From signals to stem cells and back again
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During plant development, organ morphology and body architecture are dynamically adjusted in response to a changing environment. This developmental plasticity is based on precisely controlled maintenance of primary, as well as programmed initiation of pluripotent stem cell populations during secondary- and de novo meristem formation (reviewed in [1–3]). Plant stem cells are found exclusively in specific locations that are defined by relative position within the growing tissue. It follows that stem cell fate is primarily instructed by endogenous signals that dynamically define the stem cell niche in response to tissue topography [4]. Furthermore, plant stem cell activity is strongly dependent on developmental stage, suggesting that they are sensitive to long range signaling from distant organs, including the root [5,6**]. And finally, environmental signals exert a major influence allowing plants to cope with the plethora of highly variable environmental parameters during their life-cycle [7]. Integrating tissue level positional information with long range developmental cues, as well as environmental signals requires intricate molecular mechanisms that allow to filter, classify, and balance diverse inputs and translate them into appropriate local cell behavior. In this short review, we aim to highlight advances in identifying the relevant signals, their mode of action, as well as the mechanisms of information processing in stem cells of the shoot apical meristem (SAM).

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Tissue level signaling: transcription factors, ligand-receptors systems and the cell wall
The molecular basis for stem cell identity and maintenance in the shoot is composed of a negative feedback loop between the homeodomain transcription factor WUSCHEL (WUS) and the peptide signaling factor CLAVATA3 (CLV3) (Figure 1) [1,4,7]. WUS mRNA is exclusively expressed in the stem cell niche in the deeper layers of the SAM, termed the Organizing Centre (OC). From these cells, WUS protein migrates apically via cytoplasmic bridges, called plasmodesmata, to induce stem cell fate [8–10]. Stem cells in turn express the CLV3 precursor, which is processed into a small peptide and secreted to the extracellular space [11], from where it represses WUS expression through stimulation of receptor kinase complexes (Figure 2).

Several receptors have been identified to function in CLV3 signaling to limit stem cell fate. The leucine-rich repeat receptor kinases (LRR-RKs) CLV1, the related BARELY ANY MERISTEM 1, 2 and 3 (BAM 1, 2, and 3) and the more distant RECEPTOR-LIKE-PROTEIN KINASE 2 (RPK2) receptors all function in stem cell fate restriction [12] (Figure 2). Furthermore, the heterodimer between the LRR non-kinase CLV2 and the pseudo-kinase CORYNE (CRN) is required for stem cell signaling. Redundancy between these receptor complexes is demonstrated by the ability of BAMI to partially compensate for the loss of CLV1 although BAMI is usually repressed by CLV1 signaling [13], demonstrating substantial cross regulation between the different signaling modules. Apart from the core stem cell signaling receptors, the ERECTA (ER) family and ARABIDOPSIS HISTIDINE KINASEs (AHKs) receptors are required for proper SAM morphology by tuning cellular sensitivity to cytokinin (Figure 2). While AHKs promote cytokinin perception, ER receptors appear to restrict signaling output to deeper layers of the SAM, thus collectively defining the organizing center (OC) [14,15,16*].

Importantly, CLV2 and ER receptors appear to have additional roles in immune signaling [17,18] and BAM receptors are required to control molecular trafficking through plasmodesmata [19**], suggesting that RLKs have not only functionally diverged, but are able to execute multiple context dependent roles. The fact that more than 600 RLKs are encoded by the Arabidopsis genome [20], and because many of them act in immune signaling via the recognition of defined Pathogen Associated Molecular Patterns (PAMPs), which include small peptides [21], makes it likely that additional ‘dual use receptors’ with roles in stem cell control may exist. The observations that MAPK and Ca²⁺-signaling are putative downstream effectors of CLV signaling also supports this hypothesis [22,23], since they are important downstream effectors in immune signaling as well. Taken together these observations imply that in addition to CLV3, other molecules might be sensed by stem cell regulatory
receptor complexes. This would allow for signal integration at the receptor level by diverse mechanisms, including spatial partitioning, co-receptor interaction or control of receptor abundance. The idea of information processing at the receptor level is supported by several independent findings: First, CLV3 signaling is buffered through internalization of CLV1 upon ligand exposure and different clv1 and crn alleles have divergent, yet additive effects on carpel development and BAM repression, respectively [24,25**]. Second, FEA2, the CLV2 orthologue in maize, can sense multiple ligands and confers downstream signaling specificity by differential interaction with either ZmCRN or the alpha subunit of the heterotrimeric G-protein COMPACT PLANT 2 [26**]. And third, RLKs are differentially recruited into microdomains upon stimulation [27].

