Review

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Cytokinin–auxin crosstalk in cell type specification

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Auxin and cytokinin affect cell fate specification transcriptionally and non-transcriptionally, and their roles have been characterised in several founder cell specification and activation contexts. Similarly to auxin, local cytokinin synthesis and response gradients are instructive, and the roles of ARABIDOPSIS RESPONSE REGULATOR 7/15 (ARR7/15) and the negative cytokinin response regulator ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6, as well as auxin signalling via MONOPTEROS/BODENLOS, are functionally conserved across different developmental processes. Auxin and cytokinin crosstalk is tissue- and context-specific, and may be synergistic in the shoot apical meristem (SAM) but antagonistic in the root. We review recent advances in understanding the interactions between auxin and cytokinin in pivotal developmental processes, and show that feedback complexity and the multistep nature of specification processes argue against a single morphogenetic signal.

Founder cell specification: concepts and considerations

Cell type specification (see Glossary) is the reversible acquisition of fate by a single or a group of founder cells that then are activated to undergo coordinated cell division to form a primordium for organs or tissues, which finally undergoes morphological differentiation [1]. Specification is distinguishable from activation, and both are genetically separable in roots, where not all specified founder cells develop into lateral roots (LRs) [2], and in floral organs, where primordium identity is labile and determination occurs developmentally considerably later than specification and activation [3]. Because activation is based on the criterion of cell division, it is histologically observable; however, establishing the precise timing of specification in many developmental contexts (i.e., the identity of founder cells) is hindered by the absence of visible morphological changes and by a lack of molecular markers for the specification process. Specification is possibly the result of many molecular steps, which involve incremental developmental competencies or priming, as in LR specification [4]. These steps are imprecisely elucidated for many specification contexts, as well as in the literature, and a consistently precise terminology or conceptual framework that differentiates between the timing of cellular specification and determination of cell fates is often lacking.

Various plant hormones directly or indirectly collaborate in cell fate decisions and cooperate differentially in many developmental contexts [5], especially by affecting cell proliferation and expansion within webs of responses [6–8]. Auxin is the best-characterised instructive signal, and live imaging has outlined how sites of lateral organ initiation correlate with auxin signalling, as determined via reporter gene expression from DR5, a synthetic auxin responsive promoter [2,9], and how targeted auxin accumulation occurs via polar transport [10,11] or local auxin synthesis [12,13]. The presence of auxin response maxima *per se* has often led to the logical fallacy that correlation proves causation and that auxin response maxima alone provide an instructive signal for organ initiation or specification. However, there are several developmental contexts in *Arabidopsis* where, instead of a maximum, the

Glossary: conceptual terminology in cell fate determination, network characteristics, and reporters

Cell fate processes

Activation: the initiation of regulated cell division of founder cells to give an organ initial that generates a primordium or tissue by further cell divisions.
Differentiation/determination: the irreversible acquisition of cell fate.
Founder cell(s): a cell(s) that gives rise to a tissue type or organ by clonal proliferation.
Organ/tissue anlage: an early-stage primordium (organ) or initials (tissue) resulting from activation.
Priming: the acquisition of competence by cells to respond to specification signals.
Specification: the reversible acquisition of a novel cell fate by a founder cell.
Stem cell: a pluripotent cell that is undifferentiated.

Network characteristics

Coregulation: outputs from two or more hormone pathways that independently affect the same process.
Crosstalk/cross-regulation: crosstalk is a generic term for signal integration from multiple hormone inputs within a response network that affects a common biological output. Cross-regulation is an alternative term that avoids the negative connotations of undesired signals by analogy to electrical circuitry.
Feedback regulation: where the output of a pathway regulates the input to increase or attenuate the output.
Feed-forward loop: one pathway component regulates another and both either negatively or positively modulate the output.
Morphogen: a molecule that affects cell differentiation along a concentration gradient.

Transcriptional reporters

DII-VENUS: synthetic auxin-responsive promoter that reports the degradation of Aux/IAA proteins.
DR5: synthetic auxin-responsive promoter that reports auxin response factor transcription following the degradation of Aux/IAA proteins.
Two component system (TCS): synthetic cytokinin-responsive promoter.

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presence of an auxin minimum is required for cell type specification, including valve margin separation [14], axillary meristem initiation in leaf axils [15,16], competence for LR specification [17], and also for polarity establishment in leaf primordia [18].

The fundamental antagonistic roles of auxin and cytokinin in differentiation was seminally demonstrated by the production of roots from callus by a high cytokinin:auxin ratio and shoot initiation by the converse ratio [19]. Since then the interdependency of auxin and cytokinin crosstalk underlying the complexity of biological transitions has become increasingly clear in shoot and root meristems and in founder cell specification contexts; however, crosstalk and coregulation have often not been distinguished in the literature. Conceptually, the association of spatiotemporal auxin response maxima alone as a single morphogenetic signal for cell type specification is perhaps simplistic given the established multi-component and multistep nature of specification processes.

