



# Cytokinin signal perception and transduction

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In the past few years, enormous progress has been made in understanding cytokinin perception and signalling. Three cytokinin receptor proteins, which are hybrid histidine kinases, have been identified in *Arabidopsis*. These receptors may transduce signals in a quantitative rheostat-like fashion, thus permitting long-lasting and continuously variable signalling that is directly dependent on the hormone concentration. Evidence has been provided that downstream signalling is transmitted through a His-to-Asp phospho-relay involving phosphotransmitter and response regulator proteins, typical of two-component systems. On the basis of mutant analysis, protein-protein interaction studies and target gene identification, a cellular network is emerging that links cytokinin activity to both developmental and physiological processes.

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## Abbreviations

<b>AHK</b>	<i>Arabidopsis</i> histidine kinase
<b>AHP</b>	<i>Arabidopsis</i> histidine phosphotransfer protein
<b>ARR</b>	<i>Arabidopsis</i> response regulator
<b>CHASE</b>	cyclases/histidine-kinase-associated sensory extracellular
<b>CK</b>	cytokinin
<b>CKI</b>	CYTOKININ INDEPENDENT
<b>CRE1</b>	CYTOKININ RESPONSE 1
<b>Hpt</b>	histidine phosphotransfer protein
<b>nCI</b>	mitochondrial respiratory chain complex I
<b>TCS</b>	two-component system

## Introduction

Cytokinins play a major role in many different developmental and physiological processes in plants, such as cell division, regulation of root and shoot growth and branching, chloroplast development, leaf senescence, stress response and pathogen resistance [1]. Additionally, it has been proposed that this class of plant hormones has a function in nutrient starvation and recovery response [2,3]. Until recently, virtually nothing was known about cytokinin signalling except that there were several poorly understood cytokinin binding proteins and a couple of examples of cytokinin-induced changes in protein or

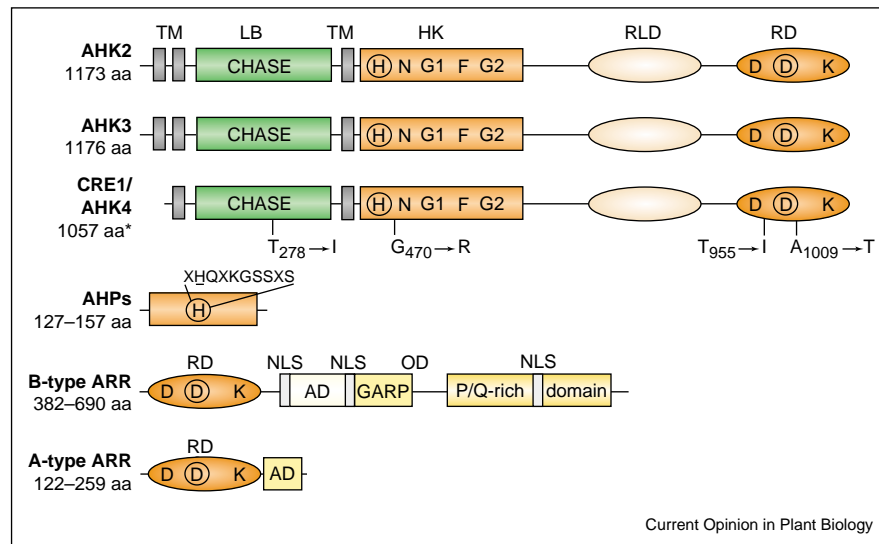
transcript abundance. This situation started to change when activation tagging experiments identified *CKI1*, a receptor histidine kinase gene of *Arabidopsis* whose over-expression induces typical cytokinin responses [4]. These initial experiments, and the identification of response regulator genes as the first primary response genes of cytokinins [5,6], indicated that histidine kinases and their downstream partners may play a role in cytokinin signalling. Finally, in 2001, *CRE1/AHK4*, another histidine kinase of *Arabidopsis*, was the first cytokinin receptor to be identified [7<sup>\*\*</sup>,8<sup>\*\*</sup>,9<sup>\*</sup>]. Identification of other cytokinin receptors (*AHK2* and *AHK3*) as well as downstream transmitters (*Arabidopsis* histidine phosphotransfer proteins [AHPs] and *Arabidopsis* response regulators [ARRs]; see Figure 1) was greatly accelerated by the availability of the *Arabidopsis* genomic sequence [10]. Various experiments demonstrated that the cytokinin signal is perceived and transmitted by a multi-step phosphorelay system through a complex form of the two-component signalling (TCS) pathway that has long been known in prokaryotes and lower eukaryotes [11<sup>\*\*</sup>]. Among higher eukaryotes, the TCS is unique to plants. In this signalling system, outlined in Figure 2, a membrane-located receptor kinase with an extracellular ligand-recognition domain (sensor) dimerises upon binding a ligand and autophosphorylates a histidine within its cytoplasmic transmitter domain. The phosphoryl group is first transferred to an aspartate residue within the receiver domain at the C terminus of the receptor and then from there to a histidine phosphotransfer protein (Hpt), which ultimately phosphorylates and thus activates a response regulator at a central Asp residue [12–14].

