1/LIGHT-DEPENDENT SHORT HYPOCOTYLS3* (*OBO1/LSH3*) and *OBO4/LSH4* genes were the only known direct targets of *NAM/CUC* genes (Cho & Zambryski, 2011; Takeda *et al.*, 2011). They are specifically expressed in the boundary zones of shoot organs. Ablation of OBO1-expressing cells leads to defects in SAM and lateral organ formation (Cho & Zambryski, 2011). Recently, CUC2 was found to bind to the *LAS* promoter, positively regulating its expression (Tian *et al.*, 2014; Fig. 3). In the same study, *SPL9* and *SPL15*, belonging to the *SQUAMOSA PROMOTER-BINDING PROTEIN-LIKE* (*SPL*) family, were

shown to act as negative regulators of *LAS* expression. The *spl9 spl15* double mutant develops accessory meristems in its leaf axils (Tian *et al.*, 2014; Fig. 3). Interestingly, these two genes were not described as boundary genes, even though their orthologs in rice (Jiao *et al.*, 2010; Miura *et al.*, 2010) and maize (*Zea mays*; Chuck *et al.*, 2010) have such function.

Taken together, these findings suggest that all these genes are implicated in numerous developmental processes in the plant lifecycle of a broad range of species. Their functions are well conserved through evolution and have been used in a modular way for the development of various structures in the plant body. The common feature is that they contribute to the establishment of a critical zone, called the boundary zone, which is delimiting the development of different morphological structures, such as cotyledons, leaves, leaflets, floral organs and ovules. Currently, it is an open question whether or not expression of one of these genes is sufficient to initiate boundary formation.

3. Brassinosteroids set the limits

Brassinosteroids (BRs) modulate cell division, cell elongation and cell differentiation in boundary zones. High BR concentrations promote cell enlargement, whereas low BR concentrations cause a reduction in cell size. This seems to be at least in part mediated by the BR-activated transcription factor BRASSINAZOLE-RESISTANT1 (BZR1), which represses directly the expression of the boundary-specific *CUC* and *LOF1* genes. In wild type, BZR1 proteins accumulate to high levels in the central meristem and organ primordia, limiting the expression of a specific set of genes, including *CUC* genes, to the boundary (Gendron *et al.*, 2012). In Arabidopsis, the boundary-specific transcription factor *LOB* negatively regulates BR concentrations in organ boundaries by directly activating a BR-inactivating enzyme, PHYB ACTIVATION TAGGED SUPPRESSOR1 (BAS1). Ectopic expression of *LOB* causes a reduced BR response, and expression of *BAS1* under the control of the *LOB* promoter can rescue the organ fusion defects of the *lob* mutant. Furthermore, *LOB* expression is regulated by BRs (Bell *et al.*, 2012). In summary, *LOB* expression and BR concentrations form a negative feedback loop to regulate BR concentrations in the boundaries, promoting a reduction of growth in this specific region. This implies that BR signaling is a crucial tool to keep a check on cell division activity and cell size in organ boundaries.

4. Boundary zones constitute corridors of low auxin signaling

Local maxima of the plant hormone auxin convey important positional information with respect to the formation of specific morphological structures during plant development. Auxin regulates transcript accumulation of target genes by activation of AUXIN RESPONSE FACTORS (ARFs) (Liscum & Reed, 2002; Leyser, 2006). ARF activity is down-regulated by interaction with AUXIN/INDOLE ACETIC ACID (AUX/IAA) proteins (Liscum & Reed, 2002; Leyser, 2006), which are targeted for degradation by the proteasome in the presence of auxin (Dharmasiri *et al.*, 2005; Kepinski & Leyser, 2005). Auxin response maxima instruct, for example, the position of leaf primordia at the shoot apex (Reinhardt *et al.*, 2003; Bayer *et al.*, 2009) and the pattern of leaflets during complex leaf development (Barkoulas *et al.*, 2008; Koenig *et al.*, 2009; Ben-Gera *et al.*, 2012). In contrast, Sorefan *et al.* (2009) showed that a regulated auxin minimum is required to establish the valve margin, a boundary zone that is essential for Arabidopsis fruit opening. Similarly, the adaxial boundary of leaf primordia is characterized by low auxin concentrations, as suggested by the local patterns of PIN-FORMED1 (PIN1) polarization and auxin response (Wang *et al.*, 2014a,b). How is this low auxin response zone established? Leaf primordium initiation is correlated with auxin accumulation resulting from PIN1-mediated polar auxin transport (Reinhardt *et al.*, 2003; Bayer *et al.*, 2009) and local auxin biosynthesis (Cheng *et al.*, 2007). Subsequently, when the leaf primordium starts to grow out, PIN1 polarity at the adaxial boundary reverses and is directed towards the center of the SAM. This shift in auxin transport direction leads to auxin depletion and enables boundary zone formation between the leaf primordium and the meristem (Wang *et al.*, 2014a,b).

