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The shoot apical meristem: the dynamics of a stable structure

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The shoot apical meristem (SAM) is a group of proliferating, embryonic-type cells that generates the aerial parts of the plant. SAMs are highly organized and stable structures that can function for years or even centuries. This is in apparent contradiction to the behaviour of their constituent cells, which continuously proliferate and differentiate. To reconcile the dynamic nature of the cells with the stability of the overall system the existence of elaborate signalling networks has been proposed. This is supported by recent work suggesting that the exchange of signals between cells, rather than a rigidly predetermined genetic program, is required for the establishment and functioning of an organized meristem. Together these interactions form a stable network, set up during embryogenesis, that assures the coordination of cell behaviour throughout development. Besides meristem-specific signalling cascades such as the CLAVATA receptor kinase pathway, which controls meristem size, these interactions involve plant hormones. In particular, cytokinins and auxins are implicated in the maintenance of meristem identity and phyllotaxis, respectively.

Keywords: shoot apical meristem; signalling; hormones; phyllotaxis

1. INTRODUCTION

Plants generate organs and tissues throughout their life cycle. This is achieved by groups of proliferating embryonic-type cells called meristems, which play an essential role in the establishment of plant architecture. Since meristems are able to alter their activity in response to both internal and external cues, they provide the developmental flexibility required to deal with continuous environmental changes.

Meristems can function for years or even centuries and thus exhibit an astonishing stability. This is in apparent contradiction to the behaviour of their constituent cells, which continuously grow, divide and differentiate, cellular differentiation being in equilibrium with cell production. How the highly dynamic behaviour of the cells can be reconciled with the stability of the overall system is a major question in plant developmental biology and will be the focus of this article. Henceforth we will concentrate on a particular meristem, the SAM, which is localized at the tip of stems and branches and in the axils of lateral organs, where it initiates the aerial parts of the plant. Since SAM structure can differ significantly amongst species, we will limit our discussion to the angiosperms, which have been the best characterized. In addition, because many insights into the apical meristem have come via studies on floral meristems, we will cover the relevant features of floral development.

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2. MERISTEM ORGANIZATION

(a) *Layers and zones*

The SAM should not be considered as a population of randomly dividing, undifferentiated cells. Instead, it is a highly organized, stable structure, divided into cytologically and functionally distinct domains. Cytohistological examination (for reviews, see Steeves & Sussex 1989; Lyndon 1998) of a wide range of species has revealed that the SAM is partitioned into at least two sets of overlapping multicellular domains (figure 1*a*,*b*). An obvious feature, easily observed in microscopical sections, is the presence of distinct cell layers. The surface layer, or tunica, remains separated from the underlying layers, or corpus, by the strict anticlinal orientation of the division planes of its proliferating cells. In dicots, the tunica itself usually comprises two layers, called L1 and L2. The corpus itself has no clearly defined stratification.

Superimposed on the layered organization of the meristem is a division into zones. This partitioning was initially described by Foster in 1938 for the ginkgo apical meristem (for a review, see Steeves & Sussex 1989) and is the basis of a zonal model that seems to be common to all (higher) land plants. At least three zones can be defined: the CZ, the PZ and the RZ, which supposedly are all also functionally distinct. The CZ, at the meristem summit, spreads out over part of the tunica and corpus. According to the prevailing model the CZ is involved in meristem maintenance, providing a continuous source of stem cells. It is surrounded by the PZ where the primordia are initiated. Inside the meristem, subtending the CZ and PZ, is the RZ. This zone is characterized by the presence of cell files, which generate the internal parts of the stem. Within the past few years genetic analysis has identified a number of regulators involved in meristem function. The analysis of

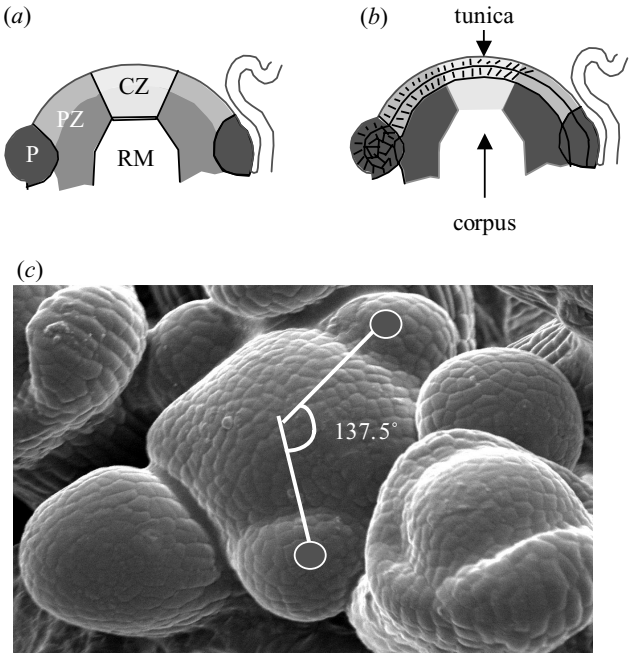

Figure 1. (*a*,*b*) Organization of the meristem in a typical angiosperm. The shoot apical meristem can be divided into overlapping zones and layers. (*a*) Division into zones. The CZ at the summit of the meristem is responsible for meristem maintenance. Descendants of the CZ cells move to the PZ where the primordia (P) are initiated on to the rib meristem (RM) where the internal parts of the stem are generated. (*b*) Represents the division into layers. The surface layer or tunica remains separated from the inner domain (corpus) because the cells divide only in anticlinal orientations. The tunica in angiosperms is again divided into sublayers (called L1 and L2). The L2 cells divide in different orientations when the primordia are initiated. (*c*) Phyllotaxis at the inflorescence meristem in *Arabidopsis*. The flower buds are initiated in a spiralled fashion. The divergence angle between successive primordia is *ca*. 137.5°.

mutant phenotypes and expression studies of the corresponding genes have helped to elaborate further the existing models for meristem organization.

