REVIEW PAPER

Meristematic sculpting in fruit development

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Abstract

The diversity of shape in life is astounding, and this is particularly vivid when the varied forms observed in our fruit bowls are examined. How some of the tissues of the Arabidopsis fruit are moulded is starting to be understood, revealing how plants may sculpt plant form by modulating the degree of meristematic properties. In this fruit the KNOX I and BLH meristem identity genes promote medial tissue proliferation by maintaining these tissues in a ‘quasi-meristematic’ fate. The action of these genes is opposed by ASYMMETRIC LEAVES activity that promotes valve formation together with JAGGED/FILAMENTOUS FLOWER and FRUITFULL activities. This is reminiscent of the function of these genes in the shoot apical meristem and in leaf development. In this review, the aim is to present the medial tissues of the Arabidopsis fruit as a modified meristem and extrapolate our knowledge from other plant organs to fruit development.

Key words: ASYMMETRIC LEAVES, REPLUMLESS/BELLRINGER, fruit development, gynoecium, JAG/FIL, KNOX I, quasi-meristem, replum, SAM, tissue patterning.

Introduction

To understand the end, we must return to the beginning and, for most plants, the beginning is the meristem. The meristem is a tissue in which cells are actively dividing and giving rise to relatively undifferentiated cells (Oxford English Dictionary, Online edition http://dictionary.oed.com). Plant meristems can be thought of as specific regions of pluripotent cells (or stem cell niches) and are commonly regarded as generating fountains of new cells. However, recent work suggests that some plant organs are sculpted by creating zones of intermediate or ‘quasi-meristematic’ activity.

Various types of meristematic tissue have been defined in plants, all of which contribute to the overall structure/function of the plant. The establishment and maintenance of root and shoot apical meristems (respectively, RAM and SAM) have been the subject of intense studies. These primary meristems develop during embryogenesis and the entire biomass of the plant can be traced back to them. Although these meristems are vital to increase plant size, secondary meristems are essential for producing specialized tissues. One example is the (pro)cambium, which is important for vascular development and increasing stem diameter. Although secondary meristems function like meristems, generating a source of new cells, a molecular description was required to reveal that they are indeed related to primary meristems (Baucher et al., 2007). The primary and secondary meristems are indeterminate in nature because the organs they produce can vary in size and shape depending on local environments (Sablowski, 2007a).

Unlike indeterminate meristems, determinate meristems produce new cells for a predetermined period and form organs and tissues of predictable size and form, such as the floral meristem. Another group of determinate meristems are found within organs. Their fuzzy and fleeting nature makes them difficult to define and, like the procambium, require molecular markers to help to reveal their presence. The cells of these meristems are partially differentiated, but they have meristematic characteristics allowing prolonged proliferation. These ‘quasi-meristems’ are important for leaf development and have recently been shown to be important for development of the central tissues of the fruit (Donnelly et al., 1999; Ori et al., 2000; Hay and Tsiantis, 2006;
Alonso-Cantabrana et al., 2007). In this review, research on Arabidopsis that supports the notion that the final morphology of the fruit is dependent on its initial meristematic and quasi-meristematic qualities is highlighted.

**Fruit and meristem morphology**

The shoot apical meristem (SAM) is a complex structure that has been extensively described (reviewed by Carles and Fletcher, 2003, andSablowski, 2007b). In most dicots, including Arabidopsis, it consists of three distinct layers: the external L1 and L2 layers, which compose the tunica, and the inner L3 layer corresponding to the corpus. The tunica layers are characterized by anticlinal cell divisions, whereas cells of the corpus can divide in all planes. Superimposed on this layer structure, the SAM can be divided into three functional zones. The central zone is maintained by a low cell division rate that generates cells for the peripheral zone and the rib meristem. The peripheral zone is responsible for the formation of lateral organ primordia, whereas the rib meristem sustains stem growth, thus pushing the SAM upward (Sablowski, 2007a).