In the inflorescence meristem, polar auxin transport via PIN1 leads to local auxin maxima at positions where new flower primordia will form (Heisler *et al.*, 2005). Once a flower primordium is initiated, PIN1 intracellular polarization in the adaxial boundary reverses, from towards the flower primordium to towards the meristem. Therefore, the boundary between the floral meristem and the inflorescence meristem also constitutes a low-auxin environment (Heisler *et al.*, 2005; Vernoux *et al.*, 2011). Furthermore, the boundaries between cotyledons (Furutani *et al.*, 2004) and between developing leaflets (Koenig *et al.*, 2009) have been correlated with low auxin concentrations. Taking into account the strong similarities in histology and gene expression patterns in different boundaries of the plant body, we speculate that low auxin signaling is a general feature of boundary zones.

How is PIN1 re-localization mediated? It is known that the AGCIII kinase PINOID (PID) modulates intracellular localization of PIN proteins by phosphorylation. In *pid* mutants, normal PIN1 polarization is disrupted, resulting in abnormal auxin distribution (Wang *et al.*, 2014a). Heisler *et al.* (2010) showed that intracellular PIN1 localization in the SAM is highly correlated with the orientation of cortical microtubules. However, re-orientation of PIN1 and re-orientation of microtubule arrays do not directly depend on each other, but seem to be regulated both by an unknown upstream mechanism. A detailed mechanistic explanation for intracellular re-shuffling of PIN proteins is still missing.

An earlier study demonstrated a link between *CUC* gene expression and auxin-dependent organ initiation (Furutani *et al.*, 2004). During embryogenesis of *pin1 pid* double mutants, intracellular PIN1 localization is compromised, leading to a disturbance of auxin distribution in the embryonic shoot apex. As a consequence, *CUC* gene expression is not restricted to the boundary region between the two cotyledons, but expands throughout the top of the embryo and represses cotyledon formation. In *pin1 pid cuc1 cuc2* mutants, cotyledon formation is restored. In addition, auxin application to the *cuc1* single mutant significantly enhanced the cotyledon fusion phenotype, corroborating that auxin represses *CUC2* expression. Beside PIN1-mediated auxin transport, the

alternative auxin transporter ATP-BINDING-CASSETTE B19 (ABCB19) plays an important role in boundary establishment. *abcb19* mutants displayed increased auxin concentrations in boundaries of the inflorescence meristem, whereas *CUC2* and *LOF1* expression was found to be decreased (Zhao *et al.*, 2013). In *cuc3 abcb19* double mutants, organ fusion defects were more severe. Taken together, these results demonstrate a sophisticated interplay between auxin signaling and *CUC* gene expression during boundary development. Low auxin signaling in boundary zones may be required to maintain boundary cells at a low differentiation level.

III. Boundaries shape the leaf

Recent studies revealed that several mechanisms involved in leaf primordium formation do also play an important role in leaf development. Leaves of many plant species develop a serrated leaf margin, with a characteristic pattern of protrusions and indentations. In Arabidopsis, a combination of genetic approaches and computer modeling suggested that a sequence of interspersed auxin maxima and *CUC2* expression domains pre-pattern the leaf margin (Bilsborough *et al.*, 2011). This patterning is dependent on PIN1 and CUC2 activity, as *pin1* and *cuc2* mutants develop smooth leaf margins. CUC2 promotes the formation of auxin maxima through its action on PIN1 re-orientation and in return auxin down-regulates *CUC2* expression, forming a robust feedback loop (Bilsborough *et al.*, 2011). *CUC2* expression conditions a repression of growth, whereas elevated auxin concentrations promote outgrowth of the leaf margin. As a result, small cells accumulate in the sinuses of the leaf margin, whereas adjacent cells in leaf teeth adopt an elongated shape (Kawamura *et al.*, 2010). In summary, patterning of the leaf margin is achieved by an alternating sequence of promotion and retardation of growth.