In this review we will first report the recent discoveries about cytokinin signal perception and transduction, then give a brief description of the components of the transduction pathway, and finally discuss the developmental and physiological relevance of these findings. The focus will be on *Arabidopsis* as most of the available data are from this model plant.

## Cytokinin perception

Map-based cloning of an *Arabidopsis* gene that, when mutated, conferred cytokinin resistance, and systematic functional testing of histidine kinase genes identified *in silico* were the two experimental strategies that led independently to the identification of the hybrid histidine kinase *CRE1/AHK4* as a cytokinin receptor [7<sup>\*\*</sup>,8<sup>\*\*</sup>,9<sup>\*</sup>]. Functional evidence for cytokinin sensing by the receptor was obtained in elegant complementation experiments in yeast and *Escherichia coli*, which rendered these heterologous hosts cytokinin-sensitive. *CRE1/AHK4*-expressing

Figure 1



Structures of cytokinin receptors and other proteins of the cytokinin signalling pathway. Amino acids that participate in the phosphorelay are circled. Other characteristic consensus motifs are also indicated. Mutations that lead to loss of function in CRE1/AHK4 are shown below the CRE1/AHK4 structure [17\*\*,20\*\*]. Abbreviations: aa, amino acids; AD, acidic domain; CHASE, cyclases/histidine kinases associated sensory extracellular; GARP, DNA-binding motif; HK, histidine kinase; LB, putative ligand binding domain; NLS, nuclear localisation signal; OD, output domain; RD, receiver domain; RLD, receiver-like domain; TM, transmembrane domain. Domains are according to [12,27,34\*\*,38]. \*A longer open reading frame of CRE1 coding for additional 23 amino acids at the N-terminal end was also identified [7\*\*].