The Arabidopsis siliqua is one of the best understood fruit. It is vital for seed protection before maturity, and enables seed dissemination as it breaks apart when mature. Its structure is conserved in many species of the Brassicaceae family, including Cardamine hirsuta and the crop species Brassica napus (oilseed rape). It is composed of the gynophore at its base, the ovary, the style, and the stigma at the top (Fig. 1A, B). At the end of development, the ovary is composed of three external tissues with distinct morphologies. The valves are the largest part of the fruit and their epidermis has long wide irregular cells interspersed with stomata. The two valves are separated by two medial repla, composed of long rectangular cells. The valves and repla are divided by the valve margins, composed of a few rows of small cells that later differentiate into the dehiscence zone. The dehiscence zone is responsible for regulating the timing of valve separation from the replum, and subsequent seed dispersal. Internally, the two congenitally fused carpels are separated by the septum, which joins one replum to the other (Fig. 1B, C). The septum and repla (medial tissues) remain attached to the plant after pod shattering.

Much of the patterning of the fruit is established at the onset of gynoecium development, although it is still not clear precisely from where the different tissues arise. The layered structure of the gynoecium and the multipotent nature of its cells, which produce ovules, suggest it has meristematic qualities (Pautot et al., 2001). The gynoecium is made of two congenitally fused carpels that arise from the terminating floral meristem after initiation of the other flower organ whorls. It appears as a circular roll of cells enclosing a small depression [stage 6 of flower development; the stages have been defined by Smyth et al. (1990) and nicely reviewed by Roeder and Yanofsky (2006)]. It then grows to form a cylinder (stage 8) and becomes closed at its tip (stage 10). Interestingly, the layered structure of the SAM is conserved in the growing cylinder, suggesting they are mechanistically similar (Pautot et al., 2001). At stage 8, the presumptive repla give rise to two medial ridges on their adaxial side (i.e. inside the tube) flanked on both sides by placental tissues. The medial ridges will grow towards each other and post-genitally fuse at stage 9 in the centre of the gynoecium. This internal structure will later form the septum, and the ovule primordia will develop from the placenta. The style and stigma, which form when the gynoecium post-genitally fuses at its tip, are also derived from the medial tissues. Therefore, the meristematic activity of the early repla is essential for development of all the marginal tissues of the fruit (medial tissues, style, and stigma).

The valves and valve margins are the only fruit tissues that develop independently of this meristematic activity. Interestingly, the valves have a leaf-like structure with an adaxial/abaxial polarity and the presence of stomata. As it is widely accepted that the carpels are modified leaves, the gynoecium can thus be seen as two modified leaves (the presumptive valves) fused to two modified meristems (the presumptive repla).

Many of the key regulators of fruit development have now been identified (Gu et al., 1998; Ferrandiz et al., 2000a; Liljegren et al., 2000, 2004; Rajani and Sundaresan, 2001; Roeder et al., 2003; Dinneny et al., 2005; Dinneny and Yanofsky, 2005). Several of them have roles in patterning both the SAM and fruit medial tissues, or leaves and fruit lateral tissues, supporting the notion that these organs have a common origin (Fig. 2A, B) (Gu et al., 1998; Ferrandiz et al., 2000b; Balanza et al., 2006; Roeder and Yanofsky, 2005).
**Fig. 2.** Comparison between the genetic and molecular pathways controlling SAM identity and medio-lateral patterning of the fruit in *Arabidopsis*. (A) Genetic pathways in the SAM (longitudinal section). STM promotes SAM identity by excluding leaf primordia factors, JAG/FIL activity, AS1 and AS2. CUC and KNAT6 maintain the boundaries of the stem cell niche. An auxin maximum (purple) predicts the position of the leaf primordia where STM is excluded. (B) Genetic pathways of medio-lateral patterning in *Arabidopsis* fruit (transverse section). Valves are shown in green (V), replum in red (R), valve margins in blue (VM), and septum in yellow (S). The purple zone represents an auxin maximum. The gene interactions that have been demonstrated are designated with solid lines and possible genetic interactions are indicated with a dashed line. See text for details.

2006; Alonso-Cantabrana et al., 2007; Østergaard, 2008). Understanding how these genes function in the SAM and leaves has provided insight into their function in fruit development.