In compound-leaf species, such as *C. hirsuta* and tomato, leaflet formation requires PIN1-mediated polar auxin transport. Auxin is transported through the epidermis of leaf primordia forming convergence points, as visualized by the DR5 auxin response reporter, at positions where leaflet primordia initiate (Barkoulas *et al.*, 2008; Koenig *et al.*, 2009; Ben-Gera *et al.*, 2012). In the *C. hirsuta pin1* mutant, both leaf primordium initiation at the shoot apex and lateral leaflet formation are disrupted (Barkoulas *et al.*, 2008). In tomato, blocking of polar auxin transport results in development of less compound leaves (Koenig *et al.*, 2009; Ben-Gera *et al.*, 2012). In contrast, auxin application leads to the formation of ectopic leaflets and lamina outgrowth, suggesting that auxin triggers leaflet initiation and lamina growth (Barkoulas *et al.*, 2008; Koenig *et al.*, 2009; Ben-Gera *et al.*, 2012). Leaflet separation is strongly compromised in the tomato AUX/IAA mutant *entire* (*e*). Young *e* mutant leaf primordia show a broad auxin response at the leaf margin, corroborating that the interspersed pattern of auxin response maxima in wild-type tomato plants is a prerequisite for the observed pattern of compound leaf development (Koenig *et al.*, 2009; Ben-Gera *et al.*, 2012).

Besides auxin transport and signaling, different boundary-specific transcription factors play important roles in shaping a complex leaf. In several compound-leaf species, *CUC/GOB* orthologous genes are expressed in the boundary of leaflets and down-regulation of their expression results in simplification of leaf architecture (Blein *et al.*, 2008; Berger *et al.*, 2009; Cheng *et al.*, 2012; Fig. 2d). Similar to their activity at the margin of Arabidopsis leaves, *CUC/GOB* genes restrict growth in the boundary zones between initiating leaflets and promote, thereby, the development of separated units in a complex leaf. A second transcriptional regulator, called *Potato leaf* (*C*), acts redundantly with Gob in tomato (Fig. 2e). In comparison to wild-type, *c* mutants develop less complex leaves (Busch *et al.*, 2011), with a reduced number of leaflets and a smooth leaflet margin. A combination of both loss-of-function alleles in a *c gob* double mutant results in tomato plants that form simple leaves (Fig. 2a–f). *Potato leaf* is expressed in the boundaries between initiating leaflets and represses growth in these regions (Busch *et al.*, 2011). Taken together, these results demonstrate that the activity of boundary-specific regulators, mediating a retardation of growth between initiating leaflets, is strictly required for complex leaf development.

Furthermore, other endogenous compounds modulate complex leaf development, such as the plant hormone cytokinin (CK). In tomato, misexpression of the CK biosynthesis gene *ISOPENTENYL TRANSFERASE* (*IPT*) from the *FILAMENTOUS FLOWER* (*FIL*) promoter, which drives expression throughout the young leaf primordium, leads to an increase in leaf complexity, whereas the CK degradation gene *Cytokinin dehydrogenase* (*CKX*), when expressed from the same promoter, conditions a strong reduction in complexity (Shani *et al.*, 2010). These data suggest that high CK concentrations promote leaflet formation, while low CK concentrations restrict it. In these transgenic plants, auxin response patterns, as indicated by the DR5::VENUS and PIN1::PIN1-GFP reporters, remained unchanged, suggesting that manipulation of CK concentrations does not interfere with auxin distribution (Shani *et al.*, 2010).

Recently, the REDUCED COMPLEXITY (RCO) homeodomain protein was identified as an important regulator of compound leaf development in *C. hirsuta*. Brassicaceae contain two *RCO*-like genes, whereas *RCO* is absent in Arabidopsis. It is supposed to inhibit growth between developing leaflets, thus promoting leaf complexity, and does not affect the auxin response pattern (Vlad *et al.*, 2014). A second study analyzed leaf dissection in two *Capsella* species (*Capsella rubella*, *Capsella grandiflora*) and also identified RCO as an important modulator of leaf shape (Sicard *et al.*, 2014). *RCO* transcripts accumulate at the base of terminal and lateral leaflets, but are absent from the meristem–leaf boundary, suggesting that it does not represent a typical boundary-specific gene (Vlad *et al.*, 2014). However, it will be interesting to see how RCO interacts with boundary-specific regulators during leaf development.

IV. Boundary zones are launching pads for meristem formation

1. Boundary zones initiate meristems

Beside the separation of parts of the plant body and the shaping of leaves, boundary zones initiate the formation of meristems (Fig. 4). In dicotyledonous plant development, a boundary zone is established between the two cotyledons, which subsequently gives rise to

formation of the SAM (Aida *et al.*, 1999). The primary SAM initiates at the bending-cotyledon stage from a small group of cells (Barton & Poethig, 1993) and bulges into the characteristic dome-shaped structure (Fig. 4a,d). After germination, leaf primordia develop from the peripheral zone of the SAM (Barton, 2010), establishing new boundary zones between leaf primordia and the SAM. Later in development, these boundary zones, at the junction between leaves and stem, initiate the formation of secondary meristems, called axillary meristems (AMs) (Fig. 4b,e). Furthermore, so-called ectopic meristems (EMs) can initiate from compound leaves in some species, for example in tomato and *C. pratensis*. The leaf margin of compound-leaved species comprises a zone of transient organogenetic activity, called the marginal blastozone (Hagemann & Gleissberg, 1996), which launches the formation of leaflets that are separated from each other by boundary zones. EMs initiate from the distal, but not from the proximal leaflet boundary (Fig. 4c,f). All these meristems produce leaf primordia developing into buds, which, depending on the plant species, environmental conditions or their position in the plant body, either stay dormant or grow out into shoots.

