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# Shoot and inflorescence branching

Gregor Schmitz and Klaus Theres

A major aspect of postembryonic plant development is the formation of secondary axes of growth: vegetative branches, inflorescence branches, or flowers. The first step in side-shoot development is the establishment of lateral meristems in the axils of leaves. GRAS-, MYB-, and bHLH-type transcription factors act as key regulators of early steps in this process. The REVOLUTA subfamily of HD-ZIP transcription factors controls the organization of lateral meristems. Whereas the development of lateral meristems into lateral buds is only poorly understood, recent studies have provided new insights into the regulation of lateral bud outgrowth. The *MORE AXILLARY GROWTH* (*MAX*) genes of *Arabidopsis* and the *RAMOSUS* (*RMS*) genes of pea are involved in the production, perception, and transduction of a signal that inhibits lateral bud outgrowth. Synthesis of this not-yet-identified hormone is positively regulated by the main shoot tip through auxin signalling.

**Addresses**
Max Planck Institute for Plant Breeding Research, Carl-von-Linné-Weg 10, D-50829 Cologne, Germany

Corresponding author: Theres, Klaus (theres@mpiz-koeln.mpg.de)

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## Introduction

The multitude of plant forms observed in nature is the result of the activities of different meristems during postembryonic development. In seed plants, the primary axis of growth, together with the primary shoot and root apical meristems, is laid down during embryonic development. During postembryonic shoot development, secondary axes of growth originate from lateral (axillary) meristems that are established in the axils of leaf primordia. In vegetative development, axillary meristems initiate the formation of several leaf primordia, resulting in an axillary bud. These buds either become dormant for periods of varying length or continue to grow. Suppression of bud outgrowth is due to an inhibitory effect of the primary shoot apex, termed apical dominance. Therefore, the pattern of vegetative shoot branching depends not only on the initiation of axillary meristems but also on the regulation of bud outgrowth. During reproductive development, lateral meristems play a crucial role in the establishment of different inflorescence structures that ultimately lead to the formation of flowers. Taken together, axillary meristems are major determinants of the architecture and the reproductive success of a plant. In this review, we focus on recent studies that have provided a better understanding of the mechanisms that regulate axillary meristem initiation, and discuss experiments that illustrate the role of novel hormone-like substances in the process of apical dominance.

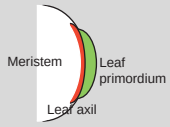
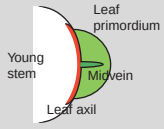
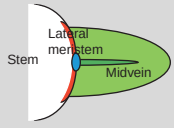
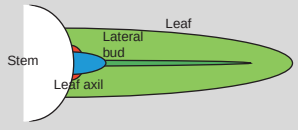
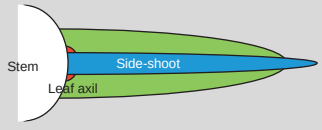
## Early steps in axillary meristem initiation are controlled by different pathways

Mutants that are impaired in early steps of axillary meristem initiation have been characterized in different plant species, including tomato [1,2], *Arabidopsis* [3], rice [4,5], and maize [6•] (Table 1). The *LATERAL SUPPRESSOR* genes from tomato [1] and *Arabidopsis* (*Ls* and *LAS*, respectively) [3] specifically regulate the formation of axillary meristems during the vegetative phase of development. After the transition to reproductive development, axillary shoots can be formed and inflorescence branching is not affected in these mutants. Introduction of a genomic fragment carrying the *Arabidopsis LAS* gene together with its regulatory sequences into the tomato *ls* mutant led to a full complementation of the mutant phenotype [3]. Rice plants that harbour mutations in the *Ls/LAS*-orthologous gene *MONOCULM1* (*MOC1*) show defects in the formation of tillers as well as a reduced number of rachis branches and spikelets [5]. The similarity of the *Ls/LAS/MOC1* mutant phenotypes and the transcomplementation experiment suggested that the *Ls/LAS/MOC1* genes encode key regulators of axillary meristem initiation that are conserved over large evolutionary distances. Differences in the mutant phenotypes might be explained by the presence of a redundant pathway that is active during inflorescence development in the eudicots.