**Valves are analogous to leaves**

Shoot apex development is governed by the mutual exclusion of SAM and leaf-promoting factors. Leaf primordia formation is promoted by a complex involving the MYB domain protein ASYMMETRIC LEAVES1 (AS1; ARP family) and the Lateral Organ Boundary transcription factor AS2 (Fig. 2A) (Byrne et al., 2000; Semiarti et al., 2001; Phelps-Durr et al., 2005; Guo et al., 2008). The AS1/AS2 complex epigenetically silences in leaf primordia the class I KNOX meristem identity genes (*KNOTTED-LIKE HOMEobox; KNOX I*). In this way, the AS1/AS2 complex promotes cellular differentiation and leaf initiation. Interestingly, this pathway is highly conserved in angiosperms, as KNOX I and ARP proteins share the same functions in *Arabidopsis*, maize, and *Antirrhinum* (Schneeberger et al., 1998; Timmermans et al., 1999; Tsiantis et al., 1999), suggesting a fundamental role for these transcription factors.

**ASI** has recently been involved in medio-lateral patterning of the fruit (Alonso-Cantabrana et al., 2007). Valve and replum width are respectively, reduced and increased in *asi* mutants, which underline a specific role of **ASI** in promoting valve initiation. Similarly to leaf specification, **ASI** is likely to act on valve formation through repression of KNOX *I* genes and replum development (Fig. 2B; see below).

Leaf development is also regulated by the JAG/FIL transcription factor families. JAGGED (JAG) acts redundantly with the related C2H2 zinc-finger NUBBIN (NUB) in promoting leaf formation and regulating leaf shape (Dinneny et al., 2004, 2006; Ohno et al., 2004). FILAMENTOUS FLOWER (FIL) acts redundantly with the related YABBY transcription factor YAB3 in promoting abaxial leaf specification (Siegfried et al., 1999). JAG/FIL activity also promotes leaf development by repressing KNOX *I* gene expression and SAM identity in the lateral organ primordia (Fig. 2A) (Kumaran et al., 2002).

In the fruit, JAG/FIL activity promotes valve and valve margin formation; the analogous structures to leaves (Dinneny et al., 2005). JAG/FIL activity positively regulates the valve-promoting gene FRUITFULL (FUL) and the valve margin identity genes SHATTERPROOF1 (SHP1), SHP2, INDEHISCENT (IND), and ALCATRAZ (ALC) (Ferrandiz et al., 2000a; Liljegren et al., 2000, 2004; Rajani and Sundaresan, 2001). JAG/FIL activity may also promote valve specification through repression of KNOX *I* genes (Fig. 2B) (Alonso-Cantabrana et al., 2007).

The replum has meristematic properties

In addition to leaf/valve specification, JAG/FIL activity may be linked to replum formation as *fil yab3* double mutants have enlarged replum. Replum development is promoted by the exclusion of JAG/FIL activity and valve margin identity genes (Dinneny et al., 2005). This exclusion from the replum is mediated by the BELL1-like homeodomain transcription factor REPLUMLESS (RPL, also known as PENNYWISE, BELLRINGER, and VAAMANA). *rpl* mutants have a reduced replum width and septum development, and strong alleles have a complete absence of outer replum (Roeder et al., 2003). This replumless phenotype is rescued when *rpl* is combined with mutations in valve and valve margin promoting genes, including JAG and FIL (Fig. 2B) (Roeder et al., 2003; Dinneny et al., 2005; Alonso-Cantabrana et al., 2007). This suggests that the role of RPL is strictly to repress valve and valve margin development, and not to induce replum formation directly.