Although meristems initiate from boundary zones, not all boundaries form meristems, as for example the proximal leaflet boundary. In addition, meristem initiation is often delayed with respect to boundary zone establishment. The developmental timing of meristem initiation depends on the type of meristem, species-specific or even accession-specific patterns of meristem formation, and environmental conditions. During vegetative development of Arabidopsis Columbia (Col-0), AM initiation can first be detected in the axils of P16 leaf primordia, using the meristem marker *SHOOTMERISTEMLESS* (*STM*) (Greb *et al.*, 2003). A similar delay of AM initiation with respect to boundary establishment was observed in tomato (cv Moneymaker), where AMs can be detected as small bulges, monitored by scanning electron microscopy, in the axils of P7 primordia (Wang *et al.*, 2014a). An extreme case of late meristem formation from boundaries is illustrated by the ectopic shoots formed on distal leaflet boundaries of adult tomato leaves, which initiate when most differentiation processes in the leaf, such as formation of vasculature and specialized leaf cells (e.g. stomata and trichomes), have already occurred. These results pose a question: From which cells do such meristems develop? The patterns of cell division and the absence of well-known differentiation markers, such as stomata and trichomes, indicate that the leaf axil and the distal leaflet boundary harbor pluripotent cell groups that retain the competence for meristem formation (Rossmann *et al.*, 2015).

2. Genes that regulate meristem formation

Several key regulators of boundary formation have been identified that share the features of a boundary-specific expression pattern and conspicuous organ fusions in their loss-of-function mutants (Fig. 3; Table 1). Many of these genes also affect the initiation of

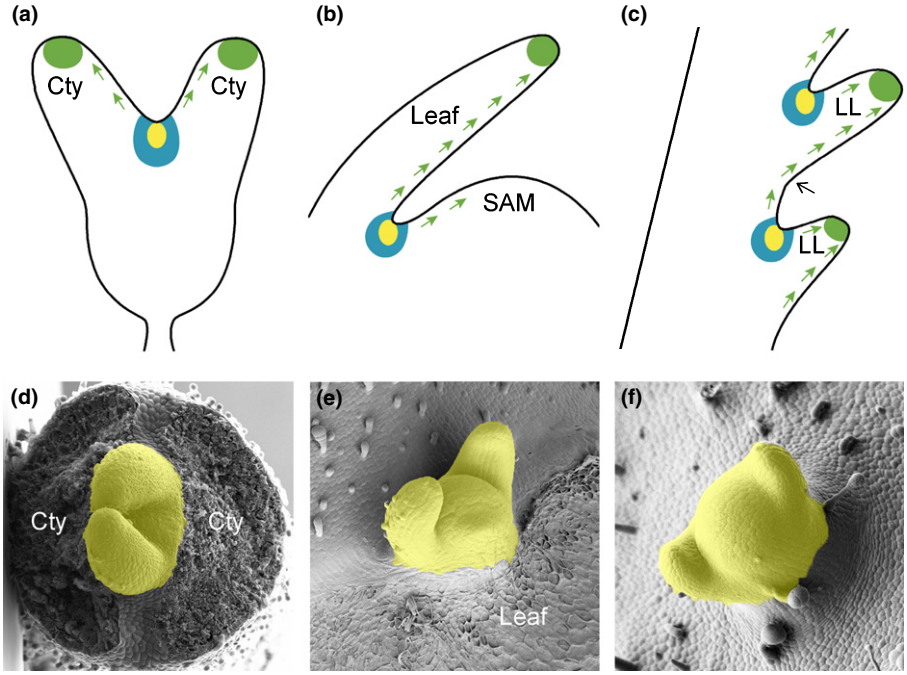

Fig. 4 Tomato boundary zones initiate shoot meristems. (a) During embryogenesis, auxin is transported out of the inter-cotyledon boundary and accumulates at the tip of cotyledon primordia (green). This results in a low-auxin zone between the cotyledon primordia (blue) in which the shoot apical meristem (SAM) initiates (yellow). (b) After leaf primordium formation, auxin is depleted from the leaf axil, leading to an auxin maximum at the primordium tip (green) and to an auxin minimum in the leaf axil (blue). Axillary meristems are formed at the boundary between developing leaves and the SAM (yellow). (c) During complex tomato leaf development, auxin is transported out from the regions between developing leaflets. Ectopic meristems are occasionally formed in a hypothetical low-auxin zone (blue) at the distal boundary between the leaflet and rachis (yellow), but not at the proximal boundary (black arrow). Green arrows indicate the direction of auxin flow. (d–f) Scanning electron microscopy (SEM) images of tomato shoot meristems formed between cotyledons (d), in the leaf axil (e) and at the distal leaflet boundary (f). Cty, cotyledon; LL, lateral leaflet. Image (f) is from Rossmann *et al.* (2015), with kind permission of *The Plant Journal*.