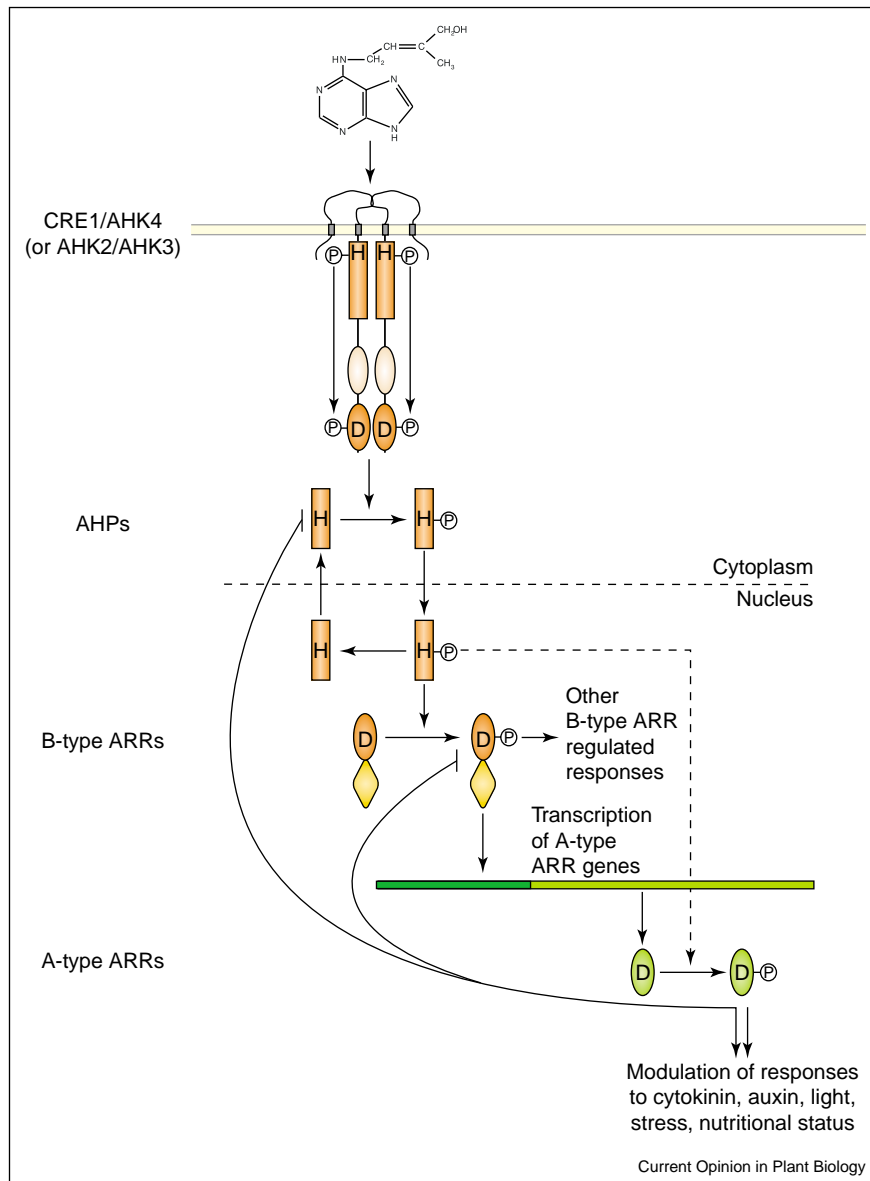
*E. coli* responded to cytokinins of the isopentenyl and zeatin types, as well as to a diphenylurea derivative with cytokinin activity, thidiazuron, at concentrations as low as  $5 \times 10^{-8}$  M [8\*\*,15\*]. Biochemical analyses revealed a dissociation constant ( $K_d$ ) for isopentenyl adenine of  $4.5 \times 10^{-9}$  M, indicating a high affinity of AHK4 for the biologically active free base of the hormone [15\*]. Two other histidine kinases, AHK2 and AHK3, have been shown to be active in the same complementation test system and to render protoplasts cytokinin-sensitive [11\*\*,15\*]. Expression of the above-mentioned earlier receptor candidate CKI1 in *Arabidopsis* protoplasts or heterologous hosts led to constitutive phenotypes that were independent of cytokinin dose [11\*\*,15\*]. In *planta*, CKI1 is essential for megagametogenesis as loss-of-function mutants display a female gametophytic lethal phenotype [16\*]. Together, it is not entirely clear whether CKI1 functions as a cytokinin receptor or whether its overexpression triggers cytokinin-dependent activities that are not normally under the control of CKI1.

The three cytokinin receptor genes differ in their expression pattern. CRE1/AHK4 is mainly expressed in the roots, whereas AHK2 and AHK3 are present in all major organs [7\*\*,17\*\*,18]. It is interesting to note that in a global expression analysis during *in vitro* shoot development from root calli the expression of CRE1/AHK4 was transiently upregulated at a time that corresponded to the acquisition of competence for shoot formation, whereas the expression level of AHK3 did not change [19]. It was

also shown that cytokinin enhances CRE1/AHK4 expression, indicating the existence of a positive-feedback loop in cytokinin signalling [20\*\*].

The primary structure of all three cytokinin receptors displays two to three transmembrane domains at the N-terminal part, followed by a transmitter (histidine kinase) domain and two receiver domains (Figure 1). The predicted extracellular ligand-binding regions, which are ~270 amino acids long, have drawn special attention, as they are the putative recognition sites for cytokinins. In CRE1/AHK4 mutation of Thr278 to Ile in this domain leads to a loss of function [17\*\*]. Sequence comparison has shown the presence of this domain in other receptor-like proteins in both prokaryotes and lower eukaryotes [21,22]. Although conservation at the amino acid level is relatively low, the secondary structure is highly homologous, with a characteristic pattern of  $\alpha$ -helices and  $\beta$ -sheets. Because of its presence in a variety of functionally diverse membrane receptor proteins that recognise cytokinin-like adenine derivatives or peptide ligands and that have intracellular histidine kinase or nucleotide cyclase domains, the domain has been named the CHASE domain (cyclases/histidine-kinase-associated sensory extracellular). As the CHASE domain is absent from all sequenced archaeal genomes, it has been suggested that this domain might have been acquired through a lateral transfer from bacteria to plants via the chloroplast, which is of cyanobacterial origin [21,22]. In *Arabidopsis*, the CHASE domain is specific for AHK2, AHK3 and CRE1/AHK4.