(b) *The genetic basis of meristem organization*

(i) *Meristem initiation and maintenance: a major role for the homeobox genes*

An important breakthrough in the analysis of meristem function was the discovery of the *KNOTTED* family (*KNOX* family) of homeobox genes in maize. Homeobox genes encode transcription factors that are involved in the development of multicellular eukaryotes (for a review, see Kappen 2000), and several families have been implicated in SAM function. The first homeobox gene to be discovered in plants was *KNOTTED1* (*KN1*) (Vollbrecht *et al.* 1991). The dominant *KN1* mutants, which ectopically express the homeodomain protein in leaves, show a variety of defects in the leaf, including knots of extra cells. The gene is normally only expressed within the maize meristem and is downregulated as cells enter the primordium. The expression pattern and the phenotype have led to the idea that the KNOTTED1 protein keeps cells in an undifferentiated proliferative state (Hake *et al.* 1995). A role in meristem maintenance was further confirmed on the basis of a loss of function in *KN1* causing fewer branches and more determinate organs than in wild-type (Kerstetter *et al.* 1997).

The first loss-of-function mutant identified in a member of the *KNOTTED* family was *shootmeristemless* (*stm*) in *Arabidopsis*. The mutant is unable to develop or maintain a functional meristem during embryogenesis (Long *et al.* 1996; Long & Barton 1998). STM protein is present throughout the vegetative and inflorescence SAM as well as in the floral meristems, but is excluded from the primordia. Interestingly, STM mRNA is already present in a few cells at the top of the globular embryo, providing evidence that the SAM is specified at a very early stage of embryogenesis (Long & Barton 1998). The location of *STM* transcripts as well as the *stm* phenotype are consistent with a role in meristem formation. However, plants carrying strong loss-of-function *stm* alleles still initiate organs (i.e. cotyledons and leaves) and one could argue that they still have residual meristematic activity. Therefore, an alternative hypothesis is that *stm* mutants can initiate meristems but are incapable of maintaining them. Of the *KNOTTED* genes identified in *Arabidopsis* so far three, *KNAT1*, *KNAT2* and *STM*, have been associated with meristem function, as they are expressed in different but overlapping domains of the SAM. *KNAT1* is expressed throughout the vegetative and inflorescence meristem in an area that largely overlaps with *STM* (Lincoln *et al.* 1994). *KNAT2* transcripts have a more restricted pattern, as they can only be detected in the L3 layer in the young seedling and in the inner core of the apex within a domain that covers at least the rib meristem and possibly the entire corpus (Pautot *et al.* 2001). Later on, *KNAT2* is absent from the inflorescence meristem. It is reactivated again in the floral meristems and in part of the carpels. No loss-of-function mutants have been described for *KNAT1* and *KNAT2*. *KNAT1* overexpression induces the formation of lobes and leaf-like structures on leaves as well as ectopic shoots (Lincoln *et al.* 1994). It is therefore sufficient for meristem formation. This is different for *KNAT2* overexpression, which also causes the formation of lobed leaves but cannot induce ectopic meristems. Instead, *KNAT2* has been associated with carpel formation, as its overexpression induces carpelloid features in leaves and a homeotic conversion of ovules into carpels (Pautot *et al.* 2001).

Another homeobox gene family involved in meristem function is defined by *WUSCHEL* (*WUS*) (Mayer *et al.* 1998). Mutants lacking *WUSCHEL* are unable to maintain a functional meristem. Apparently a SAM is initiated during embryogenesis but, after having formed several organs, the cells then fail to be incorporated into primordia (Laux *et al.* 1996). New meristems continuously form but are defective and terminate prematurely in aberrant flat structures. The central zone of the meristem is not maintained since primordia initiation often occurs ectopically within the centre. Occasional new meristems are initiated in place of lateral organs and this results in a characteristic architecture, also observed in weak *stm* phenotypes. Flowers are eventually formed but these terminate prematurely in a central stamen. In the SAM, *WUS* transcripts are detectable in a few cells, probably corresponding to the basal part of the central zone. On the basis of the phenotype and the expression patterns, Mayer *et al.* (1998) proposed that cells expressing *WUS*

could specify the identity of the stem cells in the central zone.

(ii) *Control of meristem size: the* CLAVATA *genes*

Loss-of-function mutations in any one of the three *CLAVATA* (*CLV*) genes cause a phenotype that is the opposite of that of *stm* or *wus*. (Clark *et al.* 1993; Kayes & Clark 1998; Trotochaud *et al.* 2000). In *clv* mutants the meristem gradually increases in size from the embryo stage onwards. This is associated with the formation of extra leaves, flowers and floral organs. The inflorescence meristem often becomes asymmetric and grows as a line rather than a point, leading to fasciation of the inflorescence stem. Under certain conditions, this can lead to meristems being over 1 cm in width. Genetic analysis suggests that all three genes function in the same pathway, controlling meristem size. This is illustrated by the double mutant for *CLV1* and *CLV3*, which is indistinguishable from the single mutants. Whereas the role of *CLV1* and *CLV3* appears to be limited to the meristem proper, *clv2* mutants not only affect the meristem but also lateral organs, indicating a role outside the meristem (Kayes & Clark 1998).

