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Current Opinion in Plant Biology

Cytokinin action in plant development

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Cytokinin regulates many important aspects of plant development in aerial and subterranean organs. The hormone is part of an intrinsic genetic network controlling organ development and growth in these two distinct environments that plants have to cope with. Cytokinin also mediates the responses to variable extrinsic factors, such as light conditions in the shoot and availability of nutrients and water in the root, and has a role in the response to biotic and abiotic stress. Together, these activities contribute to the fine-tuning of quantitative growth regulation in plants. We review recent progress in understanding the cytokinin system and its links to the regulatory pathways that respond to internal and external signals.

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Current Opinion in Plant Biology 2009, **12**:527–538

This review comes from a themed issue on
Cell signalling and gene regulation
Edited by Jan U. Lohmann and Jennifer L. Nemhauser

Available online 7th September 2009

1369-5266/$ – see front matter

DOI 10.1016/j.pbi.2009.07.002

Introduction

During recent years, important gaps in the understanding of the metabolism and signalling of the hormone cytokinin have been filled, so that we now have a comprehensive knowledge of the genes involved in the central aspects of metabolism and signalling. Firstly, the key findings of recent years will be reported. For a more detailed insight, the reader may refer to several detailed reviews [1–7]. The focus of this article then continues by describing the functions of the cytokinin system in regulating growth in the context of internal and external cues. The different actions of cytokinins in the shoot and root act as a structural guideline.

The cytokinin system

An overview on cytokinin metabolism and the core signalling pathway is shown in Figure 1 and recent findings are summarized in the next sections.

Cytokinin metabolism

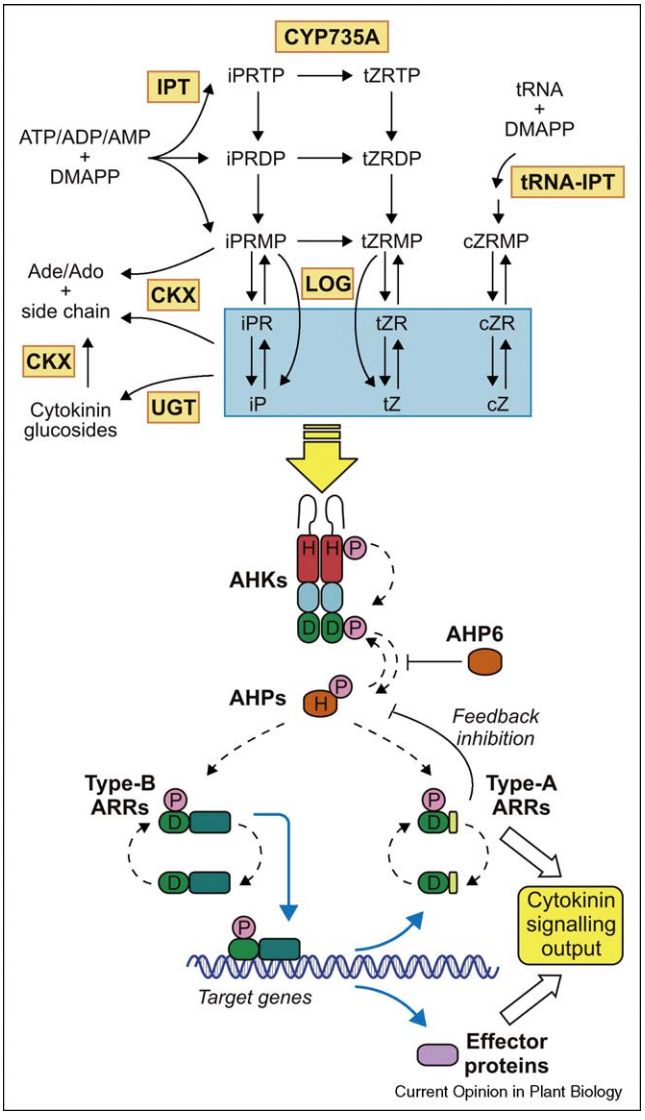
The rate-limiting step in cytokinin biosynthesis is catalyzed by isopentenyltransferases (IPTs), which exist as adenosine phosphate-IPTs and tRNA-IPTs, depending on their substrates (Figure 1). Work by Miyawaki *et al.* [8] has shown that *cis*-zeatin (cZ) formation in *Arabidopsis* depends entirely on the activity of two tRNA-IPTs, as plants mutated in the corresponding genes lacked cZ-type cytokinins. This result finally clarified the biosynthetic route for cZ and showed that ATP/ADP-IPTs are entirely responsible for the synthesis of iP and *trans*-zeatin (tZ) cytokinins. Interestingly, lack of cZ caused no gross morphological changes, raising the question of the functional relevance of this type of cytokinin in *Arabidopsis* [8]. A novel way to modulate cytokinin biosynthesis was indicated by the finding that one member of the IPT enzyme family, IPT3, can be modified by farnesylation. Farnesylation directed IPT3 to the nucleus/cytosol, whereas the nonfarnesylated protein was located in the plastids. The farnesyl acceptor side was also shown to be essential for catalytic activity [9]. A crystal structure of the agrobacterial IPT enzyme showed a structural relation to nucleoside triphosphate hydrolases [10]. An important finding has been that the release of active cytokinins from their nucleotide precursor forms may be catalyzed in a single step by a cytokinin nucleoside 5′-monophosphate phosphoribohydrolase, called LONELY GUY (LOG) [11••] (Figure 1, see also below).