Figure 2



A model for cytokinin signal transduction via a His-to-Asp phosphorelay. The structure of CRE1/AHK4 is shown as an example. Ligand binding induces receptor dimerisation and autophosphorylation. Transfer of the phosphoryl group by activated receptors activates AHPs which transport the signal from the cytoplasm to type-B ARRs in the nucleus. Type-B response regulators transcribe target genes, among them type-A *ARR* genes. Type-A response regulators may downregulate the primary cytokinin signal response via a negative feedback loop, modulate downstream activities of cytokinins in a positive or negative fashion or modulate other signalling pathways through protein-protein interaction. A more complex regulation than shown in the model may exist. Abbreviations: D, aspartate residue, H, histidine residue, P, phosphoryl group.

The conserved His residue in the cytoplasmic transmitter domain has been shown to be functionally essential [7<sup>\*\*</sup>]. Of the two receiver domains the first is referred to as receiver-like and is not believed to be functional, as the phospho-accepting aspartate residue is only present in AHK2 [12,15<sup>\*</sup>,18]. The C-terminal receiver domain of AHKs is thought to transmit the signal to the histidine phospho-transmitter proteins (AHPs). Indeed, deletion of the entire receiver domain or mutation of the conserved

Asp973 destroys the ability of CRE1/AHK4 to complement heterologous signalling mutants [7<sup>\*\*</sup>,8<sup>\*\*</sup>,11<sup>\*\*</sup>]. Furthermore, co-expressing AHK4 together with AHP2 or AHP5 in the mutant host resulted in a failure to complement, probably due to signal titration [8<sup>\*\*</sup>].

The mechanism by which signalling of the cytokinin receptors is reset is not known. Resetting is usually achieved through the intrinsic instability of the phosphoryl

group and/or via regulatory phosphatase activity of the receptor itself or of additional proteins [23]. For other histidine kinase receptors it has been shown that the external signal can control the ratio of kinase activity to phosphatase activity of the receptor, permitting them to function as an on-off switch or as a rheostat, depending on the concentration differences of the ligand [23]. Cytokinin receptors that function according to the rheostat model could make the signal strength directly dependent upon the cytokinin concentration over a broad concentration range. Furthermore, the model could explain continuous signalling over a longer period without gross changes in ligand concentration. This might be important for the mediation and maintenance of slow and complex cytokinin responses such as shoot formation. Whether cytokinin receptors function as rheostats remains to be shown.

### Cytokinin signalling

Current knowledge suggests that the downstream signalling components of the cytokinin signal-transduction pathway consist in *Arabidopsis* of five histidine phospho-transmitters (AHPs; [24]) and 22 response regulators (ARRs; [12,25–28]). It should be noted that only few of these elements have been functionally characterised and that clear evidence of a link to cytokinin signalling is missing in most cases.

### AHPs

The five *AHP* genes code for proteins of ~12 kDa, all of which contain the highly conserved XHQXKGSXS motif responsible for the His-Asp phospho-transfer ([12]; Figure 1). AHPs transmit the signal from the receptor, which is presumably localised in the plasma membrane, to ARRs, which are mostly found in the nucleus [11<sup>••</sup>,29]. Importantly, it has been shown that upon induction by cytokinin some AHPs (AHP1 and AHP2) localise specifically and transiently to the nucleus, indicating that members of this class of protein function as cytoplasmic-nuclear shuttles [11<sup>••</sup>]. Additional evidence for the function of AHPs in the phosphorelay comes from several experiments. It has been demonstrated that AHP1, AHP2 and AHP3 functionally complement a yeast Hpt mutant, and interactions between different AHPs and AHKs or ARRs have been detected using yeast, *E. coli* and *in vitro* assays ([11<sup>••</sup>,24,25,30–32,33<sup>•</sup>]; see Table 1).

### ARRs

The final output element in the TCS in plants is the response regulator, whose activity is altered by its phosphorylation state. Analysis of the *Arabidopsis* genome sequence reveals the existence of 22 predicted response regulator (*ARR*) genes, characterised by the presence of the hallmark residues DDK, necessary for phospho-accepting activity ([28]; Figure 1). The ARRs are divided into two major classes, the A- and the B-type, on the basis of their structure. The 11 type-A ARRs consist mainly of

the receiver domain with a short extension at the N- and C-terminal ends. By contrast, the 11 type-B ARRs contain a C-terminal output domain in addition to the receiver domain. The genes coding for the two types of ARR respond differently to cytokinin. A-type *ARR* genes are rapidly induced in the presence of cytokinin and fulfil the criteria of a primary response gene [34<sup>••</sup>]. The expression level of B-type *ARR* genes is not influenced by cytokinin [25,35,36]. Type-B ARRs act upstream of type-A ARRs and are discussed first.