RPL also has important roles in SAM organization and function, such as maintenance of stem cell fate, phyllotaxy establishment, internode patterning and response to floral induction (Byrne et al., 2003; Smith and Hake, 2003; Bhatt et al., 2004; Smith et al., 2004). RPL has been demonstrated to function in the SAM by interaction with KNOX *I* meristem genes. This interaction may be direct as KNOX *I*
and BELL1-like homeodomain can form heterodimers in vivo (Bellaoui et al., 2001; Chen et al., 2003; Smith and Hake, 2003; Hackbusch et al., 2005; Kanrar et al., 2006). KNOX proteins are transcription factors containing a highly conserved homeobox domain. They are named after the founder member KNOTTED1 from maize and can be subdivided into two classes (Vollbrecht et al., 1991; Kerstetter et al., 1994). The KNOX I class is important for the establishment and maintenance of meristems in all angiosperms studied so far (Hake et al., 2004). In Arabidopsis the KNOX I genes include KNAT2 (KNOTTED IN ARABIDOPSIS THALIANA2), KNAT6, BP (BREVIPEDICELLUS, also known as KNAT1), and STM (SHOOTMERISTEMLESS). Mutations in these genes lead to loss of meristematic identity whereas misexpression induces ectopic meristem formation (Long et al., 1996; Ori et al., 2000; Hake et al., 2004; Belles-Boix et al., 2006; Scofield and Murray, 2006; Scofield et al., 2007). Their expression patterns are restricted to overlapping domains of the vegetative SAM. They are repressed in leaves and leaf primordia by the action of AS1/AS2 complex and JAG/FIL activity (Fig. 2A).

It has recently been suggested that this regulatory module involving RPL, KNOX I, and ASI acts on the fruit medio-lateral patterning as it does on the SAM/primordia patterning. Alonso-Cantabrana et al. (2007) showed that ASI and the KNOX I gene BP have antagonistic effects on replum development. Whereas BP promotes replum development, ASI represses it. Analysis of the as1 mutant showed that BP expression is restricted to the replum by the repressing action of ASI in the valves (Fig. 2). BP ectopic expression, either by overexpression under the 35S promoter or in the as1 mutant, increases replum width. ASI probably acts in the valves through a complex involving AS2, since as1 and as2 mutants show a similar enlarged replum. The AS1/AS2 complex therefore has a similar role in the fruit as it has in the SAM region, promoting lateral tissues by inhibiting BP expression.

BP probably acts on replum development by interacting with RPL, but also through a separate mechanism. This is first suggested by the fact that interaction between BP and RPL proteins regulates meristem function (Smith and Hake, 2003; Bhatt et al., 2004; Kanrar et al., 2006). It is also consistent with the strong replumless phenotype of the rpl bp double mutant (Alonso-Cantabrana et al., 2007). In addition, BP ectopic expression in the valves and valve margins drives a similar expression pattern for RPL, which correlates with an enlargement of replum width. However, the replumless phenotype of rpl mutant can be rescued by the as1 mutation, and this is probably because of BP ectopic expression in this background. In other words, BP overexpression caused by as1 mutation is able to trigger replum formation in the absence of a functional RPL gene. This suggests that BP and RPL act in a partially independent manner.

Extension of the regulatory network from leaves could further explain the spatial expression pattern observed for BP. In addition to repression by ASI, BP expression in the valves could be further quenched if JAG/FIL repressed BP as it does in the leaves. High BP expression in the replum could then be promoted by JAG/FIL repression by RPL. This leads to a positive feedback loop: BP induces RPL which indirectly blocks BP repression.

Alonso-Cantabrana et al. (2007) proposed an attractive ‘overlap’ model in which the medio-lateral patterning of the fruit is organized by two opposing gradients (Fig. 3A), thus modifying the French flag model for pattern formation proposed by Wolpert (1969) on a single gradient basis. Accordingly, the valves and the replum would, respectively, be defined by high activities of valve factors (JAG/FIL) and replum factors (BP being one of these). The valve margins would form in a narrow stripe where the activities overlap, being weakly expressed in this tissue or diffusing from adjacent tissues. This is consistent with the observation of Dinneny et al. (2005) that activation of FUL and SHP require, respectively, a high and a low level of JAG/FIL.
activity, thus defining valve and valve margin territories. Furthermore, the high BP expression in the replum is likely to produce the necessary gradient through the valve margins since KNOX I proteins can diffuse via plasmodesmata (Bolduc et al., 2008).

If BP was the only replum factor, the model would predict that the bp mutant has a reduced replum, but this is not the case (Alonso-Cantabrana et al., 2007). The authors suggest that other KNOX I genes act redundantly with BP, as it has been shown for the SAM. KNAT2 and KNAT6 are unlikely candidates, as knat2 knat6 bp triple mutants exhibit no reduction of the replum (Ragni et al., 2008). Moreover, the knat6 mutant rescues the reduction of replum width of rpl and rpl bp mutants, suggesting that KNAT6 is a suppressor of replum formation. KNAT2 and KNAT6 are exclusively expressed in the valve margins. Therefore, they may be partially involved in valve margin formation occurring at the boundaries between the valves and replum. This is reminiscent of the role of KNAT6 in the establishment of the boundaries between the SAM and the cotyledons (Belles-Boix et al., 2006).

