Tortula ruralis, and the green algae *Chlamydomonas reinhardii*. This study revealed that the order of the structural domains of plant and animal/fungal Tom20 proteins is only similar when viewed in reverse. AtTom20 is anchored to the mitochondrial outer membrane by a carboxy-terminal transmembrane domain, in contrast to an amino-terminal domain in animal/fungal Tom20 (Figure 2). Furthermore, the Tom20 transmembrane domains and proximal cytosolic regions from both lineages display striking structural similarities, but only in reverse order. No genetic mechanisms are known that could generate such a reversal in the order of structural domains, strongly suggesting that the animal/fungal and plant Tom20 proteins evolved from two distinct genes after the divergence of the animal and plant lineages.

Perry *et al.* [10] present an elegant example of convergent evolution on a molecular scale, where different organisms adapted distinct proteins to fulfil a function demanded by a similar cellular environment. With only the core import pore present in the early stages of mitochondrial evolution, great selective pressures would have existed to develop a discriminating receptor protein to increase targeting fidelity and import efficiency. In various species, the hundreds of proteins that had to be imported into mitochondria, possessing similar targeting sequences obtained from the original endosymbiont, probably acted as the driving force that produced the same receptor solution twice. As the process of gene transfer progressed, there was probably an increase in both the complexity of the protein complement targeted to mitochondria and the cellular requirement for an effective protein import apparatus. Tom20 greatly enhances the process of mitochondrial protein import, thus the development of this receptor was perhaps an essential step in the evolution of the relationship between mitochondria and the host cell.

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ARC Centre of Excellence in Plant Energy Biology, MCS Building M310, University of Western Australia, 35 Stirling Highway, Crawley 6009, Western Australia, Australia.
E-mail: seamus@cyllene.uwa.edu.au

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Plant Meristems: The Fiendish SU DOKU of Stem-Cell Maintenance

Three recent studies have uncovered effector mechanisms and novel pathways in the regulation of the dynamic changes to cell behaviour that occur in plant meristems. The results show how exquisite regulation of cell-cycle mechanisms is central to root stem cell homeostasis.

Peter Doerner

After a plant seed has germinated, all new cells in the growing plant ultimately derive from pluripotent stem cells in the shoot apical meristem and root apical meristem. Stem cells are defined by their capacity for self-renewal and their simultaneous ability to generate cells destined for differentiation, but the details of these central processes are not yet well understood. Three new studies [1–3] have revealed exciting new mechanistic details of how stem cells are maintained and how they control the switch between fates, including the involvement of cell-cycle mechanisms controlling entry into S-phase.

In shoots, stem cells are located at the meristem centre, and their non-differentiated state is maintained by the expression of *WUSCHEL* (*WUS*) in an underlying domain, which thus functions as a stem cell niche for the shoot apical meristem. In roots, stem cells surround the cells of the quiescent centre, which expresses the *SCARECROW* (*SCR*) gene

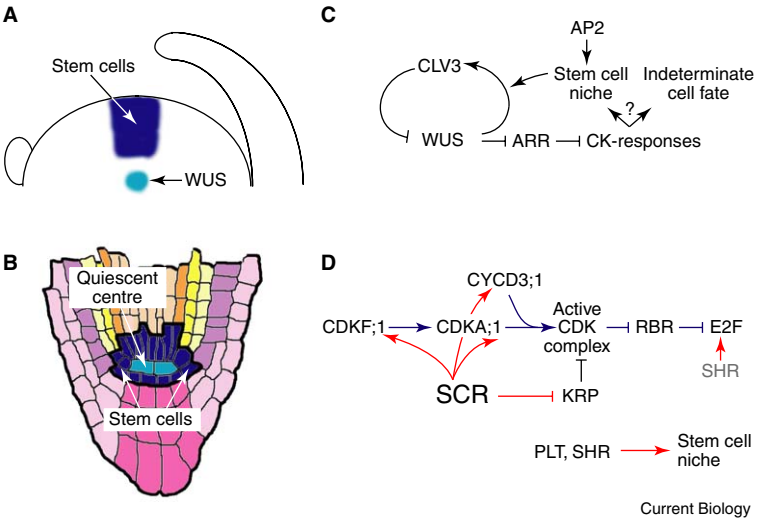

Figure 1. Regulatory mechanisms in plant meristems.