meristems (Fig. 3). This applies to formation of the primary meristem, to secondary meristems or in some species to the formation of ectopic meristems on leaves. Very important are the *NAM/CUC* genes *CUC1* and *CUC2*, required for the formation of the inter-cotyledon boundary zone and the SAM. From the globular until the torpedo stage of embryonic development, *CUC2* mRNA accumulates in the upper region of the embryo, focusing to the boundary between cotyledons (Aida *et al.*, 1999). Loss of *CUC1* and *CUC2* gene function causes a lack of embryonic SAM (Aida *et al.*, 1997). Similarly, tomato *gob-3* loss-of-function mutants do not form an embryonic SAM (Brand *et al.*, 2007). Other genes involved in establishment of the inter-cotyledon boundary, such as *LOF1*, contribute to meristem maintenance, as the meristem defect of the weak *stm-10* allele is enhanced by *lof1* (Lee *et al.*, 2009).

Furthermore, *LOF1* and its paralog *LOF2* are both specifically expressed in the adaxial leaf boundary and modulate AM formation in Arabidopsis (Lee *et al.*, 2009). In *lof1* single mutants and in *lof1 lof2* double mutants, accessory side-shoots do not develop. The orthologous gene in tomato, *Trifoliate*, regulates axillary meristem formation and compound leaf development, as the *tf* mutant phenotype shows a reduced number of side-shoots and a reduction in leaf complexity (Naz *et al.*, 2013). The proposed function of this R2R3 transcription factor is to inhibit differentiation and maintain morphogenetic competence. Another R2R3 MYB protein, called REGULATOR OF AXILLARY MERISTEMS1 (RAX1), which is also specifically expressed in the boundary region between SAM and leaf primordia, modulates AM formation during early vegetative development in Arabidopsis (Keller *et al.*, 2006; Müller *et al.*, 2006), whereas the orthologous genes in pepper (*Capsicum annuum*) and tomato regulate both AM initiation and transition to flowering (Mapelli & Kinet, 1992; Schmitz *et al.*, 2002; Jeifetz *et al.*, 2011).

Beside their involvement in SAM formation, *NAM/CUC* genes also play a very important role during postembryonic development, as loss of these gene functions leads to defects in shoot branching in Arabidopsis, snapdragon and tomato (Weir *et al.*, 2004; Hibara

Table 1 Genes involved in boundary and meristem formation

Gene in Arabidopsis	Homologous genes in other species	Protein family	Loss-of-function mutant phenotypes	Expression domain in boundary between	References
BOP1-2	*NOOT* (medicago (*Medicago truncatula*)), *COCH* (pea (*Pisum sativum*)), *Cul4* (barley (*Hordeum vulgare*))	BTB/POZ	Fused inflorescences, simpler leaves in compound leaf species	SAM and leaf primordia, within the leaf	Ha *et al.* (2004, 2007); Norberg *et al.* (2005); Couzigou *et al.* (2012); Tavakol *et al.* (2015)
CUC1-3	*Gob* (tomato (*Solanum lycopersicum*)), *CUP* (snapdragon (*Antirrhinum majus*)), *NAM* (petunia (*Petunia hybrida*))	NAC	Fusions of various organs, simpler leaves in compound-leaf species, smoother leaf margin, reduced number of AMs	SAM and lateral organs (cotyledons, leaves, floral organs), leaflet and rachis, emerging teeth of simple leaves	Souer *et al.* (1996); Aida *et al.* (1997); Takada *et al.* (2001); Vroemen *et al.* (2003); Weir *et al.* (2004); Brand *et al.* (2007); Berger *et al.* (2009)
JLO	Unknown	LBD	Fusion of cotyledons and floral organs	SAM and organ primordia	Borghi *et al.* (2007); Bureau *et al.* (2010)
LAS	*Ls* (tomato), *MOC1* (rice (*Oryza sativa*))	GRAS	Fusion of lateral branches to the main axis, reduced number of AMs, reduced number of tillers and spikelets	SAM and lateral organs, leaflet and rachis, in mature tiller bud	Schumacher *et al.* (1999); Li *et al.* (2003); Greb *et al.* (2003); Busch *et al.* (2011)
LOB	*ra2* (maize (*Zea mays*)) *ELP/PLP* (medicago) *SLP* (lotus (*Lotus japonicus*)) *APU* (pea)	LBD	Fusion of axillary branch and leaf	SAM and lateral organs	Shuai *et al.* (2002); Zhou *et al.* (2012); Bortiri *et al.* (2006); Chen *et al.* (2012)
LOF1-2	*Tf* (tomato)	MYB	Organ fusions, reduced number of AMs, simpler leaves in compound-leaf species	Stem and cauline leaves, inflorescence meristem and young floral primordia, floral organs	Lee *et al.* (2009); Naz *et al.* (2013)
LSH3/4 (*OBO1/4*)	*G1* (rice)	ALOG	Only gain-of-function phenotype described	SAM and lateral organs (cotyledons, leaves, floral organs)	Takeda *et al.* (2011); Cho & Zambryski (2011); Yoshida *et al.* (2009)
RAX1	*Bl* (tomato)	MYB	Reduced number of AMs	SAM and leaf primordia	Keller *et al.* (2006); Müller *et al.* (2006); Busch *et al.* (2011)
ROX	*ba1* (maize) *LAX1* (rice)	bHLH	Reduced number of AMs, reduced number of tillers and spikelets	SAM and leaf primordia	Komatsu *et al.* (2003); Gallavotti *et al.* (2004); Yang *et al.* (2012)