An additional KNOX I gene which could be a candidate for promoting replum development is STM. Indeed, STM is expressed in the presumptive replum at the early stages of gynoecium development, is partially redundant with BP in regulating stem cell function, and interacts both genetically and at the protein level with RPL to regulate inflorescence stem growth and stem cell fate (Long et al., 1996; Byrne et al., 2002, 2003; Bhatt et al., 2004). Until recently, a role of STM has not been shown in gynoecium patterning because most stm mutants do not develop normal inflorescences and flowers. Moreover, the study of weak stm mutant alleles has revealed that STM is involved in early floral patterning and carpel initiation which makes the study of its function in the replum even more difficult (Endrizzi et al., 1996; Pautot et al., 2001). Scofield et al. (2007) obtained inducible STM-RNAi lines to study late STM functions. In the most affected flowers, the floral meristem aborted before initiating the gynoecium, but weakly affected flowers developed two independent structures instead of the gynoecium. These structures correspond to unfused valves topped with a stigma and flanked by residual replum, placenta, and ovules. The septum is completely absent and the repla are highly reduced and divided into two parts. A related phenotype is found in the weak stm-6 mutant in which the carpels are partially unfused at the top of the replum (Endrizzi et al., 1996). STM is thus involved in the formation of medial tissues of the fruit. It is unfortunately impossible to conclude whether it is strictly necessary for replum formation, as stm-6 and the STM-RNAi lines still have some medial tissue that could be promoted by residual STM activity. In the SAM, STM functions in part by restricting ASI expression to the leaf primordia, preventing the differentiation of the stem cells (Clark et al., 1996; Endrizzi et al., 1996; Byrne et al., 2000). STM could have a similar role in the fruit, restricting ASI expression to the valves thus enabling BP expression in the replum.

The KNOX I genes BP and STM are clearly involved in replum development. However none of them have been shown to be strictly necessary as all the single mutants studied so far still have some replum tissue. In the SAM, these two genes act redundantly to maintain stem cell fate (Byrne et al., 2002), suggesting a similar mechanism in the replum. It would then be interesting to study the effect on replum development in the absence of both BP and STM. It could also be interesting to test the implication of the KNOX II genes and the newly discovered KNATM gene. The encoded proteins differentially interact in a yeast 2 hybrid experiment with KNOX I and RPL, but no developmental function have yet been reported (Hackbusch et al., 2005; Scofield and Murray, 2006; Magnani and Hake, 2008). The relative importance of the different KNOX genes could provide a difference between SAM and replum developments.

In addition to KNOX and ASI transcription factors, a key factor regulating SAM activity and leaf initiation is the phytohormone auxin (Reinhardt et al., 2000; Reinhardt et al., 2003; Furutani et al., 2004; Heisler et al., 2005). The position of lateral primordia is predicted by the production of auxin maxima and the boundaries between them are characterized by auxin minima. The ebb and flow of auxin within the meristem to generate the auxin maxima is mainly directed by the PIN-FORMED1 (PIN1) auxin efflux carrier (Petraske et al., 2006). The dynamic nature of PIN1:GFP polar localization is controlled by direct phosphorylation by the PINOID (PID) protein kinase family (Benjamins et al., 2001; Friml et al., 2004; Michniewicz et al., 2007). Disturbance of auxin transport by mutations in PIN1 and/or PID cause the loss of lateral primordia (Christensen et al., 2000; Furutani et al., 2004). A negative correlation between STM and PIN1 expression domains in the meristem has been observed (Heisler et al., 2005). Moreover, STM is ectopically expressed at the periphery of the meristem in auxin transport mutants or after auxin transport inhibitor treatment, suggesting that auxin flow is important in limiting STM expression (Scanlon, 2003; Heisler et al., 2005; Schuetz et al., 2008). PIN-dependent auxin fluxes are also required, together with ASI, to repress BP expression in leaf primordia (Hay et al., 2006). It is particularly interesting that the complete lack of lateral floral primordia in pin1 mutants may be a consequence of ectopic KNOX function, since mutations in BP or in its binding partner RPL, both involved in replum development, are known to partially rescue pin1 mutant influences (Hay et al., 2006). These data highlight the interdependency of KNOX I gene function and auxin dynamics.