(A) Schematic of the shoot apical meristem. The expression domain of *WUS* (turquoise), which maintains the stem cell niche, subtends that of the stem cells at the centre of the meristem (dark blue). (B) Schematic of the root apical meristem. The quiescent centre (turquoise) maintains the stem cell niche for the surrounding stem cells (dark blue). (C) Model for the function of the shoot apical meristem. A negative feedback loop comprising the CLV signalling complex and *WUS* maintains stem cell homeostasis. AP2-type transcription factors are also required for this. *WUS* negatively regulates A-type *ARR* genes, which in turn regulate cellular responses to cytokinin. (D) A model for the control of stem cell maintenance by the S-phase promoting pathway. Interactions between components of this pathway are shown in blue, possible conduits for *SCR* and other root stem cell niche genes are shown in red.

and defines the root stem cell niche.

An interacting network of genes is required for shoot meristem maintenance: The *SHOOTMERISTEM-LESS* (*STM*) gene is required to maintain an indeterminate state throughout the shoot apical meristem, whereas *WUS* maintains a subset of these cells at the centre of the shoot apical meristem as stem cells. The *CLAVATA1–3* (*CLV1–3*) genes function to restrict stem cell numbers by a negative feedback loop which regulates *WUS* expression to maintain stem cell homeostasis (Figure 1A,C). In roots, the stem cell niche is maintained by the quiescent centre cells, in which the expression domain of *SCR* abuts a zone of high-level auxin accumulation at the root apex [4,5]. Persistence of this niche also requires the expression of the *SHORT-ROOT* (*SHR*) and *PLETHORA* (*PLT*) genes [5,6] (Figure 1B,D).

One of the big gaps in our understanding of stem cell maintenance in shoots has been how *WUS*, which encodes a homeodomain-type transcription factor, exerts its effect on *CLV3* expression. Leibfried and collaborators [1] have now identified direct targets of *WUS* that bring us a step closer towards answering this question. To identify immediate-early targets of *WUS*, they inducibly expressed *WUS* and followed changes to gene expression by microarray analysis. They found that 148 genes were up-regulated or down-regulated following the induction of *WUS* expression, a gold mine for further exploration of the regulatory networks in the shoot apical meristem.

Strikingly, Leibfried and collaborators [1] found that several of the down-regulated genes are members of the type-A *ARABIDOPSIS RESPONSE REGULATOR* (*ARR*) family, and at least one of these is a direct *in vivo* target of *WUS*. These A-type *ARR* genes are also direct targets of the cytokinin signalling pathway, and function in a negative feedback loop to dampen cytokinin-dependent responses [7]. Cytokinin growth regulators stimulate shoot meristem activity, whilst inhibiting root meristems. Therefore, *WUS* can modify the responsivity of cells to cytokinin by acting through *ARR* genes (Figure 1C).

If A-type *ARR* genes are important conduits for *WUS* function, then their inactivation should result in meristem defects. A-type *ARR* genes are present as a small, 10-member gene family in the *Arabidopsis* genome, and are to a large extent redundant, as single loss-of-function mutants appear to be without phenotype. Leibfried and colleagues [1] had to generate a septuple *ARR* mutant to observe subtle meristem defects. But when constitutively-active *ARR7* was expressed in transgenic plants, stronger meristem phenotypes with similarities to the *wus* mutant phenotype were observed. As the constitutively-active form mimics the cytokinin-activated ARR protein, this observation suggests that *WUS* action might require cytokinin perception. Although these experiments do not yet provide a plausible mechanism for the cell non-autonomous effect on *CLV3* expression, the identification of further direct *WUS* targets will undoubtedly accelerate its discovery.

Würschum and colleagues [3] asked whether *WUS* is the only gene involved in maintaining stem cells in the shoot apical meristem. Using a mutational approach, they identified a dominant-negative allele of the *AP2* gene, *I28*, which had previously been shown to be required for floral patterning and also regulates seed size. A range of phenotypes of varying severity were observed in germinating homozygous *I28* seedlings, suggesting that the shoot apical meristem is not initiated during embryogenesis or maintained during vegetative development. Just as in *wus* mutants, the number of stem cells and the size of the shoot apical meristem were reduced in *I28* mutant plants.