Although the function of the *CLV* genes will be discussed below in detail, we will briefly mention their expression patterns, which reveal domains within the meristem that were not seen previously using cytological analysis. *CLV1* encodes a receptor kinase that is expressed in the corpus and possibly the L2 layers of the meristem (Clark *et al.* 1997), in a domain that surrounds and covers the domain expressing *WUS*. *CLV3* encodes a small peptide that could be the extracellular ligand for *CLV1* (Fletcher *et al.* 1999; Trotochaud *et al.* 2000; and see below). *CLV3* is expressed in a zone that may correspond with the L1 and L2 layers of the classical CZ. Therefore, although their analysis confirms the existence and role of the CZ, *WUS*, *CLV1* and *CLV3* define three subdomains in the SAM, which were not identified previously by cytological criteria (figure 2).

After having discussed some of the molecules involved in SAM organization and maintenance, we will now turn to the major function of the meristem, the initiation and positioning of organs. Our discussion will not concern the factors controlling the identity of the organs produced (i.e. leaves, flowers, floral organs), although some of these genes are switched on very early in the incipient primordia of all meristems. The transcription factor *FLORICAULA* (*Antirrhinum*) and its orthologue *LEAFY* (*Arabidopsis*), for instance, are upregulated as soon as the cells leave the meristem and are essential for the subsequent patterning of the flower. The organ identity issue has yielded extremely valuable information about the way differentiation patterns and domains within tissues are established and the reader is referred to recent reviews (e.g. Gutierrez-Cortines & Davies 2000; Irish 1999; Ng & Yanofsky 2000; Weigel 1998).

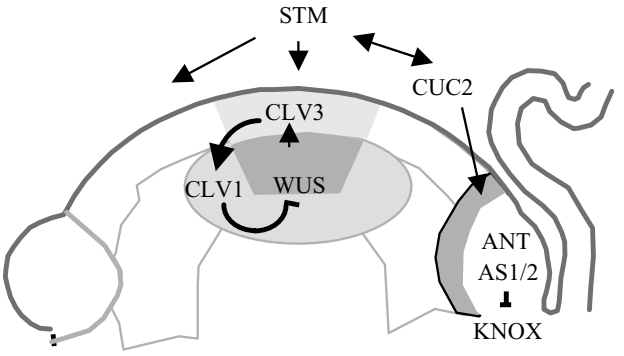

Figure 2. Interactions between meristem regulators involved in meristem function. *WUS*, expressed at the basis of the CZ, induces the production of *CLV3* which then moves to the zone surrounding the WUS domain, where the receptor kinase CLV1 is expressed. Interaction with the ligand CLV3 activates CLV1, which then restricts *WUS* expression and the size of the CZ. *STM* has a general role in the meristem and probably keeps the cells in a meristematic state. At the periphery of the meristem at organ boundaries its expression overlaps with that of *CUC2* and both genes probably positively influence each others activity. Within the primordium, ANT is involved in primordium outgrowth, whereas AS1 and AS2 downregulate some of the *KNOX* genes.

3. ORGAN INITIATION AND PHYLLOTAXIS

(a) *Introduction*

The SAM produces units called phytomers. Although variants exist, a phytomer typically consists of a stem segment (internode) and a leaf with a meristem in its axil (node). The SAM does not initiate organs in random positions. Instead, organ primordia are positioned in highly stereotypic arrangements, usually referred to as phyllotaxis. Different arrangements exist, such as whorled, spiralled, decussate and distichous (for reviews, see Steeves & Sussex 1989; Lyndon 1998). Spiralled arrangements, in which organs are positioned in intersecting spirals, or parastichies, are widespread and have fascinated scientists from ancient times onwards. In this type of phyllotaxis one organ is formed per node. When two lines are drawn between the centre of two successive primordia and the meristem centre, the angle between these lines (or the divergence angle) is constant and for most spiralled patterns approximates 137°, which corresponds to the Fibonacci angle (figure 1*c*). The complexity of the arrangements, i.e. the number of intersecting spirals, can be described as a function of the divergence angle and a variable called the PR. A plastochron is defined as the time interval between the initiation of two successive organs and the PR is the ratio of the distances between the centres of two successive primordia and the meristematic dome. To explain the highly ordered arrangements of the primordia, different models have been proposed. On the basis of microsurgery experiments, Snow & Snow (1931, 1933), for instance, proposed that a new organ is initiated as soon as there is enough space for it. Wardlaw, in 1949, on the basis of similar experiments, proposed that existing primordia themselves produce signals that influence the positioning of new ones (for reviews, see Steeves & Sussex 1989; Lyndon 1998). This and other work has led to the hypothesis that both the meristem itself and the primordia produce inhibitory fields that prevent primordium formation in their vicinity. Green suggested that phyllotaxis might be the consequence of physical constraints and stresses created by growth patterns (e.g. Hernandez & Green 1993; see also Douady & Couder 1996*a*,*b*,*c*). Although different models exist, and the processes

involved are still poorly understood, there is an overall consensus that the underlying general mechanism is relatively simple. However, though phyllotaxis might simply rely on the diffusion of single molecules or the strains created by differential growth, its translation in the living plant in terms of molecular processes remains an (at least partially) open question. It is therefore important to identify the molecules instrumental to phyllotaxis. Some of these have been isolated and are discussed in the next section.