A systematic evaluation of all cytokinin-degrading CKX enzymes (Figure 1) of *Arabidopsis* has revealed their distinct biochemical characteristics. Importantly, CKX2, CKX4 and CKX6 showed the highest activity with the free bases iP and tZ, whilst others unexpectedly preferred glucosides or nucleotides as a substrate [12•]. Whether these differences are reflected by specific differences in the three-dimensional structure of CKX enzymes has not yet been resolved [13]. Systematic testing of the activities of novel purine derivatives led to the discovery of potent inhibitors of CKX enzymes, which may become important tools in cytokinin research [14•].

Cytokinin signalling

Cytokinin signals through a complex two-component system (TCS) (Figure 1). This signalling pathway is, amongst the multicellular eukaryotes, unique to higher plants. A phylogenetic analysis revealed that the cytokinin receptors and A-type ARRs first appeared in land plants, indicating that the cytokinin signalling pathway and its negative feedback regulation were established simultaneously. In contrast, B-type ARRs (Figure 1) evolved before the conquest of land, suggesting that they

Figure 1



Schematic model of cytokinin metabolism and core steps of the cytokinin signalling pathway. Biosynthesis of iP-cytokinins and tZ-cytokinins is initiated by adenosine phosphate-isopentenyltransferases (IPTs) to form iP-nucleotides which can be converted to the corresponding tZ-nucleotides by cytochrome P450 monooxygenases (CYP735As). iPRTP, iPRDP and the corresponding tZ-nucleotides are dephosphorylated by phosphatases, and iPRMP and tZRMP can be directly converted to active free bases by cytokinin nucleoside 5′-monophosphate phosphoribohydrolases (LOGs). *cis*-Zeatin (cZ) cytokinins, which in some plant species are the major cytokinin metabolites, are synthesized in *Arabidopsis* exclusively by tRNA-IPTs which utilize tRNAs as prenyl acceptors. Biologically active cytokinins, highlighted in blue, are inactivated by cytokinin oxidases/dehydrogenases (CKXs) and by conjugation to sugar moieties through glycosyltransferases (UGTs). A histidine (H)/aspartate (D)-phosphorelay (indicated by dotted arrowed lines) through a two-component signalling cascade is initiated by the binding of biologically active cytokinin to a CHASE domain of histidine kinase receptors (AHKs) and autophosphorylation of a His-residue in the protein kinase domain (red). The phosphoryl group is transferred via the Asp-residue of the receptor receiver domain (green) to a conserved His of the histidine phosphotransfer proteins (AHPs). Non-activated CRE1/AHK4 possesses phosphatase activity that dephosphorylates AHPs. AHP6, lacking the conserved His-residue, inhibits the phosphoryl transfer, presumably by interacting with activated receptors and/or response regulators. AHP proteins relay the signal to B-type or A-type response regulators (ARRs). B-type ARRs, which contain a C-terminal DNA-binding domain (turquoise), are transcription factors regulating expression of their target genes including A-type *ARR*s. One function of the A-type ARRs is to repress signalling in a negative feedback loop. Together with other effector proteins, they determine the signalling output of the pathway. For detailed information about cytokinin metabolism and signalling refer to [1,3,4,6].

had functions independent of cytokinin which might still be preserved [15•]. Whole-genome analysis showed that the *Arabidopsis* and rice TCS are similar in architecture, with rice having more TCS proteins [16–18].

The predicted extracellular CHASE domain of the cytokinin receptors was shown to be sufficient for cytokinin binding [19]. A direct binding assay revealed different biochemical properties of cytokinin receptors and confirmed differences in the ligand preference of CRE1/AHK4 and AHK3; the latter having a significantly lower affinity for iP-type cytokinins [20]. It was proposed that auxin-mediated shifts in pH may modulate cytokinin receptor activity, and thus provide a mechanism to mediate cytokinin–auxin interactions [20]. An evolutionary proteomics approach identified conserved amino acids essential for ligand binding [19], and Miwa *et al.* [21] identified several mutations that cause a constitutive activation of CRE1/AHK4 in *E. coli*. Elucidation of the three-dimensional structure of the receptors will provide further insight into structure–function relations and the role of these specific amino acids. CRE1/AHK4 was shown to possess phosphatase activity in the absence of cytokinin, which provides a flexible mechanism to rapidly downregulate the pathway [22].

Previously described compounds with anti-cytokinin activity were shown not to act at the receptor level, but to inhibit downstream responses to cytokinin via the inhibition of cyclin-dependent kinases [23]. Recently, the first cytokinin antagonist acting at the receptor level was discovered [24•]. The compound, called PI-55, is structurally closely related to aromatic cytokinins, but carries substitutions at specific positions of the side chain that reduce cytokinin activity and confer antagonistic properties. Further developments should provide the long-missed tools of chemical interference with cytokinin activity *in planta*.

Multiple knockouts of genes encoding AHP proteins (Figure 1) displayed reduced cytokinin sensitivity, confirming their positive regulatory role in cytokinin signalling [25]. The multiple interactions of TCS proteins shown in a systematic analysis were in accordance with redundancy in the signalling pathway, and underpinned the function of AHPs as hubs that are able to interact with