Replum development in Arabidopsis is regulated by auxin action. Disruption of polar auxin transport (PAT) by pin1 or pid mutations or by auxin transport inhibitor treatment leads to an expansion of the replum and a reduction of the lateral valves (Bennett et al., 1995; Nemhauser et al., 2000; K Sorefan, unpublished data). This is similar to the floral meristem where disruptions in PAT cause an enlargement of the meristem and reduction of lateral primordia. However, there are some notable differences between the SAM and the replum. In the SAM, auxin maxima are associated with
lateral organ primordia, where ASI is highly expressed and KNOX 1 genes are repressed. By contrast, an auxin maximum can be visualized in the replum by DR5::GFP whereas the lateral valves have low GFP signals (T Girin, unpublished data). The auxin maximum in the fruit is therefore associated with a low ASI expression and a high STM and BP expression.

Other meristem-associated genes involved in fruit development

The CUP SHAPED COTYLEDON 1 (CUC1) and CUC2 genes provide another interesting link between gynoecium medial tissues and stem cell niches. These two transcription factors of the plant-specific NAC family are indispensable for the initiation of the embryonic SAM, and the separation of cotyledons (Aida et al., 1997, 1999; Takada et al., 2001; Hibara et al., 2003). CUC1/2 are expressed in the presumptive embryonic SAM, and initiate SAM formation by activating STM expression. Their expression is later restricted to the SAM boundaries, maintaining the cells in an undifferentiated state. Accordingly, the SAM fails to form in the cuc1 cuc2 double mutant, and the stem cells are used to produce fused cotyledons. Ishida et al. (2000) studied their role in late developmental stages by producing cuc1 cuc2 plants from calli. The mutants have a defect in the growth of the gynoecium medial ridges, thus failing to form a septum. Interestingly, the fruit also often lack the repla in their upper parts. The CUC1 expression pattern in the gynoecium is currently unknown. CUC2 is expressed at stage 7 in the presumptive medial ridges, then at the tip of these ridges until stage 11 (after the fusion that forms the septum). Accordingly, CUC genes are believed to maintain the presumptive septum cells in an undifferentiated state.

An intriguing question is how cuc1 cuc2 mutations can affect the replum development since CUC2 is not expressed in this tissue. This could be explained by CUC1 activity or by a putative transitory expression of CUC2 in the presumptive replum at an early stage of gynoecium development. The mutant phenotype could then result from a lack of KNOX 1 activation by CUC1/2 in the replum, as for the embryonic SAM.

To our knowledge, the only known mutant that completely lacks replum tissues is the ant lug double mutant (Liu et al., 2000). ANT encodes a protein of the AP2/ERF transcription factor family. It is involved in cell proliferation in lateral organ primordia and is also linked to meristem maintenance as ant mutants have smaller floral meristems. It is thought to regulate cell proliferation by maintaining meristematic activity during organogenesis (Krizek, 1999, 2003; Long and Barton, 2000; Nole-Wilson and Krizek, 2006). The LUG transcriptional co-repressor is involved in the growth of lateral organ primordia, but its role is less well understood (Cnops et al., 2004). Both ANT and LUG were first identified for their role in floral organ identity and growth (Elliott et al., 1996; Klucher et al., 1996; Chen et al., 2000; Conner and Liu, 2000).

Quasi-meristem as key modulators of plant development

The presence of meristematic activity outside proper meristems is not specific to the gynoecium/fruit, as many meristematic regions in organs have been described. It is proposed that these regions are named ‘quasi-meristems’. A quasi-meristem corresponds to a zone of active cell proliferation within an organ, giving birth to specialized and determinate tissues. Contrary to true-meristems, all the cells of quasi-meristems will eventually differentiate.