(b) *Genes involved in organ initiation and separation*

Genetic analysis has defined at least four separable processes involved in organ initiation, including the inactivation of meristematic identity, the establishment of organ boundaries, the establishment of organ identity and organ outgrowth. Although we do not know the exact sequence of events, one of the first steps in organ initiation is the loss of meristematic identity, as demonstrated by the inactivation of STM/KN1 expression in the organ founder cells as soon as they leave the meristem. The inactivation of meristematic identity in the incipient organ primordia requires myb-like transcription factors encoded by the homologous *ROUGH SHEATH 2* (*RS2*) (Timmermans *et al.* 1999), *PHANTASTICA* (*PHAN*) (Waites *et al.* 1998) and *ASYMMETRIC LEAVES 1* (*AS1*) (Byrne *et al.* 2000) in maize, *Antirrhinum* and *Arabidopsis*, respectively. *PHAN*/*AS1* are expressed in PZ cells which will become part of the leaf primordial; *RS2* becomes active slightly later. If they are inactivated, at least some of the meristematic *KNOX* genes are ectopically expressed in the primordia (Ori *et al.* 2000; Schneeberger *et al.* 1998; Sinha 1999; Taylor 1997; Timmermans *et al.* 1999; Semiarti *et al.* 2001). In *Arabidopsis*, genetic analysis has shown that *AS1* is expressed throughout the apex of *stm* mutant embryos, suggesting that STM is required to inactivate *AS1* in the SAM. Conversely, *AS1* also seems to downregulate *STM* expression, although the effects are more subtle than with *KNAT1*, *KNAT2* and *KNAT6*. This suggests a scenario where the inactivation of *STM* leads to the subsequent activation of *AS1* which, in turn, further maintains *STM* at a low level and downregulates *KNAT1*, *KNAT2* and *KNAT6* (figure 2).

After their isolation from the meristem the primordium founder cells start to grow more rapidly than the surrounding tissue. A gene associated with this outgrowth from the very early stages onwards is *AINTEGUMENTA* (*ANT*). This gene encodes a potential transcription factor, related to *APETALA2*, and is a marker for primordium initiation throughout development (Long & Barton 1998). *ant* mutants have obvious defects during floral and seed development as floral organs are reduced in size and integument outgrowth is severely perturbed (Elliott *et al.* 1996; Klucher *et al.* 1996; Mizukami & Fischer 2000). Vegetative development is only slightly perturbed, although the cell numbers in leaves are reduced, further suggesting a role in cell proliferation in the primordia. As the reduced cell number is compensated for by an increase in cell size, the overall leaf size is only slightly reduced.

The definition of an organ primordium also requires the establishment of its boundaries. Mutations in either of the two related putative transcription factors *CUP SHAPED COTYLEDON* (*CUC1* and *CUC2*) genes have very mild phenotypes but, if both are inactivated, cotyledons partially fuse to produce a funnel-shaped structure (Aida *et al.* 1997; Takada *et al.* 2001; Ishida *et al.* 2000). The *CUC* genes are expressed in a zone between the cotyledons, consistent with the idea that they act as a local growth suppressors. Later in development, the genes are expressed at all organ boundaries. In addition, if both *CUC1* and *CUC2* are inactivated, a meristem is not formed in the embryo and *STM* is not expressed. Conversely, *STM* is required for the correct spatial expression of the *CUC2* gene, at least during embryogenesis (Aida *et al.* 1999; figure 2). Similar observations were made for the related *NO APICAL MERISTEM* (*NAM*) gene, which is absolutely required for the presence of an apical meristem in *Petunia* (Souer *et al.* 1996). Together the results suggest a link between *CUC* and meristem formation, as well as a link between *STM* and organ boundary formation.

4. INTEGRATION OF CELLS WITHIN THE MERISTEM

(a) *Dynamic stability of the SAM*

Whereas the ordered structure of the SAM and the stereotypic phyllotactic patterns illustrate the stability and ordered nature of the system, even a superficial look at the meristematic cells, which divide, elongate and differentiate, gives a much more dynamic picture. To reconcile the highly dynamic behaviour of the individual cells with the stability of the overall system different scenarios can be proposed. Stability could be provided by a very strict spatial control of each cell's fate and behaviour. Certain aspects of plant development, such as the control of division plane alignment, appears to hint at a mechanism in which the position of every cell and its descendants is very precisely predetermined. However, careful studies involving clonal analysis have shown that cell fate does *not* depend on cell lineage (for discussion, see e.g. Dawe & Freeling 1991; Szymkowiak & Sussex 1996). In these studies the visualization of descendants of individual cells, using different genetic markers, showed that the offspring of cells in the L1 layer of the meristem were not located uniquely in the epidermis, as might be expected from the apparently very strict anticlinal orientation of the division planes in this layer. Instead, descendants could also be found in other tissues. This implies that cells can readapt their differentiation status when aberrant division plane alignment causes the abnormal positioning of a daughter cell. This is even more dramatically illustrated by the *tonneau* and *fass* mutants. Both are perturbed in division plane alignment from embryogenesis onwards, and cells apparently divide in random orientations (Torres-Ruiz & Juergens 1994; Traas *et al.* 1995). Nevertheless, cell differentiation patterns, taking into account the cellular phenotype, are relatively normal. This confirms that a model where the strict programming of cell fate through cell lineage would be the basis of meristem stability and structure is not very plausible.

An alternative explanation is based on the notion that cells do not act autonomously in the meristem but continuously interact. Here, the combination of interactions would create a stable network and guarantee the maintenance of the overall system. Such interactions could imply

physical properties such as tension within tissues. This was proposed by Green and co-workers, who were able to influence both organ identity and position by applying a directed physical constraint on growing sunflower SAMs (Hernandez & Green 1993). Although this type of experiment suggests that biophysical processes are important in the establishment of developmental patterns, their interactions with molecular and cellular processes have not been characterized, and will not be discussed here in detail.