AM, axillary meristem; *APU*, *APULVINIC*; *ba1*, *barren stalk1*; *Bl*, *Blind*; *BOP*, *BLADE-ON-PETIOLE*; *COCH*, *COCHLEATA*; *CUC*,*CUP-SHAPED COTYLEDON*; *Cul4*, *Uniculm4*; *CUP*, *CUPULIFORMIS*; *ELP/PLP*, *ELONGATED PETIOLULE/PETIOLULE-LIKE PULVINUS; G1*, *LONG STERILE LEMMA1*; *Gob*, *Goblet*; *JLO*, *JAGGED LATERAL ORGANS*; *LAS*, *LATERAL SUPPRESSOR*; *LAX1*, *LAX PANICLE1*; *LOB*, *LATERAL ORGAN BOUNDARIES*; *LOF*, *LATERAL ORGAN FUSION*; *Ls*, *Lateral suppressor*; *LSH*, *LIGHT SENSITIVE HYPOCOTYLS*; *MOC1*, *MONOCULM1*; *NAM*, *NO APICAL MERISTEM*; *NOOT*, *NODULE ROOT*; *OBO*, *ORGAN BOUNDARY*; *ra2*, *ramosa2*; *RAX*, *REGULATOR OF AXILLARY MERISTEMS*; *ROX*, *REGULATOR OF AXILLARY MERISTEM FORMATION*; SAM, shoot apical meristem; *SLP*, *SLEEPLESS*; *Tf*, *Trifoliate*.

et al., 2006; Raman *et al.*, 2008; Busch *et al.*, 2011). Transcript accumulation of *CUC1* and *CUC2* is modulated by microRNA *miR164*, fine-tuning the number of secondary meristems per leaf axil (Laufs *et al.*, 2004; Raman *et al.*, 2008). Another important regulator of AM formation expressed in the leaf axil has been studied through characterization of orthologous genes in Arabidopsis (*LAS*; Greb *et al.*, 2003), tomato (*Ls*; Schumacher *et al.*, 1999) and rice (*MOC1*; Li *et al.*, 2003). Mutations in *LAS/Ls* strongly compromise AM formation, leading to a complete loss of side-shoots during vegetative development in tomato and Arabidopsis, whereas the rice gene *MOC1* modulates AM formation in both vegetative and reproductive stages. Loss of MOC1 function conditions a lack of vegetative side-shoots (tillers) as well as a reduction in reproductive rachis branches and spikelets (Li *et al.*, 2003).

LAX PANICLE1 (*LAX1*) and *BARREN STALK1* (*BA1*) were characterized as key regulators of shoot and inflorescence branching in the monocots rice and maize, respectively (Komatsu *et al.*, 2003; Gallavotti *et al.*, 2004). *lax1* mutant plants display a defect in AM formation during vegetative development and formation of panicle branches is severely reduced (Oikawa & Kyozuka, 2009). Similarly, maize *ba1* mutants are compromised in lateral meristem initiation at all stages of development (Ritter *et al.*, 2002; Gallavotti *et al.*, 2004). *LAX1* and *ba1* encode orthologous bHLH proteins expressed at the adaxial boundary of developing lateral organs. The LAX1 protein moves from the boundary region towards the site where the lateral meristem will be formed, to enhance cell proliferation (Oikawa & Kyozuka, 2009). *REGULATOR OF AXILLARY MERISTEM FORMATION* (*ROX*) is the ortholog of *LAX1* and *BA1* in Arabidopsis. In contrast to *lax1* and *ba1*, the *rox* mutant does not display any branching defects during reproductive development. However, during early vegetative development axillary bud formation is compromised in this mutant (Yang *et al.*, 2012). Similar to *LAX1* and *ba1*, *ROX* is expressed in the adaxial boundary between leaf primordia and the SAM.