In addition to mechanisms acting on the level of the receptor molecule, regulation of the apoplastic space, or extracellular matrix, is important for receptor-ligand interactions. The cell wall is the outermost interface for signal perception and integration for all plant cells. It acts as a selective barrier to many biomolecules and it confers mechanical strength, while at the same time preventing migration of cells within or between tissues. Therefore, in developing tissues like the SAM, mechanisms are required that enable coordinated remodeling of the cell wall to maintain tissue and cell-integrity. Since differentiation of plant cells is usually accompanied by changes in cell wall composition and mechanical properties [28], it is not surprising that these aspects seem to be intimately linked with cell fate decisions, as demonstrated by the ability of cell wall remodeling enzymes to induce organ initiation in the periphery of the SAM [29]. Strikingly, even the expression domain of the stem cell factor SHOOT MERISTEMLESS (STM) is controlled by mechanical forces present at the boundary of the dome shaped SAM [30,31]. STM is an important regulator of meristematic cell identity by activating expression of enzymes for CK biosynthesis in the center of the SAM [32] promoting proliferation, while at the same time suppressing differentiation through repression of ASYM-METRIC LEAVES1 [33]. In addition, cell wall synthesis is highly controlled in the SAM [34] leading to differential stiffness of cells even within the meristem [35]. The coupling of cell fate progression and cell wall remodeling, implies a feedback regulatory mechanism between the molecular networks controlling stem cell fate and the signaling modules controlling cell wall integrity (CWI). With regard to CWI, the methyl-esterification status of the cell wall molecule pectin, which is the major component of primary walls, seems to be actively sensed by several plasma-membrane localized receptor complexes [36–38]. Galacturonic acid oligomers, a class of cell wall derived molecules and major constituent of pectin, inhibits the developmental transition from skotomorphogenesis to photomorphogenesis [39**] which is accompanied by SAM activation [40**]. While most studies on cell wall
signaling so far have not focused on the SAM, the fact that even within the meristem substantial differences in cell stiffness exist [35] and that the expression of one of the core transcriptional regulators is strongly influenced by mechanical forces [30], suggest that cell wall signaling and membrane tension sensing [41] will play an important role for meristem integrity (Figure 2).