Another means of interaction involves the exchange of molecules that act as signals. Depending on their position within the tissue, the cells will receive specific signals, behave accordingly and produce position-dependent signals themselves. Once set up, such a system would automatically maintain itself. Although the signalling pathways and sensing mechanisms are still poorly defined, some of the molecules involved and the routes they take in the SAM have been identified.

(b) *Signalling in the meristem*

(i) CLAVATA *signalling and meristem size*

One of the best characterized signalling mechanisms in the meristem is the CLV pathway involved in the regulation of meristem size (see § 2b(ii)). CLV3 is a small peptide which potentially acts in the extracellular space (Fletcher *et al.* 1999; Brand *et al.* 2000; Trotochaud *et al.* 2000). The use of periclinal chimeras derived from transposon-induced alleles showed that CLV3 activity in one cell layer is sufficient to complement the absence of the peptide in other layers (Fletcher *et al.* 1999). It was hypothesized that CLV3, which is produced in the outer layers of a central domain in the meristem, could move to underlying cell layers, maybe in the form of a 25 kDa multimeric complex (Trotochaud *et al.* 2000). Here, it could interact more particularly with CLV1, an RLK of about 105 kDa, with a putative extracellular domain of 21 tandem leucine-rich repeats, a membrane-spanning sequence, and a presumed intracellular serine/threonine protein kinase domain. Biochemical analyses have shown that CLV1 binds directly *in vitro* and *in vivo* to KAPP (Stone *et al.* 1998), an interaction requiring the (auto)phosphorylation of CLV1. The 65 kDa KAPP is able to bind to multiple plant RLKs and is potentially involved in several kinase signalling pathways. If the levels of KAPP transcripts are reduced by means of cosuppression, the *clv1* mutant phenotype is partially complemented, the degree of repair being correlated with the level of cosuppression. Conversely, overexpression of KAPP mimics certain aspects of the *clv* phenotype, such as the club-shaped siliques. This would imply that KAPP is a negative regulator of CLV and is possibly required to modulate the signal strength needed to stimulate the CLV receptor complex.

CLV complexes isolated from cauliflower meristems have been size-fractionated, showing that CLV1 is present in at least two different complexes: one of 185 kDa, another of 450 kDa. Besides CLV1 the 185 kDa complex contains another, smaller protein. A good candidate for this partner is CLV2 (Kayes & Clark 1998), a receptor-like protein necessary for the assembly of the CLV1 complex. The 450 kDa complex contains a number of additional proteins, including KAPP and a Rho GTPase-related protein. The 450 kDa complex also requires CLV3 for its formation and the current model proposes that interaction with the extracellular CLV3 ligand allows the CLV1/CLV2 heterodimer to become active and to interact with additional proteins such as KAPP and a Rho-like protein, potentially involved in intracellular signal transduction (Trotochaud *et al.* 1999). It must be noted that the binding of CLV3 to CLV1 requires the kinase activity of the latter (Trotochaud *et al.* 2000).

An important target of CLV signalling is the WUS homeodomain protein. The *wus* mutation is epistatic over *clv*, indicating that WUS functions downstream of CLV (Laux *et al.* 1996). However, the ectopic expression of WUS, for instance under the control of a primordium-specific promoter, also induces CLV3 expression ectopically. Based on this evidence, it was proposed that meristem maintenance is controlled by a loop in which WUS induces CLV3 in the CZ. Subsequently, CLV3 will move to the CLV1 domain and activate the receptor kinase complex, which in turn will inhibit WUS. Thus, increased WUS activity will cause increased CLV3 levels, which in turn will lead to a higher inhibitory capacity of CLV1 (Schoof *et al.* 2000; see also Brand *et al.* 2000). Conversely, reduced levels of WUS would lead to reduced CLV1 activity.

Such a mechanism, where different levels of CLV would counterbalance conditions leading to an increased or reduced meristem size, would improve meristem stability (figure 2). At this moment a number of important questions remain. For instance, it is unclear how WUS activates CLV3, nor is it known what intracellular signalling pathways lead to the inactivation of WUS. Further work is required to elucidate the interactions between CLV and WUS, as well as between CLV and other meristem regulators such as STM.

(ii) *Hormone signalling and meristem function*

Several hormones have been associated with the structure and function of the meristem proper. Recent work has revealed some of the links between meristem regulators and two of these hormones, cytokinin and gibberellin. These results are discussed in more detail below.

There is little doubt that cytokinins are involved in meristem function although their precise mode of action at the meristem is not well defined. Classically, cytokinins have been thought of as positive regulators of cell proliferation (Mok & Mok 2001). Different links between cytokinins and the cell cycle have been proposed, currently most clearly in the case of D-cyclin activation. D-Cyclins are cell cycle regulators supposedly involved in the transition from G1 to S and the expression of at least one isoform in *Arabidopsis* appears to be directly regulated by the level of cytokinins. This was shown for cell suspension cultures, but the addition of exogenous cytokinins also induces the expression of the cell cycle regulator *in planta* (Soni *et al.* 1995). A relation between cytokinin and meristem function is also suggested by the *abnormal meristem mutant 1* (*amp1*) in *Arabidopsis* (Riou-Khamlichi *et al.* 1999). This mutant has abnormal levels of cytokinins combined with an enlarged meristem which produces primordia at a higher rate than normal. Interestingly, the *amp* mutant also has increased levels of D-cyclin transcripts.