Quasi-meristems are important for determining the structure of plants. Such zones are important for leaf
morphogenesis, where the marginal meristem initiates the leaf blade before the plate meristem expands it (Donnelly et al., 1999). They are also found at the base of monocotyledon leaves, and determine the final size of the leaf blade. Ovule primordia arise and develop from a meristematic zone (Brambilla et al., 2007). To some extent the rib meristem can be considered as a quasi-meristem, which drives stem growth. It is often considered part of the SAM but was initially defined as an independent structure within the stem (Ruonala et al., 2008). All its cells will differentiate rapidly and its apparent indeterminate activity is only due to the provision of new cells from the SAM.

These quasi-meristems share key regulatory genes with true-meristems, but also require some specific genes. The SAM and the replum both require KNOX I gene function, whereas the SAM and ovule primordia both require WUSCHEL activity. ANT and LUG on the other hand are specifically required for quasi-meristem activity in the leaf and the medial part of the fruit.

Another good example of quasi-meristems is illustrated by dissected leaf species. Some of the molecular mechanisms for SAM identity have been diverted in these species to control organ morphology. In the Arabidopsis relative Cardamine hirsuta, which has a dissected leaf form, ChBP was expressed not only in the SAM, as in Arabidopsis, but also in leaf primordia. ChBP is first repressed in leaf founder cells, but this repression is not maintained later in leaf primordia. This re-establishment of KNOX I expression is thought to maintain a degree of meristematic activity, promoting leaflet outgrowth and repressing precocious differentiation (Champagne and Sinha, 2004). The molecular mechanisms of KNOX I gene regulation have been investigated. The chasl mutant had additional leaflets and the ChBP was ectopically expressed (Hay and Tsiantis, 2006). Auxin maxima predict the positions of lateral leaflet formation. Disruption of auxin transport with chemical inhibitors or mutations in the ChPIN1 gene blocks the auxin maxima and reduces lateral leaflet formation. Conversely, application of exogenous auxin induces auxin maxima and leaflet production. The exclusion of STM expression from positions of auxin maxima in the SAM is also maintained in leaflet formation of C. hirsuta (Barkoulas et al., 2008). The establishment of a quasi-meristem with partial meristematic activity is thus a key regulatory mechanism to drive leaf morphology in some species.

The establishment of the quasi-meristem allows for the timing of tissue differentiation to be fine-tuned. In the SAM, developmentally ‘simple’ primordia can form in a matter of hours. Morphologically ‘complex’ leaves and fruit on the other hand can take days and weeks to develop. Therefore, the prolonged quasi-meristematic state may allow for the organ to modulate growth during this time.

Quasi-meristems as targets for crop improvement

An important goal for plant biology is to translate our fundamental knowledge from model plants to crops. Quasi-meristems are clearly important for leaf and fruit development and may be involved in stem growth. Controlling the degree of meristematic identity may allow the moulding of desirable plant traits in crops. For example, premature pod shattering in oilseed rape crops is a major cause of yield loss. In some Brassica cultivars, the replum is reduced and the valves become partially fused, which is similar to the rpl-3 mutant in Arabidopsis. These plants have much reduced pod shatter. Therefore, it may be possible to modulate pod shatter in Brassica crops by manipulating replum meristematic activity, possibly by reducing Brassica RPL or KNOX I gene function. It has already been demonstrated that knowledge from Arabidopsis can be transferred to other Brassicaceae. It is possibly not surprising that modulating FUL transcription in Brassica juncea abolished valve margin development and therefore pod shattering as it does in Arabidopsis (Østergaard et al., 2006).

What is more exciting and valuable is that soft fruit species may regulate fruit development through shared mechanisms with Arabidopsis but this is something that needs more attention (Seymour et al., 2008).

Research has focused on the beginning and ends of cell-type differentiation, and it is indeed much more difficult to analyse the intermediate states. Ground-breaking work on plant growth using computer models is now allowing us to record these transient stages of development. Petal formation in Antirrhinum, for example, can now be replayed to demonstrate how small localized changes in growth rate, perhaps by quasi-meristems, help to mould the final petal shape (Coen et al., 2004). The discovery of quasi-meristems in leaves and fruit highlights the importance of understanding the journey from stem cells to terminally differentiated cells, if we are to fully understand plant development.

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