In the *I28* mutants, the accumulation of *WUS* and *CLV3* transcripts was found to be reduced, but when the *I28* mutation was combined with strong *clv3*

alleles, which in an otherwise wild type background lead to a dramatic expansion of the stem cell population, the shoot apical meristem defect was masked. By contrast, progressive reduction of *WUS* activity in an *I28* background exacerbated the *I28* mutant phenotype, suggesting that the two genes affect the same process in stem cell maintenance (Figure 1C).

As *I28* is a dominant-negative allele of *AP2*, it is not entirely clear which other AP2-like putative transcription factors function in maintaining the stem cell population of the shoot apical meristem. As revealed by expression analysis and ability of *I28* to suppress stem cell maintenance when expressed in individual sub-domains of the shoot apical meristem [3], these genes might function not only in the organising centre defined by the expression domain of *WUS*, but in the stem cell niche itself.

Stem cell maintenance is not only defined by the ability to self-renew, but also by the ability of stem cells to generate progeny with the capacity for differentiation. For individual cells, this involves the transition from one dynamically stable state (self-renewal) to another (differentiation). Wildwater and collaborators [2] have now found a plausible mechanism that mediates this transition and affects the balance of stem cells to differentiated progeny: the regulation of *RETINOBLASTOMA-RELATED* (*RBR*) gene activity.

The single *RBR* gene in *Arabidopsis* is essential for gametophytic development, therefore Wildwater and colleagues [2] selectively inactivated *RBR* expression in the root apex by root meristem-specific RNA interference to study its function in post-embryonic development. They found that reduction of *RBR* expression led to the accumulation of supernumerary tissue-specific stem cells surrounding the quiescent centre. But *RBR* down-regulation had little effect on the rapidly dividing cells in the proximal meristem, suggesting that a key target for *RBR* is absent in these cells. Satisfyingly, when *RBR* was inducibly over-expressed, the expression of stem cell-specific markers was rapidly quenched and stem cells were not maintained, but this did not occur when a defective form of *RBR* was expressed.

In an *scr* mutant background, stem cells of the root apical meristem are gradually depleted, but strikingly, down-regulation of *RBR* can rescue the stem cell defect in these roots. These cells do not properly differentiate, however, as they are locked in cycles of self-renewal. *RBR* downregulation could not alleviate the stem cell defect in *shr* or *plt* mutant plants.

RBR is part of a pathway that controls entry into S-phase (Figure 1D). Wildwater and colleagues [2] examined whether other components of the RBR pathway could also modify stem cell behaviour similar to when RBR was up-regulated or down-regulated. They found that over-expression of cyclin D3 and the S-phase transcription factors E2F and DP promoted stem cell fate, while increased expression of KRP, a cyclin-dependent kinase inhibitor, had the opposite effect. This is consistent with earlier observations that down-regulation of an upstream kinase necessary for activation of the cyclin D–CDK complex also led to loss of stem cell maintenance [8].

These experiments reveal a crucial role in stem cell maintenance for regulatory genes that control S-phase entry. *RBR* plays a key role in this process: as an enforcer of the decision to enter S-phase, it not only targets the S-phase transcription factor E2F, but also interacts with many other targets, including components of the chromatin re-modelling machinery [9]. By establishing links between cell cycle control and other regulatory pathways, RBR is ideally positioned to chaperone changes in stem cell behaviour and then to insure that these become fixed. Recent analysis of differentiation in *Arabidopsis* roots shows that such chromatin modifications can occur rapidly and are involved in differentiation [10]. But the experiments reported by Wildwater and collaborators [2] do not yet reveal how behavioural change is regulated: whether through transcriptional control of components of the S-phase pathway, or by reversible phosphorylation. It will be interesting to examine stem cell behaviour in shoots as well after modulating RBR levels by precise cell type- and domain-specific expression.

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Institute of Molecular Plant Sciences, School of Biological Sciences, University of Edinburgh, Mayfield Road, Edinburgh EH9 3JH, UK.
E-mail: Peter.Doerner@ed.ac.uk

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