### B-type ARRs

The C-terminal output domain of type-B ARRs contains the ~60-amino-acid GARP motif (so-called after the founding members Golden2, ARR and Psr1; it is also termed B motif or ARRM) (Figure 1), which is distantly related to the Myb repeat of transcription factors [33<sup>•</sup>,37,38]. The predicted secondary structure of this domain, as determined by NMR spectroscopy, contains three  $\alpha$ -helices and recognises the major groove of DNA with residues located in a helix-turn-helix variant motif in essentially the same way as homeobox proteins. Structural analysis suggested that type-B ARRs might bind DNA as a monomer [39<sup>•</sup>]. The DNA motif optimal for binding is 5'-(A/G)GAT(T/C)-3' with the GAT motif in the middle being of special importance [33<sup>•</sup>,38,39<sup>•</sup>]. 5'-AGATT-3' was found to be optimal for ARR1, ARR2 and ARR10 [38,39<sup>•</sup>], whereas ARR11 bound preferentially to 5'-GGATT-3' [40]. Because these recognition motifs occur frequently, it was suggested that additional factors are needed to increase the specificity of DNA recognition *in planta* [38]. In addition to the GARP domain, and consistent with a function as transcription factors, the C-terminal parts contain nuclear localisation sequences and P/Q-rich, acidic domains. The latter domain has been demonstrated to be sufficient for transactivation in the case of ARR1, ARR2 and ARR11 [36,38,40]. As predicted by the presence of the nuclear localisation sequences, B-type ARRs are found in the nucleus [11<sup>••</sup>,12,33<sup>•</sup>,38].

Two reports showed that ARR1, ARR2 and ARR10 are able to activate the transcription of several A-type ARRs directly, independently of exogenous cytokinin, although activation is further enhanced by additional cytokinin [11<sup>••</sup>,41<sup>••</sup>]. Furthermore, overexpression of ARR1, ARR2 or the C-terminal parts of ARR1 or ARR11 (the regulatory N-terminal part was removed) mimics some cytokinin effects in transgenic plants (disordered cell division and ectopic shoot formation) and renders plants more sensitive to cytokinin. Collectively, these data suggest that B-type ARRs could play a central, rate-limiting role in cytokinin signalling [11<sup>••</sup>,40,41<sup>••</sup>]. Evidence further indicated that the N-terminal receiver has a suppressor function in the absence of cytokinin [11<sup>••</sup>,41<sup>••</sup>]. Interestingly, overexpression of the C-terminal part of ARR1 (ARR1-C) upregulated several type-A *ARR* genes whereas ARR11-C overexpression did not. However, the developmental