Recently, it was discovered that the distal leaflet boundary of complex tomato leaves is a region where EMs frequently initiate. This EM formation correlates with and is dependent on specific expression of the transcriptional regulators *Ls* and *Gob*. *NAM/CUC3* gene expression in the distal leaflet boundary is evolutionarily conserved in many compound leaved species, such as *Aquilegia caerulea, S. tuberosum, C. hirsuta*, *P. sativum* and *C. pratensis* (Blein *et al.*, 2008; Berger *et al.*, 2009; Rossmann *et al.*, 2015). *In tomato,* the complete lack of EMs in *gob-3* loss-of-function plants and an enhancement of EM formation in *Gob-4d* gain-of-function mutants support the role of *Gob* in this process. In addition, the GRAS gene *Ls* is expressed in a domain very similar to that of *Gob* (Rossmann *et al.*, 2015). Loss of *Ls* function also results in a complete block in EM formation. Genetic experiments and expression studies suggest that the two genes act in hierarchical order, with *Ls* being downstream of *Gob*, to initiate new meristems at distal leaflet boundaries (Rossmann *et al.*, 2015).

3. Plant hormones trigger meristem initiation

Beside their role in boundary establishment and maintenance, plant hormones have also a function in meristem initiation. In maize, the *sparse inflorescence1* (*spi1*) and *vanishing tassel2* (*vt2*) mutants display strong branching defects (Gallavotti *et al.*, 2008a; Phillips *et al.*, 2011). *spi1* promotes AM formation during both vegetative and reproductive development, whereas *vt2* is only active in inflorescence development. *spi1* encodes a flavin monooxygenase related to the Arabidopsis *YUCCA* genes (Gallavotti *et al.*, 2008a), while *vt2* is the ortholog of the Arabidopsis *TRYPTOPHAN AMINOTRASNFERASE OF ARABIDOPSIS1/TRYPTOPHAN AMINOTRASNFERASE RELATED* (*TAA1/TAR*) genes (Phillips *et al.*, 2011). Mutations in either one of these auxin biosynthesis genes strongly compromise AM formation, underpinning a crucial role for local auxin biosynthesis in this process.

In addition to local auxin biosynthesis, polar auxin transport was shown to influence AM formation in maize. Application of the auxin efflux carrier inhibitor N-1-naphthylphthalamic acid (NPA) not only prevents initiation of AMs in the maize inflorescence, but also affects spikelet development (Wu & McSteen, 2007). Moreover, AM formation is strongly compromised in the polar auxin transport mutant *barren inflorescence2* (*bif2*) (McSteen & Hake, 2001; McSteen *et al.*, 2007). *bif2* encodes an AGC serine/threonine kinase orthologous to the Arabidopsis PINOID protein. Compared with wild type, maize *bif2* mutants develop shorter tassel branches and a reduced number of spikelets. Besides its role in inflorescence development, *bif2* affects the formation of both primary and secondary tillers (McSteen *et al.*, 2007). Like PINOID, BIF2 regulates intracellular polarization of the auxin efflux carrier PIN1 (Skirpan *et al.*, 2009). Differential polarization of PIN1 polar auxin transporters leads to local auxin maxima, which are associated with AM formation in maize. (Gallavotti *et al.*, 2008b).

At first glance, the role of auxin in AM formation seems to differ between maize and Arabidopsis. As mentioned above, two studies (Wang *et al.*, 2014a,b) revealed that, during Arabidopsis vegetative development, the boundary zone between a leaf primordium and the SAM contains low auxin concentrations. In addition, ectopic auxin synthesis in the leaf axil compromises AM formation, while repression of auxin signaling partially rescues the branching defect of the polar auxin transport mutant *pid-9* (Wang *et al.*, 2014a). These results suggest that a low auxin environment is required for AM formation in Arabidopsis, whereas in maize auxin maxima are associated with the formation of AMs. How to explain the different requirements for auxin in AM formation of maize and Arabidopsis? One important difference between vegetative Arabidopsis development and maize inflorescence development is the developmental timing of AM initiation. In vegetatively growing Arabidopsis plants, AM initiation is delayed with respect to formation of the corresponding leaf primordium. During this transition phase, cells in the leaf axil, which later initiate AMs, are kept in a competent state, as indicated by expression of the meristematic marker *STM* (Greb *et al.*, 2003). The low auxin concentrations in the boundary are very likely part of a safeguard mechanism that prevents early differentiation of these cells. This view is compatible with the detached meristem concept, which describes the situation in ferns where lateral buds originate from pluripotent cells tracing back to the SAM (Wardlaw, 1943). However, during maize inflorescence development, such a safeguard mechanism is probably not needed,