Despite the contributions of other hormones, which are important in many different developmental contexts, in view of recent advances in understanding auxin and cytokinin roles in various cell fate contexts it is appropriate to focus on these two hormones that are differentially distributed and act via different signalling pathways, and to review how they converge on a single output of coordinated cell division. These auxin and cytokinin interactions are tissue- or context-specific, and act via transcriptional and non-transcriptional responses and across gradients of synthesis and response. This review summarises our current view on auxin and cytokinin in cell type specification by focusing on their established key roles in several cell fate decisions contexts in the root or the shoot apical meristem, and highlights commonalities and differences in their cross-regulation as a guide for further research.

Specification contexts

The three specification contexts that are the best-characterised in terms of their auxin and cytokinin interactions during *Arabidopsis* development all reside in the root.

Root-pole specification: the fate of a single cell

The specification of a single cell is exemplified by the hypophysis, the upper suspensor cell of the embryo, which is the root meristem founder cell that divides asymmetrically to generate precursors of two cell types that determine the root stem cell niche: the upper, lens-shaped cell generates the quiescent centre, and the lower cell, the columella stem cells. Hypophysis specification is dependent on auxin in three different ways: auxin response in the suspensor [20], auxin accumulation by basipetal polar transport from the proembryo [21], and the basipetal movement of the auxin-inducible basic helix-loop-helix (bHLH) transcription factor TARGET OF MONOPTEROS 7 (TMO7) from the adjacent pro-embryo cells into the hypophysis [22]. The upper and lower daughter cells after hypophysis division are re-specified to generate two distinct stem cell pools. Whereas the upper lens-shaped cell maintains cytokinin signalling and shows low auxin response, the lower cell shows high auxin response, which transcriptionally upregulates the A-type negative cytokinin *ARABIDOPSIS RESPONSE REGULATOR* 7 (*ARR7*) and *ARR15* that concertedly suppress cytokinin signalling [23] (Figure 1). Embryo lethality resulting from combined loss of *ARR7* and *ARR15* function [23] was shown to probably be due to genomic rearrangements in these insertion mutants [24]. However, conditional loss of *arr7* function via RNA interference in the *arr15* mutant background caused changes in the stereotypic cell division pattern, suggesting that ectopic cytokinin signalling is crucial for the establishment of the root stem cell niche. Furthermore, constitutive overexpression of either *ARR7* or *ARR15* resulted in a defective root pole during somatic embryogenesis [25], and repression of cytokinin signalling throughout the globular embryo, by converting the positive B-type cytokinin response regulator ARR10 into a dominant-negative regulator, resulted in cell division patterning defects [23], confirming that appropriate cytokinin signalling in the basal cell and upper cell is necessary for their re-specification. Thus, mutually exclusive and antagonistic roles of auxin and cytokinin are required at the transcriptional level in adjacent single cells to generate distinct stem cell populations at the root pole (Table 1).

LR initiation: the pairwise specification of founder cells

The second example concerns LR initiation from pairs of xylem-pole pericycle cells, which are primed by oscillating waves of auxin response maxima that move upwards from the basal root meristem with a periodicity of 6 h [26]. Cellular competence to respond to this oscillating auxin signal and the location of LR founder cell specification are distal to the root stem cell pool and are restricted to the transition zone, where the auxin concentration is at a minimum [17], and are also radially confined to the lateral periphery of both xylem poles. In *Arabidopsis*, the molecular events from LR founder cell specification to activation and anlagen are well characterised [27,28]. An auxin maximum in a single or pair of pericycle cells is sufficient for LR initiation [2], which can be induced by local auxin synthesis or by polar auxin transport via PINFORMED3 (PIN3) from the endodermis [29]. The priming of pre-branch sites or the specification of founder cells is followed by nuclear polarisation of two adjacent pericycle cells, which divide anticlinally and asymmetrically to give rise to two large cells and two inner small daughter cells, to form a stage I LR primordium, before periclinal activation generates the two cell layer stage II primordium. The activity of discrete auxin response factor (ARF) and Aux/IAA (auxin/indole-3-acetic acid-responsive) pairs accompanies founder cell specification, nuclear polarisation, and asymmetric cell division [30]. Founder cell specification is dependent on transcription of the GATA23 transcription factor by INDOLE-3-ACETIC ACID INDUCIBLE 28 (IAA28) and ARF7/19 [31], whereas nuclear polarisation and asymmetric cell division include bimodal auxin signalling pathways: comprising BODENLOS (BDL)/IAA12 and MONOPTEROS (MP/ARF5), or SOLITARY ROOT/IAA 14 and ARF7/19 [32]. Auxin promotes cell cycle progression at the G1–S checkpoint via *LATERAL ORGAN BOUNDARY* gene induction by ARF7 and 19 [33,34], which in turn activates D-type cyclins. In addition, CYCLIN-DEPENDENT KINASE A and D-TYPE CYCLIN 2;1 (CYCD2;1)