Table 1

## Features of cytokinin receptors and components of the signalling pathway.

Gene name	MIPS designation	Features
<b><i>Arabidopsis</i> cytokinin receptors</b>		
<i>AHK2</i>	At5g35750	Increases CK dependent <i>ARR6</i> promoter activity [11**]; expression in roots, leaves, stem, flowers [58].
<i>AHK3</i>	At1g27320	Increases CK dependent <i>ARR6</i> promoter activity [11**]; expression in roots, leaves, stem, flowers [18].
<i>CRE1/AHK4</i>	At2g01830	Loss of function mutants are CK resistant [7**,20**]; increases CK dependent <i>ARR6</i> promoter activity [11**]; localised in the plasma membrane in yeast [8**,15*]; interacts with AHP2/AHP5>AHP3 >AHP18; downregulated by Pi starvation, upregulated by CK [20**]; upregulated during shoot induction [19]; expressed predominantly in root vascular tissue [7**,17**,18].
<b><i>Arabidopsis</i> histidine phosphotransfer protein gene family (AHP)</b>		
<i>AHP1</i>	AT3g21510	Interacts with ARR1 [24], ARR2 [33*], ARR4, ARR9 [58] and ARR10 [25]; translocates from cytoplasm to nucleus upon CK treatment [11**], expression in roots [30] and seedlings [31].
<i>AHP2</i>	AT3g29350	Interacts with ARR1, TCP10 [24], ARR2 [33*], ARR10 [25], CKI1 and AHK1 [32]; translocates from cytoplasm to nucleus upon CK treatment [11**]; expression in roots, leaves, stems, siliques [30] seedlings [31].
<i>AHP3</i>	AT5g39340	Interacts with ARR1, ARR10 [25], CKI1, ARR9 [32], TCP10 [24]; expression in roots, leaves, stems, siliques [30] and seedlings [31].
<i>AHP4</i>	AT3g16360	Weak expression in roots and leaves [29].
<i>AHP5</i>	At1g03430	No translocation from cytoplasm to nucleus upon CK treatment [11**]; expression in roots and leaves [29].
<b><i>Arabidopsis</i> type B response regulator genes (type B ARR)</b>		
<i>ARR1</i>	At3g16857	Activates transcription of CK response genes [11**,38]; interacts with AHP2 [24]; nuclear localisation [38]; ESTs from leaves [1]; overexpression causes aberrant cell proliferation [41**].
<i>ARR2</i>	At4g16110	Activates transcription of CK response genes; overexpression promotes cell proliferation and shoot growth [11**,33*,38]; interacts with AHP1 and AHP2 [24,33*]; nuclear localisation [11**,38]; expression in pollen [33*].
<i>ARR10</i>	At4g31920	Activates transcription of <i>ARR6</i> [11**]; nuclear localisation [39*]; interacts with AHP1, AHP2, AHP3 [25]; expression in root, leaves, flowers and siliques [25].
<i>ARR11</i>	At1g67710	Binds DNA specifically [40] and activates transcription [36]; interacts with AHP2 [40]; expressed in roots; overexpression causes aberrant cell proliferation [40].
<i>ARR12</i>	At2g25180	ESTs from developing seeds and roots
<i>ARR13</i>	At2g27070	No EST
<i>ARR14</i>	At2g01760	ESTs from leaves
<i>ARR18</i>	At5g58080	EST from developing seeds
<i>ARR19</i>	At1g49190	No EST
<i>ARR20</i>	At3g62670	No EST
<i>ARR21</i>	At5g07210	No EST
<b><i>Arabidopsis</i> type A response regulator genes (type A ARR)</b>		
<i>ARR3</i>	At1g59940	ESTs from leaves
<i>ARR4</i>	At1g10470	Represses CK induced transcription of <i>ARR6</i> [11**]; renders tissues more CK sensitive [48*]; interacts with PhyB [44**], AHP1 [32], AtDBP1 and AtDBP2 [52]; expressed in leaves, stem, flowers, roots and induced by osmotic stress and CK [5,34**,44**,45*,46].
<i>ARR5</i>	At3g48100	Represses CK induced transcription of <i>ARR6</i> [11**]; expressed in shoot and root meristems; induced in leaves, flowers, stem, roots, siliques by CK, osmotic stress and during shoot induction [5,19,34**,45*,46].
<i>ARR6</i>	At5g62920	Represses CK induced transcription of <i>ARR6</i> [11**]; nuclear localisation [11**]; expression induced by CK [34**,11**,45*].
<i>ARR7</i>	At1g19050	Represses CK induced transcription of <i>ARR6</i> [11**]; nuclear localisation [43]; expression induced by CK [34**,45*].
<i>ARR8</i>	At2g41310	Renders transgenic overexpressors CK insensitive [48*]; expression in roots induced by CK and osmotic stress [35,46].
<i>ARR9</i>	At3g57040	Interacts with AHP1 and AHP3 [32]; expression in flower, seeds, leaves, roots [46], induced by CK [35].
<i>ARR15</i>	At1g74890	Nuclear localisation [45*]; expression in stele of root tips [45*] and induced by CK [34**].
<i>ARR16</i>	At2g40670	Cytoplasmic localisation [45*]; expression in endodermis of root tips [45*]; induced by CK [34**].
<i>ARR17</i>	At3g56380	No EST
<i>ARR22</i>	At3g04280	EST from etiolated seedlings

EST, expressed sequence tag.

consequences of overexpression were similar in both cases, suggesting at least partially different signalling pathways for these two type-B ARR proteins [40,41\*\*]. An *arr1* loss-of-function mutant had longer roots than the wild type and was partially resistant to cytokinin, characteristics shared by cytokinin-deficient plants [41\*\*,42]. However, *arr1* mutant plants did not show other gross

phenotypic alterations, suggesting redundancy among type-B ARR proteins [41\*\*].