The *Arabidopsis LAS* gene is expressed in a band-shaped domain at the adaxial boundary of all primordia derived from the shoot apical meristem (SAM) [3]. This expression pattern is very similar to those of the *CUP-SHAPED COTYLEDON* (*CUC*) genes *CUC1–CUC3* and *LATERAL ORGAN BOUNDARIES* (*LOB*). Transcripts of all of these genes are detected at the boundary between the SAM and new leaf primordia [7–10]. This suggests that the axillary region has a specific identity. On the basis of the onset of the accumulation of *LAS*, *CUC1–CUC3*, and *LOB* transcripts, axil identity is established very early in develop-

**Table 1**

**Model of the molecular events leading to side-shoot development.**

| Stages of side-shoot development | | Genetic regulators in *Arabidopsis* | in other plants | Activities |
|---|---|---|---|---|
| (1)   | Establishment of axil identity | CUC1–3 [7–9]<br>LOB proteins [10]<br>LAS [3] | CUP [12•]<br>Ls, MOC1 [1,5] | Transcript accumulation of *CUC1–CUC3*, *LOB* and *LAS* at the new border region [3,7–10]. |
| (2)   | Maintenance of meristem formation competence in leaf axils | LAS [3]<br>MYB[a] | Ls, MOC1 [1,5]<br>Bl[c] [2] | Co-expression of STM and LAS in leaf axils [3,45]. |
| (3)   | Organization of lateral meristem | REV, PHB, PHV [16–20]<br>bHLH[a]<br>MYB[a] | LAX, ba1 [4,6•]<br>Bl[c] [2] | Downregulation of LAS in the center of leaf axils [3].<br>Focused expression of STM in the organizing meristem [3,45,46].<br>REV expressed in the organizing meristem [3,17]. |
| (4)   | Formation of lateral bud | STM[b]<br>WUS[b] [47]<br>CLV1,3[b] [48]<br>CUC[b] | | Downregulation of STM at the flanks of the axilary meristem [45,46]. |
| (5)   | Lateral bud outgrowth | MAX1–MAX4 [32]<br>TCP[a] | RMS1–RMS6 [30,31]<br>DAD1–DAD3 [49]<br>D3, D10, D14, D17 and D27 [42]<br>tb1 and OsTB1 [43,44] | Elongation of internodes in the bud [32,46]. |

Schematic drawings represent top views of nodes undergoing progressive steps in lateral-meristem and side-shoot development (red: tissue with leaf-axil identity; green: tissue with leaf identity; dark green: midvein of leaf; blue: lateral meristem and side-shoot). Genes regulating lateral-shoot development that have been identified in *Arabidopsis* are compared to genes identified in other organisms. [a]No homologous function has been identified in *Arabidopsis* (homologs of the *Bl*, *LAX/ba1* and *tb1* genes). [b]The involvement of a gene function in a specific step is only hypothesized (*STM*, *WUSCHEL* [*WUS*], *CLAVATA* [*CLV*] and *CUC*). [c]A gene function cannot be assigned to a specific step (*Bl*).

ment, probably together or immediately after the formation of the corresponding leaf primordium (Table 1). Several studies have shown that the *CUC* genes play an important role in boundary formation [11]. Phenotypic analysis of the *Antirrhinum cupuliformis* (*cup*) mutant, which carries a mutation in a *CUC1/CUC2* homologue, provides a strong indication that axil specification by *CUC*-like genes is a prerequisite for lateral meristem formation. Shoots of *cup* plants develop misformed leaf axils without lateral buds [12•].

The *Ls/LAS/MOC1* genes encode putative transcriptional regulators of the plant-specific *GRAS* family [13]. In *Arabidopsis*, *LAS* and the meristem marker gene *SHOOT MERISTEMLESS* (*STM*) are co-expressed in leaf axils from the $P_1$ stage until the formation of the new axillary meristem, which is indicated by a focussing of *STM* expression [3]. In *las-4* mutants, this focussed *STM* expression is not found. Expression of the early meristem marker gene *OsH1* is abolished in the axillary regions of the rice *moc1* mutant, where lateral meristems are initiated in wildtype plants [5]. These findings suggest that the *LAS/MOC1* genes are involved at an early step in the pathway leading to lateral meristem formation, probably ensuing that the meristematic character of leaf axil cells is retained until axillary meristem formation (Table 1).

Double-mutant combinations of *ls* and *blind* (*bl*) in tomato had additive effects, suggesting that at least two independent pathways control the initiation of axillary meristems [2]. In the tomato *bl* mutant, the formation of all lateral shoot and inflorescence meristems is compromised. *Blind* encodes a MYB transcription factor. Comparison of mutant phenotypes indicates that the rice genes *MOC1* and *LAX PANICLE* (*LAX*) [4] might be components of independent pathways. In rice plants that are homozygous for strong *lax* alleles, panicle branches are severely reduced and the formation of lateral spikelets is completely suppressed. In addition, *LAX* regulates the