**Inter-regional signaling: plant hormones**

Since stem cells and organ initiation sites in the SAM are spatially distinct, continuous interregional communication is required to coordinate developmental decisions with proper cell behavior. These processes are mainly driven by interacting hormone signaling pathways, most notably auxin and cytokinin. During embryogenesis, shoot identity and SAM domain architecture are laid down through the activity of the WUS homolog WUSCHEL-RELATED HOMEobox2 (WOX2), whereas WUS itself seems to be dispensable for the initiation of shoot stem cells [42**]. At early embryo stages, WOX2 promotes the expression of HD-ZIP III family genes to repress auxin signaling in the stem cell progenitor cells, which in turn prevents differentiation and promotes the formation of a central cytokinin signaling domain. This is in line with findings from *in vitro* shoot regeneration studies: Here, cytokinin signaling output via the pioneering type-B ARABIDOPSIS RESPONSE REGULATORS (ARRs) transcription factors is essential to de-differentiate the chromatin landscape. Type-B ARRs promote accessibility of the WUS locus, inter alia, by direct binding to upstream regulatory regions and thereby enable *de novo* SAM formation [[51**],3,43*,44–46]. During homeostatic growth, cytokinin inhibits differentiation throughout the SAM and promotes stem cell proliferation in the center, while auxin stimulates differentiation in the periphery. Consequently, stem cells in the center of the SAM seem to be devoid of auxin signaling as indicated by expression minima of the DRS synthetic auxin response element [47,48]. In contrast, cytokinin dependent gene activity is mostly confined to the organizing center, likely because of spatially restricted expression of CK receptors, the AHKs [49]. This effect is further enhanced by the repression of Type-A ARRs, negative feedback components in the cytokinin signaling system, by WUS [50]. In addition, signaling output is controlled by ligand availability at the SAM periphery, with the cytokinin importer PURINE PERMEASE 14 (PUP14) sequestering active cytokinin into the cell, away from the AHK receptors at the plasma membrane (Figure 2) [51**]. Emerging organ primordia furthermore express AHP6, a dominant negative component of the signal transduction system, thereby suppressing cytokinin response locally [52]. Importantly, auxin and cytokinin pathways intersect at multiple levels bringing about a complex signaling network. For example, type-B ARRs promote auxin biosynthesis by inducing *L-TRYPTOPHANE AMINOTRANSFERASE 1* transcription in Arabidopsis seedlings and YUCCA1, 4 and **PINFORMED7** expression in the central zone of the gynoecium, consequently enhancing auxin signaling [53,54]. Intriguingly, type-B ARRs were shown to negatively regulate *YUCCA* genes during shoot regeneration [44], whereas the AUXIN RESPONSE FACTOR3 represses many members of the LONELY GUY and ISOPEPTYL TRANSFERASE gene families during floral meristem determinacy and reduce the expression of the cytokinin receptor *AHK4*, A-type and D-type cyclin genes, thereby attenuating cytokinin signaling and proliferation [55]. This resembles the inhibitory effect found for the auxin response factor MONOPTEROS (MP) on *ARR15* and *ARR7* expression [56], however, since type-A ARRs, including ARR7 and ARR15, act as negative feedback regulators of cytokinin, the net effect here is to promote cytokinin signaling output. In addition, HECATE (HEC) bHLH transcription factors control the rate at which cells progress from the central zone towards the periphery of the SAM by enhancing cytokinin signaling in the center, while attenuating auxin signaling in the periphery, presumably by physical interaction with MP [57*]. The diverse cross-regulatory interactions between auxin and cytokinin are highly context dependent and can substantially vary between different tissues, cell types, developmental stages, or environmental conditions. Thus, it seems highly likely that the regulatory network is not limited to auxin and cytokinin, but will integrate signals from other hormone pathways as well. One important convergence mechanism may be provided by DELLA proteins. Initially described as regulators of gibberellic acid signaling, interactions with signaling components from several hormone signaling pathways, including cytokinin, have been reported and their importance for SAM regulation is undisputed [58*,59–61].

**Environmental signaling**

As organogenesis requires large amounts of energy and building blocks for cellular components, it seems obvious that stem cells need to adjust their proliferative activity in accordance with changing nutritional states. Again, plant hormones represent important convergence points for sensing and integrating whole organism physiology with developmental decisions. Cytokinin is a good example since its biosynthesis is strongly dependent on environmental signals: nitrate availability in roots or light exposure of leaves trigger cytokinin accumulation, which in turn acts as a quantitative systemic signal to the SAM where *WUS* expression and thus stem cell activity are adjusted accordingly [5,6*,40**,62,63]. Apart from the activity of plant hormones, it has emerged that the evolutionary conserved TARGET OF RAPAMYCIN (TOR) kinase complex plays an important role in integrating energy-signaling and environmental signaling in plants [40**,64]. TOR is well characterized as core regulator of translation, protein biosynthesis and proliferation across all kingdoms of life. Multiple studies provide evidence that TOR activity is required for stem cell
Diverse signaling pathways converge on the promoters of key meristem regulatory genes. The TOR kinase complex integrates metabolic, light and hormonal signals and is essential for activation of WUS expression after germination. Cytokinin (CK) signaling induces WUS RNA expression, which in turn is limited by the CLAVATA (CLV) receptor module. Cell wall integrity (CWI) signaling provides positional and mechanical information by so far mostly uncharacterized signal transduction pathways. In addition, plasma membrane localized transporters regulate the abundance of ligands in the apoplast. Dashed lines indicate hypothetical or complex interactions.