#### A-type ARRs

In addition to the receiver domain, six of the eleven A-type ARR proteins (ARR3, ARR4, ARR7, ARR8, ARR9, ARR15) contain a short acidic C-terminal extension

[34\*\*] (Figure 1). The extension was shown to be responsible for nuclear localisation in the case of *ARR7* [43]. The subcellular localisation of different A-type ARR is cytosolic, nuclear, or both, depending on the gene and on conditions [11\*\*,43,44\*\*,45\*].

The expression of A-type ARRs was highest in roots, but transcripts were detected in all adult organs of plants with variations between the different genes [34\*\*,46]. Transcript abundance of most if not all A-type ARRs increases rapidly (within 10 min) in response to cytokinin [34\*\*]. A strong response was obtained for *ARR4*, *ARR5*, *ARR6*, and *ARR7*. *ARR8*, *ARR9*, *ARR15* and *ARR16* showed a weak and somewhat delayed response. Transcripts for *ARR3* and *ARR17* were not identified on RNA blots [34\*\*,35,45\*]. Nuclear run-on transcription analysis showed that the increase of transcript abundance is at least partially regulated on the transcriptional level [34\*\*]. Plants carrying an *ARR5::GUS* fusion gene showed expression mainly in the shoot and root apical meristems, which are sites where cytokinins exert regulatory functions [34\*\*,42]. Upon induction by cytokinin, GUS enzyme activity accumulated in most cells of transgenic seedlings, indicating that almost all cells are competent to respond to cytokinins [34\*\*,47]. By contrast, the cytokinin-responsiveness of *ARR15::GUS* and *ARR16::GUS* transgenes was limited in seedling roots to the stele containing the vasculature and the endodermis close to the root tip, respectively [45\*]. This indicates that the cytokinin responsiveness of *ARR5*, *ARR15* and *ARR16* in the root is mediated by at least partially different components of the cytokinin signalling pathway. The simplest explanation is that each gene requires a specific combination of signalling elements for activation that is present only in the responsive cells. In the same vein, transcript accumulation of *ARR15* and *ARR16* in response to cytokinin was absent in the roots of *cre1* mutants, whereas the accumulation of all other type-A ARR gene transcripts was not inhibited [45\*]. This indicates redundant cytokinin receptor functions in the root but also the existence of specific pathways.

The expression characteristic of type-A ARR genes suggests that they are likely to be mediators of cytokinin responses within the cell, but little is known about their molecular functions. Transient overexpression of *ARR4*, *ARR5*, *ARR6* and *ARR7* in protoplasts resulted in the repression of a *ARR6::LUC* reporter gene, indicating negative-feedback control of the cytokinin signalling pathway [11\*\*]. This conclusion is supported by diminished cytokinin responses in stable transgenic *ARR8* overexpressers. However, in contrast to the general idea of a negative feedback function of type-A ARRs, stable overexpression of *ARR4* in transgenic plants enhanced cytokinin responsiveness [48\*]. Together, these data indicate that type-A ARRs can be positive or negative regulators, depending on the gene and the output reaction analysed [11\*\*,48\*]. This hypothesis is consistent with specific

combinations of the signalling components mediating different responses.

### Developmental and physiological significance

Few data are available to place the cytokinin signalling components in a functional context. Results have been mainly obtained with CRE1/AHK4, for which extensive analyses of loss-of-function mutants indicate roles in embryonic cell proliferation, shoot regeneration *in vitro* and regulation of the phosphate starvation response [7\*\*,17\*\*,20\*\*]. Additional information about functional links of the cytokinin signalling pathway was obtained from the identification of target genes and from protein-protein interaction studies.

CRE1/AHK4 is expressed in the four innermost cells of the globular embryo, which are the precursors to the vascular tissue, and remains associated with the root vasculature throughout embryogenesis and in the primary root [17\*\*]. Consistent with the expression pattern, in the *wooden leg* mutant, which proved to be allelic to *CRE1/AHK4*, the number of vascular initials is reduced and these subsequently form xylem but not phloem cells [17\*\*]. This finding establishes an important role for cytokinins in embryonic developmental patterns and, more specifically, in vascular morphogenesis [17\*\*,49]. Moreover, cytokinin resistance of *cre1* mutants in a root elongation test indicates a role for CRE1 and the TCS in mediating cytokinin signals to the root meristem, whose activity is controlled by cytokinin [7\*\*,42].