Recently, cytokinins have been directly associated with the activity of meristem regulators such as *STM* and other

KNOTTED-like genes. If expressed from a senescence-responsive promoter, KN1 delays cell death in old leaves (Ori *et al.* 1999). Cytokinin levels are increased in these tissues. Other *KNOTTED*-type homeobox genes, when ectopically expressed, can also increase the levels of cytokinin without inducing meristem formation (Tamaoki *et al.* 1997). These results place KN1 above cytokinin in the hierarchy of meristem regulation, but other studies suggest that *KNOTTED*/*STM* transcript levels are regulated by cytokinin levels (Rupp *et al.* 1999), which would place the hormone upstream of the homeobox genes. In addition, the ectopic expression of the cytokinin biosynthesis gene *ipt* delays senescence, alters leaf shape and promotes ectopic meristems (Estruch *et al.* 1991; Li *et al.* 1992; see also Chuck *et al.* 1996; Williams-Carrier *et al.* 1997). Together the results would suggest the existence of a loop where cytokinins and *KNOX* genes would mutually stimulate each other's activity. Such a loop would contribute to the overall stability and maintenance of the meristem.

Another hormone which has been associated with homeobox genes in the meristem is gibberellin. Using an inducible system, Sakamoto *et al.* (2001) were able to show that the activation of the homeobox gene 15 (*NTH15*) in tobacco immediately suppresses the expression of the biosynthetic gene GA 20-oxidase (*NTc12*); this repression even occurs when protein synthesis is blocked using cycloheximide. Furthermore, recombinant *NTH15* binds to a sequence in the first intron of *Ntc12*. The homeobox gene is expressed in the corpus of the tobacco SAM, and together the results suggest that NTH15 downregulates GA synthesis in the meristem. The functional significance of the downregulation of GA in the meristem (and of its upregulation in young primordia) is not known at present.

(iii) *Auxin and phyllotaxis*

Auxins are phytohormones involved in a wide range of processes, ranging from cell division and cell expansion to cell differentiation (e.g. Bartel 1997). The principal form of auxin in higher plants, IAA, is synthesized in as yet undefined young tissues and is then thought to be transported actively from cell to cell via the apoplast (Delbarre *et al.* 1996; for a review, see Ding *et al.* 1999). IAA transport involves auxin influx and efflux carriers which supposedly create a directional flow of auxin throughout the plant, associated with local differences in hormone concentration.

Both physiological and genetic studies have provided evidence that auxin plays a role in primordium initiation and in phyllotaxis. When applied to young primordia, NPA, an inhibitor of auxin transport, induces their lateral extension (Meicenheimer 1981). A similar effect was obtained on primordia of *Phaseolus* using the synthetic auxin 2,4-D (Pereira & Dale 1982). In both cases it was proposed that the treatment caused the over-accumulation of auxin in and around the incipient primordia, resulting in an increase in the number of cells to be recruited. Genetic analysis has further confirmed a role for auxin and local differences in auxin accumulation. In particular the analysis of the *pin-formed1* (*pin1*) mutant in *Arabidopsis* has shed new light on the issue. Loss of *PIN1* activity leads to reduced organ numbers and organ fusion throughout development (Okada *et al.* 1991). The most dramatic effect of the mutation is observed during flowering. In strong alleles, a naked inflorescence stem is formed, which occasionally produces flowers with fused organs (figure 3*a*,*b*). *PIN1* encodes a 67 kDa transmembrane protein with homologies to transporter proteins in a wide range of organisms and it was proposed that the protein might act as a catalytic auxin efflux carrier (Gälweiler *et al.* 1998).

Although this remains to be proven, several observations argue in favour of this hypothesis. Certain aspects of the phenotype, in particular the incapacity of the inflorescence to produce flowers, are mimicked by inhibitors of auxin transport such as NPA and it was shown that the mutants have a reduced ability for polarized auxin transport. When auxin is applied at the periphery of the *pin1* apex, primordia are induced at the site of application (Reinhardt *et al.* 2000; figure 3*c*). This suggests that organ initiation and/or outgrowth requires the local accumulation of auxin and that this process is impaired in the mutant. *In situ* hybridization further confirmed a role of *PIN1* in organ initiation, as the gene is strongly upregulated in the very young primordia, even before they start to grow out (Vernoux *et al.* 2000). This suggests a scenario where cells, as soon as they leave the meristem, immediately start to accumulate auxin, thereby increasing the local growth rate and causing the primordium to bulge out.

Interestingly, the PIN protein it is not uniformly inserted into the plasma membrane. Instead it is localized in a polar manner within the cell. This was shown at least for cells in the vasculature and embryo, where the localization of PIN protein at the distal or proximal ends of the cells suggests auxin flows in specific directions through cell files in particular tissues (Gälweiler *et al.* 1998; Steinmann *et al.* 1999). This localization of PIN at cross walls sheds a new light on division plane alignment, as the protein is apparently deposited in an asymmetric fashion during cell plate formation. In this context it is noteworthy that the *fass*/*tonneau* mutant showing abnormal division plane alignment (see § 4a) also exhibits abnormal auxin levels (Fisher *et al.* 1996) and increased sensitivity to the inhibition of auxin transport (J. Traas and T. Vernoux, unpublished data). It is not known, at this stage, whether such a precise localization along cell files is also found at the SAM.