function in the SAM and is involved in the transcriptional regulation of WUS (Figure 2) [40**,65,66]. During germination, WUS expression is additively induced by energy and light-signaling and TOR activity is essential for both inputs [40**]. Mechanistically, light signaling likely induces auxin biosynthesis by inhibiting the E3 ligase CONSTITUTIVE PHOTOMORPHOGENIC 1 [67]. Auxin then activates the small Rho-like GTPase ROP2, which in turn activates TOR [65,68*]. During later developmental stages, Trehalose-6-phosphate (T6-P) has been proposed to be an important signal for plant sucrose status by inhibiting the TOR antagonist sucrose-non-fermenting-1 (SNF1) Related Kinase1 (SnRK1) and thus controlling the transition to flowering [69,70,71]. Several recent reports in addition suggest a role for TOR as an integrator of hormone signaling status. It has been shown that during stress induced abscisic acid (ABA) reception, for example under starvation conditions, PYR1-LIKE (PYL) receptors and the Phosphatase PP2C activate SnRK2 which then phosphorylates RAPTOR, a regulatory subunit of the TOR complex required for its kinase function. Phosphorylation causes dissociation of RAPTOR from TOR, thus inhibiting kinase activity. Under favorable conditions, TOR in contrast phosphorylates PYL receptors and prevents SnRK2 activity [72**]. In addition, TOR activity impacts on many plant hormone pathways, either by controlling the translation of core regulators of auxin signaling like for example, ARF3 [68*], or by orchestrating large transcriptional networks involved in hormonal regulation. Specifically, ABA, jasmonic acid and salicylic acid pathways are negatively regulated by TOR activity [73,74,75]. The example of ABA induced TOR complex dissociation provides a mechanistic model to quantitatively balance opposing signals: Stress or favorable conditions continuously shift the equilibrium between the TOR complex and the PYL-PP2C-SnRK2 complex. Given the emerging complexity in metabolic signaling, our current list of interaction partners and interacting pathways of the TOR complex is likely far from complete. Future research into the mechanistic details of how complex composition and activity output is modulated by different signaling pathways will be required to identify context specific functions of TOR and other pathway integrators, especially in the SAM.

**Conclusion**

While an increasing number of stem cell regulatory signals is reported in the literature, our understanding of how these inputs are integrated and translated into the appropriate cell behavior is still very limited. One emerging theme certainly is that signal integration is achieved
through modulation of complex multilayered networks that converge on a fairly small number of transcriptional master regulators, such as WUS. It is thought that these networks provide robustness while at the same time allowing specific context dependent variations in stem cell activity. To decode these networks and the associated downstream genetic programs, a thoughtful combination of environmental stimulation with high resolution readouts at the single cell level will be required. Thus, while forward genetic screens have identified the major regulators of plant stem cells almost 30 years ago, the advent of in vivo system genetics may finally open new avenues for understanding their developmental plasticity.

References


In this elegant study it is demonstrated how WUS expression correlates with nitrate availability from the soil due to long distance cytokinin signals from roots, priming the SAM for elevated leaf organogenesis.


In a hypothesis driven silico approach the authors predict that epidermis derived signals can be sufficient to maintain SAM architecture and to establish the underlying molecular pattern that scale with tissue size.


Groundbreaking study showing that BAM1, a receptor known for its function in CLV signaling, regulates spread of RNAi through plasmodesmata supporting the ‘dual use’ hypothesis.


Classical genetics and imaging are used to decipher the redundant and specific functions of the different CLV signaling modules CLV1, CLV2/ CRN and BAM1.


A study in maize demonstrates differential downstream effector signaling of the CLV receptor FASCIATED EAR2 in response to different CLE peptides, addressing a long standing question of how signaling specificity in CLE peptide signaling is achieved.


A very thorough biochemical characterization demonstrating how auxin acts as an activation signal for TOR via ROP2.


Using a challenging phosphoproteomics approach, the authors uncover reciprocal interactions between abscisic acid receptor complexes and the TOR complex, thus providing a model for quantitative integration of favorable and stress conditions.