formation of vegetative branches (tillers) in a redundant fashion together with the *SMALL PANICLE* (*SPA*) gene. Mutations in the *LAX*-orthologous maize gene *barren stalk1* (*ba1*) [6•] affect the formation of all lateral meristems during vegetative and reproductive development. *LAX* and *ba1* encode basic helix–loop–helix (bHLH) proteins. The patterns of transcript accumulation of *LAX* and *ba1* are very similar: transcripts are initially detected in the boundary region between the main shoot apex and the presumptive lateral meristem. At later stages of development transcripts accumulate in the upper part of the new meristem [4,6•]. The onset of *LAX/ba1* transcript accumulation seems to be later than that of *LAS/MOC1* (Table 1). Because the described *myb* and *bhlh* mutants have been identified in different plant species, it is currently unclear whether or not the respective genes are involved in the same or in different pathways of axillary meristem formation. It is tempting to speculate, however, that interactions between MYB and bHLH transcription factors might also play a role in the regulation of axillary meristem formation, as is the case in processes such as trichome and root-hair development [14].

## Axillary meristem initiation and vascular differentiation

Several lines of evidence suggest that axillary meristem formation is intimately related to vascular differentiation [15]. In the *Arabidopsis revoluta* (*rev*) mutant, lateral meristems in the axils of rosette and cauline leaves are often not initiated and flowers frequently fail to develop [16,17]. The *REVOLUTA* gene, which belongs to a subfamily of Homeodomain-Leucine-Zipper transcription factors, has also been independently identified as the *INTERFASCICULAR FIBERLESS1* (*IFL1*) gene, which is needed for the correct patterning of the vascular system in *Arabidopsis* stems [18]. *REV* is expressed in vascular bundles as well as in the SAM and axillary meristems. Comparative expression studies in wildtype and *las-4* plants have demonstrated that *REV* acts downstream of *LAS* in the initiation of axillary meristems [3]. Dominant mutations in the two closest *REV* relatives, *PHABULOSA* (*PHB*) and *PHAVOLUTA* (*PHV*), lead to a complete adaxialisation and radialisation of leaves and to the formation of ectopic meristems at the abaxial leaf base [19,20]. The activity of *REV*, *PHB*, and *PHV* is restricted by microRNAs, and dominant *PHB* and *PHV* alleles contain point mutations in their miRNA target sites that render them insensitive to miRNA-regulated degradation [21,22]. Analogous gain-of-function mutations in the *REV/IFL1* gene (i.e. *rev-10d* and *amphivasal vascular bundle1* [*avb1*]) lead to a radialisation of vascular bundles [23,24•]. Although leaf polarity and shoot branching are not affected by the *rev-10d* mutation in the Landsberg erecta ecotype [23], the same base substitution in the *avb1* mutant (ecotype Columbia) was reported to lead to changes in leaf polarity and to the formation of ectopic meristems on the inflorescence stem [24•]. Currently, it is not clear, if the *REV*, *PHB*, and *PHV* genes control the formation of lateral meristems directly or by their influence on the development of vascular elements (Table 1). An indirect effect via the vascular system is indicated by the observation that the development of misdirected veins along the stem surface is correlated with the formation of ectopic meristems in a dominant tobacco *phv* mutant [25•].

## Newly discovered hormones play a role in the control of bud outgrowth

The outgrowth of axillary buds into side-shoots is influenced by environmental conditions and by hormonal signals [26]. In many plant species, the development of lateral shoots is inhibited by signals that are derived from the main shoot tip, a phenomenon named apical dominance or correlative inhibition [27]. Auxin produced in the main shoot tip was believed to be the main repressor and cytokinin produced in the roots was considered to be the main activator of lateral bud (shoot) development. In many plant species, the inhibitory effect of the main shoot can be relieved by decapitation, but apical application of auxin can restore apical dominance. Direct application of cytokinin to lateral buds promotes their outgrowth. The influence of both auxin and cytokinin was confirmed in isolated *Arabidopsis* inflorescence nodes [28]. However, the observation that apically applied auxin does not enter lateral buds suggested that a second signal is involved in the repression of lateral shoot outgrowth [29].