Two hybrid screens revealed an interaction between AHP1, AHP2 and AHP3 and the putative transcription factor TCP10 [24]. The TCP domain is found for example in TB1 (Teosinte Branched 1) of *Zea mays*, which represses growth of lateral branches, and CYC (Cycloidea) and DICH (Dichotoma) of *Antirrhinum majus*, which regulate flower symmetry [50,51]. This interaction indicates that AHPs may also transmit their signal to other transcription factors than type-B ARRs.

Members of a class of genes that is likely to be regulated by ARR2 code for several components of the mitochondrial respiratory chain complex I (nCI) from *Arabidopsis*, which generates the proton-motive force of ATP synthesis [33\*]. ARR2 is strongly expressed in pollen and binds to a *cis*-acting DNA sequence in the promoter of *nCI* genes, which contains the so-called pollen box and is necessary for upregulating gene expression during sporogenesis. This suggests that cytokinins may be involved in controlling the capacity for ATP synthesis during sporogenesis [33\*].

*ARR4* has been shown to accumulate in response to light and to interact with the extreme N terminus of phytochrome B [44\*\*]. This interaction results in the stabilisation

of the physiologically active form of phytochrome B, and plants overexpressing ARR4 display hypersensitivity to red light. This interaction provides, for the first time, a mechanistic basis for the long-known cross-talk between cytokinin and light signal transduction pathways [27]. ARR4 also interacts with AtDBP1 and AtDBP2 [52], DNA-binding proteins previously identified as auxin-response genes [53]. This interaction could provide a link between auxin and cytokinin signalling.

Low phosphate availability triggers several adaptive changes including the induction of specific genes (Pi genes). Cytokinin represses the expression of some Pi genes in response to phosphate deprivation [2]. Several mutant alleles of *CRE1/AHK4* were identified in a screen for mutants that showed a reduced repression of Pi genes in the presence of cytokinin. This indicates that the hormone has a role in Pi starvation signalling [20\*\*]. Consistent with this finding, it was shown that the expression level of *CRE1/AHK4* decreased during Pi starvation [20\*\*].

It has been proposed earlier that cytokinins have a role in coordinating root and shoot development by carrying information about the nutritional status of the root to the shoot. It was recently shown that nitrogen treatment of nitrogen-starved plants leads to increased cytokinin export from the root. The cytokinin is then transported via the xylem to the leaves, where it causes enhanced expression of type-A response regulator genes [3,6,54,55]. Thus cytokinin may represent a long-distance signal for the relief of nitrogen starvation that is able to trigger downstream events in shoot tissues. It was also proposed that cytokinins and the TCS may be involved in mediating nitrogen-dependent root development [49].

The expression of some *ARR* genes of the A- and B-type in response to abiotic stimuli such as salt, drought and low temperature indicates cross-talk between the signalling pathways of these abiotic stresses and cytokinin [46]. This finding is consistent with the fact that AHK1, another member of the *Arabidopsis* His-kinase family, possibly functions as an osmosensor [56].

Transgenic plants overexpressing *ARR2* could overcome the glucose repression response, thus mimicking the mutation in the glucose-response mutant *gin2* and indicating an intimate link between cytokinin signalling and the HXK1 glucose-signalling pathway [57].

## Conclusions

The identification of the components of cytokinin perception and signal transduction has occurred at a breathtaking pace. Within a very short time, cytokinins have gone from being powerful but enigmatic molecules to one of the best understood plant hormones. The fact that genes for all proteins of the TCS, including His-kinases containing a CHASE domain, have been found in several

other plant species suggests that the pathway elucidated in *Arabidopsis* may be of a general nature.

The TCS has the necessary complexity to mediate different cytokinin responses in different tissues and at different developmental stages. Characteristically, multiple steps providing regulatory checkpoints and relay stations for cross-talk are used for the fine-tuning of the signal. Elucidation of the pathway is at a very early stage. Detailed knowledge about the tissue specificity of expression of individual genes, analysis of knockout phenotypes and the identification of more interacting proteins and target genes is needed and will be achieved rapidly with genomic tools. Data should be obtained from the whole plant, as non-physiological interactions are likely to occur in heterologous hosts and transient test systems. Certainly different biochemical characteristics of the receptors and other components will add an additional level of complexity to cytokinin signal perception and transduction. Last but not least, although it is tempting to think that the TCS provides all the components for cytokinin signalling, the possibility that alternative pathways are used should not be forgotten. In fact, pharmacological experiments indicate that they may exist [47].

## References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
  - of outstanding interest
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