What is the effect of perturbed auxin transport on pattern formation at the shoot apex? To answer this question, Vernoux *et al.* (2000) looked at the expression patterns of several markers for organ initiation in *pin1* mutants. This revealed that cells outside the *pin1* meristem activate both *ANT* and *LFY*. However, the cells are not recruited in discrete primordia, which then fail to grow out. Instead, the mutant meristem is surrounded by a ring of cells, all expressing the two primordium markers. Interestingly, the same cells also express the organ boundary marker *CUC2*, suggesting that the *pin1* meristem is surrounded by a ring of cells having a hybrid identity (figure 4*a*,*b*). The local accumulation of auxin would be required to separate these two identities, allowing primordia to form (figure 5). The results obtained by Reinhardt *et al.* (2000), as well as the earlier experiments using the synthetic auxin 2,4-D on pea, suggest that all the cells in the ring have the same competence to form a primordium and that the local auxin

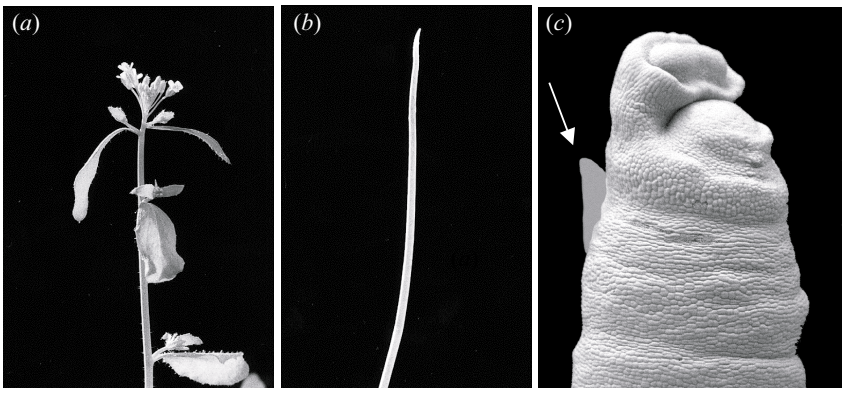

Figure 3. (*a*) Wild-type inflorescence apex. (*b*) *pin1* inflorescence apex. No flowers or cauline leaves are formed. (*c*) When auxin (arrow) is applied at the periphery of the naked *pin1* meristem a primordium is initiated, suggesting that a local accumulation is required for organ initiation in the wild-type.

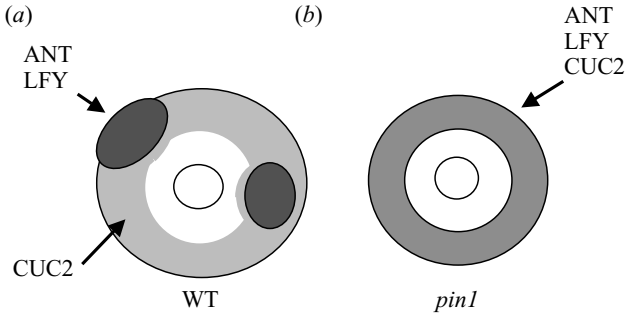

Figure 4. Expression patterns of markers at the periphery of wild-type (WT) and *pin1* inflorescence meristems. *ANT* and *LFY* are expressed in the primordia; *CUC2* is expressed at the boundaries. In *pin1* meristems the meristem has a normal structure and is surrounded by a hybrid zone where cells express both organ primordium and organ boundary markers.

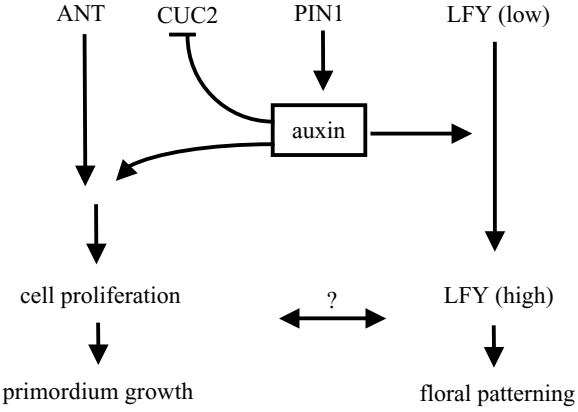

Figure 5. Model for gene interactions during organ initiation. As soon as the cells leave the meristem centre they have the potential to express *CUC2*, *PIN1*, *LFY* and *ANT*. The expression of *PIN1* in the incipient primordium, however, causes the local accumulation of auxin, which in turn leads to a downregulation of *CUC2*. Besides consolidating primordium identity, auxin also promotes organ outgrowth and floral patterning. Note that outgrowth and patterning are not necessarily independent processes. It could be, for instance, that auxin promotes patterning via its effect on outgrowth.

concentration will determine how many cells will be recruited in the incipient organ (Pereira & Dale 1982; Reinhardt *et al.* 2000). Indeed, if auxin is applied on top of the *pin1* meristem, all cells in the competent zone receive sufficient hormone and a ring-like primordium grows out. It should also be noted that the site of organ initiation appears to be limited to the ring-like competent zone.

In summary, the results suggest that the competence to form a primordium does not depend on functional *PIN1*. In contrast, within the population of competent cells it appears to be the local amount of auxin, accumulated via PIN1 activity, that will define how many cells actually participate in the initiation of an organ primordium. This model needs further testing; the short-term effects of auxin on the primordium initiation genes need to be evaluated and other elements in the network controlling phyllotaxis have to be identified. In this context another gene associated with auxin transport, the *PINOID* or *PID* gene, appears to be highly relevant. The *PID* gene encodes a serine/threonine kinase (Christensen *et al.* 2000). Although the phenotypic effects of its inactivation are less pronounced during the vegetative phase, the inflorescence of *pid* mutants is an exact phenocopy of *pin1*.