A new view on the regulation of apical dominance arose from the analysis of mutants that have enhanced shoot branching phenotypes, such as *ramosus* (*rms*) mutants in pea [30,31] and *more axillary growth* (*max*) mutants in *Arabidopsis* [32]. These recessive mutations cause a premature and enhanced outgrowth of lateral shoots in combination with modest pleiotropic effects. No significant differences in the amounts of the branching regulators auxin and cytokinin could be detected that would explain the phenotypes of these mutants [33]. Reciprocal grafting experiments suggested that several of the gene products that correspond to these mutations (e.g. *MAX1*, *MAX3*, *MAX4*, *RMS1*, *RMS2* and *RMS5*) are involved in the synthesis of a new mobile signal that can move acropetally from the rootstock or a stem section to a grafted scion to repress shoot branching [33,34,35,36•,37•]. On the basis of several series of grafting studies between the different *max* and *rms* mutants, *MAX3*, *MAX4*, *RMS1* and *RMS5* have been placed at the beginning of the pathway leading to signal synthesis, and *MAX1* and *RMS2* in an intermediate position [33,37•,38••]. *max2*, *rms3*, *rms4*, and *rms6* scions are not affected by grafting to wildtype rootstocks. Therefore, MAX2, RMS3, RMS4 and RMS6 are thought to be components of signal perception or transduction. Because double mutants between the different *max* mutants or the different *rms*

mutants resemble the single mutants, it seems that most or all of the genes act in a single pathway [37•].

The four *MAX* genes have been cloned [32,35,36•,37•]. Both *MAX3* [36•] and *MAX4* [35] encode members of a chloroplast-localized subclass of dioxygenases. The MAX3 protein cleaves different carotenoids, especially β-carotene, at the 9,10-position [36•,39•]. The C27-product of the MAX3 reaction is subsequently cleaved by MAX4 to form 13-apo-β-carotenone and a C9-dialdehyde [39•]. One of the two cleavage products might be the new mobile branching-inhibiting molecule or its precursor. *MAX1* encodes a cytochrome P450 family member that is thought to activate an already mobile precursor of the signalling molecule [37•]. The F-box protein MAX2, which is also involved in the regulation of leaf senescence [32,40], probably plays a role in a signal-dependent protein degradation process. The pea *RMS1* [35] and *Petunia DECREASED APICAL DOMINANCE1* (*DAD1*) [41] genes have been identified as functional homologs of *MAX4* and the rice gene D3 as homologue of *MAX2* [42], demonstrating an evolutionary conservation of this pathway. It is not clear how the activity of the *teosinte branched1* (*tb1*) gene is connected to the *MAX/RMS* signalling pathway. High expression levels of the *tb1* gene are correlated with a suppression of lateral bud outgrowth in maize and rice [43,44], and a loss-of-function allele of *tb1* leads to enhanced shoot branching [44].

The expression patterns of the *MAX* genes and their homologs match the expectations derived from the grafting experiments. Transcript accumulation of *MAX3* and *MAX4/RMS1* is highest in roots [35,36•,38••]. *MAX1* is expressed in the vascular elements of many different plant organs. Expression of *MAX4/RMS1* is positively controlled by auxin [35,38••]. Transcription of *RMS1* is downregulated by decapitation, and this downregulation can be counteracted by apical auxin application. *RMS1* transcript accumulation can also be inhibited by the application of 3,4,5-triiodobenzoic acid (TIBA), an auxin transport inhibitor [38••]. These findings suggest that the branching-inhibiting signal is regulated by a feedback control mechanism that involves auxin.

## Conclusions

Recent analysis of shoot and inflorescence branching has focussed on two steps, the formation of lateral meristems and the regulation of lateral bud outgrowth. By contrast, the developmental phase that connects these two steps, the formation of lateral buds, is not well understood because specific mutants are not available. Early steps in shoot branching require the establishment of a specific identity of the leaf axil region, which involves the activity of the *CUC* genes. Maintenance of meristem formation competence and the subsequent initiation of axillary meristems is regulated by a set of transcription factors: Ls, LAS and MOC1, which are GRAS proteins; Bl, which is a member of the R2R3-type MYB family; and LAX/ba1, which belongs to the bHLH-group proteins. Meristem organization seems to depend on a cross-talk between the presumptive axillary meristem cells and the vascular system, which involves the HD-ZIP genes *REV*, *PHB*, and *PHV*. It will be interesting to find out which targets translate these transcription factor activities into an organized lateral meristem.

With respect to the regulation of lateral bud outgrowth, the exciting new development is that several gene products (e.g. MAX1–MAX4) have been identified that act in the synthesis, perception and transduction of an unknown branching inhibiting molecule. It is unclear how TB1, a transcription factor that negatively regulates lateral-bud outgrowth in maize and rice, is integrated into this control mechanism. The analysis of mutants that have similar phenotypes in different plant species suggests that these regulatory pathways are highly conserved among flowering plants. Because model species differ considerably with respect to their branching patterns and even their wildtype forms might have been selected for reduced shoot branching, it is not surprising that mutations in other regulatory genes, such as *Bl*, *REV*, *LAX/ba1* or *tb1*, were only identified in a subset of species. In the future, reverse genetic and transgenic approaches will make it possible to compare the effects of homologous genes in different species and to analyze interactions between different regulators within one species.

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