because AMs derive rapidly from an existing meristem. In this scenario, the observed auxin maximum may be associated with meristem initiation. Such a trigger can also not be excluded in the vegetative phase of Arabidopsis development.

CKs are known to promote cell division in the shoot and to positively regulate SAM size and activity (Barton, 2010). Do CKs also play a role in axillary meristem formation? Recently, it was shown that in Arabidopsis leaf axils a CK pulse precedes AM formation and that CK perception and signaling mutants are compromised in AM development (Wang *et al.*, 2014b). The Arabidopsis *supershoot* mutant displays increased CK concentrations compared with wild type and produces more AMs in leaf axils, leading to a highly branched shoot architecture (Tantikanjana *et al.*, 2001). It was also shown that CK biosynthesis is required for axillary bud formation, as AM initiation is strongly reduced in the early vegetative phase of *ipt3 ipt5 ipt7* triple mutants (Müller *et al.*, 2015). Similarly, ectopic CK biosynthesis in leaf axils can rescue AM initiation in the Arabidopsis *rax1* mutant, which fails to form AMs at the early vegetative stage (Wang *et al.*, 2014b). In tomato, ectopic expression of the *LONELY GUY 1* (*LOG1*) gene, encoding a CK activation enzyme, suppresses the branching defects of *blind* and *trifoliate* mutants (Eviatar-Ribak *et al.*, 2013). In addition, ectopic CK synthesis leads to misplaced organ formation, for example in *IPT7*-expressing tomato plants it results in development of meristems and flowers at the adaxial side of the leaf rachis (Shani *et al.*, 2010). Similarly, the Arabidopsis *ap1* mutant forms extra flowers in the boundary zone of sepals, as a consequence of ectopic CK accumulation (Han *et al.*, 2014).

In light of these findings, the current view is that both auxin and CK strongly impact on the process of AM formation. In situations, such as prolonged vegetative development, when AMs are not initiated in close proximity to the SAM, boundary cells are kept in a competent state, to maintain the ability for later meristem formation. In this scenario, a low-auxin environment is needed to avoid differentiation of these cells. Later, both an auxin and a CK pulse are probably needed to trigger AM initiation.

V. Conclusions/future perspectives

In the last couple of years, it has become evident that boundaries established in different regions of the plant body show similar properties. In tomato, this is for example reflected by the patterns of gene expression in boundary zones established in the leaf axil and between leaflets during compound leaf development. Similar gene modules, involving two paralogous MYB genes (*Bl* and *C*), *Gob* and *Ls*, are utilized in both boundaries and seem to be required for full functionality of these zones. At this point, we know very little about molecular interactions of the respective gene products. In addition, the axillary boundary is characterized by low auxin and brassinosteroid signaling, and, taking into account the other observed similarities, this may also hold for other boundary zones. It remains to be determined whether and how plant hormone signaling and specific gene expression profiles affect each other, to establish and maintain proper boundaries.

A second feature often associated with boundaries is the initiation of meristems, leading to the formation of new axes of growth. Although it had already been known for a while that the primary SAM and secondary shoot meristems originate from boundaries, a new study has demonstrated that this connection is more widespread than anticipated. Ectopic meristems on tomato leaves initiate from the adaxial edge of the distal leaflet boundary. Are all cells in boundary zones competent to form new meristems, and when in development are such competent cell groups established? One idea discussed in this context is that competent cell groups are derived from existing meristems, which at a later stage can be triggered to form new meristems. It seems to be feasible to test for a cell lineage that may precede, for example, the formation of AMs in the leaf axil, using live imaging and marker gene expression patterns.

Currently, we know very little about the physical properties of boundary zones. Changes in cell shape, as observed in boundary zones, are usually associated with changes in the physical properties of the region. It may be informative to study the physical properties of boundary zones, using live imaging in conjunction with computer modeling, and to relate these features to the molecular profiles observed in these regions.

Finally, we have to enhance our understanding of the triggers that ultimately initiate meristem formation. We have some initial information on the roles of the plant hormones auxin and CK in meristem initiation, but we do not know how this important step is connected to other developmental processes, such as vascularization of the stem or the onset of flowering.

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