Work on the *PID* gene also points to a role in auxin signalling. *PID* overexpression leads to phenotypic alterations that are reminiscent of auxin-resistant plants. 35S::PID plants, for instance, show reduced gravitropism of the roots and produce fewer lateral roots (Christensen *et al.* 2000). This would imply that PID is a negative regulator of auxin action or transport, but some caution is required as the aerial phenotypes would rather suggest that both PIN1 and PID play similar roles. In this context, it is important to note that more recently Benjamins *et al.* (2001) provided compelling evidence that PID does have an auxin-transport-stimulating function. In any case, although the exact role of PIN1 and PID remains to be established, there is now strong evidence that the distribution of auxin is involved in primordium initiation and phyllotaxis.

(c) *The symplast: the direct transfer of molecules via plasmodesmata*

Signals like auxin and CLV3 are thought to travel via the extracellular matrix or apoplast. Another route is provided by the symplast, where molecules travel directly

from cell to cell via plasmodesmata. Plasmodesmata are narrow connections between the cytoplasms of neighbouring cells. These structures are laid down after cytokinesis, during phragmoplast formation, but so-called secondary plasmodesmata can also be formed in mature walls. Although the process is not understood it must involve the local degradation of the cell wall and the fusion of the neighbouring plasma membranes and the ER. Finally, besides the formation of new connections, the cell also has the possibility to close existing ones and even to remove them (for an extensive review, see Ding *et al.* 1999). The precise molecular structure of plasmodesmata has not been elucidated but ultrastructural studies show that they contain ER and plasma membranes, as well as structural spoke-like components, most probably proteinaceous in nature.

Molecules travelling from cell to cell via plasmodesmata have different possibilities. They can employ the membrane systems, the ER lumen, or the space between the membranes created by the spokes. It must be noted, however, that not all molecules can freely move via plasmodesmata. In particular, there is a limit which excludes molecules above a certain size. These so-called size exclusion limits are highly variable and subject to active modification.

Several studies have pointed to the potential importance of symplastic connections in SAM function. Rinne & Van der Schoot (1998), by injecting a membrane-impermeant probe, showed that cells at the periphery of the birch SAM are coupled to each other in a field that is separated from the central zone (see also Van der Schoot & Rinne 1999). In addition, the same authors showed a symplastic separation between cells in the tunica and the corpus. It therefore appears that the zonal and layered organization of the SAM corresponds precisely with a partitioning into symplastic domains. Some of these domains are maintained automatically as cells do not divide in random orientations. For example, cells in the tunica preferentially divide anticlinally, forming sheets of cells that are rich in primary plasmodesmata, which are exclusively formed during cytokinesis. In contrast, the interface between tunica and corpus contains only secondary plasmodesmata, which may differ in their transfer capacity and specificity. In other cases the symplastic discontinuity between the fields must be actively regulated, for instance in the case where cells in the tunica transit from the centre to the periphery (Van der Schoot & Rinne 1999).

What type of signals can be transported via the symplast? Recent efforts have concentrated in particular on protein traffic. Some of the meristem regulators themselves can move from cell to cell via plasmodesmata and may be regarded as signals influencing distant or neighbouring cells. For example, the *KNOTTED1* homeobox gene from maize is expressed only in the corpus, but the protein is also detected in the tunica, suggesting that it is transported from one layer to the other, most probably via the symplast (e.g. Lucas *et al.* 1995).

Several studies on floral development have used chimeras to study the behaviour of floral meristem regulators. Perbal *et al.* (1996) used periclinal chimeras in which either the L1, the L2 or the L3 (corpus) layer lacked DEFICIENS (DEF) or GLOBOSA, two transcription factors in *Antirrhinum* involved in floral organ identity. If the genes were expressed in the L2 and L3, protein could also be detected in the epidermal layer, indicating transport via an as yet unidentified mechanism. Diffusion could play a role, but several observations also suggest an active mechanism involving directional and selective transport. The movement of DEF, for example, is probably polar because in chimeras expressing DEF in the L1 only, the protein and mRNA remain confined to this layer (Perbal *et al.* 1996). Likewise, Sessions *et al.* (2000) examined the movement of two transcription factors in *Arabidopsis*, LFY and APETALA1 (AP1), both also implicated in floral patterning. The approaches used involved the creation of chimeras as well as the activation of gene expression in the L1 layer of the floral meristems. This work showed that LFY protein is transported throughout the meristem, even when its mRNA is produced in a subdomain only. This is apparently not the case, however, for AP1, suggesting a differential regulation of protein traffic between cells. The precise role of these protein movements remains to be established. It could be, as suggested by Hake (2001), that cells within a particular compartment need to receive similar levels of certain regulators to assure the integrity of the domains concerned. In such a scenario, the effect of the cytoplasmic connections would be to synchronize the differentiation state of groups of cells.

5. CONCLUSION

Genetic analysis combined with expression studies have improved our view of meristem structure and function and revealed the importance of cell–cell interactions. The exchange of signals between cells, rather than a rigidly predetermined genetic program, seems to be required for the establishment and functioning of an organized meristem. Together these interactions form a stable network, set up during embryogenesis, that assures the coordination of cell behaviour throughout development. These interactions involve at least some plant hormones, in particular cytokinins and auxins implicated in the maintenance of meristem identity and phyllotaxis, respectively. Although important progress has been made, it will be a challenge to unravel further the links between these signalling molecules and other factors involved in setting up differentiation patterns.

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GLOSSARY

2,4-D: 2,4-dichlorophenoxyacetic acid
CZ: central zone

ER: endoplasmic reticulum
IAA: indoleacetic acid
GA: gibberellic acid
KAPP: kinase-associated phosphatase
NPA: *N*-1-naphthylphthalamic acid
RLK: receptor-like kinase
RZ: rib zone
PR: plastochron ratio
PZ: peripheral zone
SAM: shoot apical meristem