Plant Cell Rep (2012) 31:789–799
DOI 10.1007/s00299-012-1229-9

REVIEW

Advances in upstream players of cytokinin phosphorelay: receptors and histidine phosphotransfer proteins

Xiuling Shi · Aaron M. Rashotte

Received: 9 December 2011 / Revised: 13 January 2012 / Accepted: 14 January 2012 / Published online: 15 February 2012

Abstract Cytokinins are a class of plant hormones that have been linked to numerous growth and developmental aspects in plants. The cytokinin signal is perceived by sensor histidine kinase receptors and transmitted via histidine phosphotransfer proteins (HPts) to downstream response regulators. Since their discovery, cytokinin receptors have been a focus of interest for many researchers. Ongoing research on these transmembrane receptors has greatly broadened our knowledge in terms of cytokinin–receptor interaction, receptor specificity, receptor cellular localization, and receptor functions in cytokinin related growth and developmental processes. This review focuses on the recent advances on the cytokinin receptors and HPt proteins in *Arabidopsis.*

Keywords Cytokinin · Plant hormone · Cytokinin receptors · Phosphotransfer proteins

Introduction

Cytokinins are a class of plant hormones that are principally N^6-substituted adenine derivatives. Over 50 years of study has shown that cytokinins play a myriad of roles in biological processes including cell division, seed germination, apical dominance, shoot meristem initiation and maintenance, leaf and root differentiation, stress tolerance, and senescence in plants (Gan and Amasino 1995; Haberer and Kieber 2002; Hwang and Sheen 2001; Mok and Mok 2001; Werner and Schmülling, 2009). In the past decade, genetic and molecular studies have led to the establishment of a well-defined cytokinin signaling model which resembles the two-component systems (TCSs) widely used by bacteria and some fungi (Beier and Gross 2006; Catlett et al. 2003; Ferreira and Kieber 2005; To and Kieber 2008). In *Arabidopsis*, cytokinin signaling starts with perception of the cytokinin molecule by a sensor histidine kinase (AHK). The signal is then relayed by a histidine phosphotransfer protein (AHP) through phosphorylation to the response regulators (ARRs) in the nucleus (Fig. 1). A branch pathway has also been identified in *Arabidopsis* that includes AHKs, AHPs, and cytokinin response factors (CRFs) (Rashotte et al. 2006).

The perception and relay of a cytokinin signal through this type of cytokinin signaling pathway appears to be conserved across plant species as cytokinin receptors (HKs), phosphotransfer proteins (HPts), response regulators (RRs), and even CRFs have been identified in increasing number of species, such as maize, rice, *Medicago sativa*, soybeans, and tomato (Yonekura-Sakakibara et al. 2004; Ito and Kurata 2006; Pareek et al. 2006; Gonzalez-Rizzo et al. 2006; Pils and Heyl 2009; Rashotte and Goertzen 2010; Le et al. 2011). This level of conservation indicates the essential role that cytokinin signaling plays in plants. This review focuses on the research advances on the cytokinin receptors and HPt proteins of the cytokinin signaling pathway in *Arabidopsis*.

Communicated by R. Reski.

X. Shi · A. M. Rashotte (✉)
Department of Biological Sciences, Auburn University, 101 Rouse Life Sciences Bldg., Auburn, AL 36849, USA
e-mail: rashotte@auburn.edu

Cytokinin receptors

Nearly half a century after the discovery of cytokinin, its receptors were first identified in *Arabidopsis* as *ARABIDOPSIS* HISTIDINE KINASE 4 (AHK4)/WOODENLEG

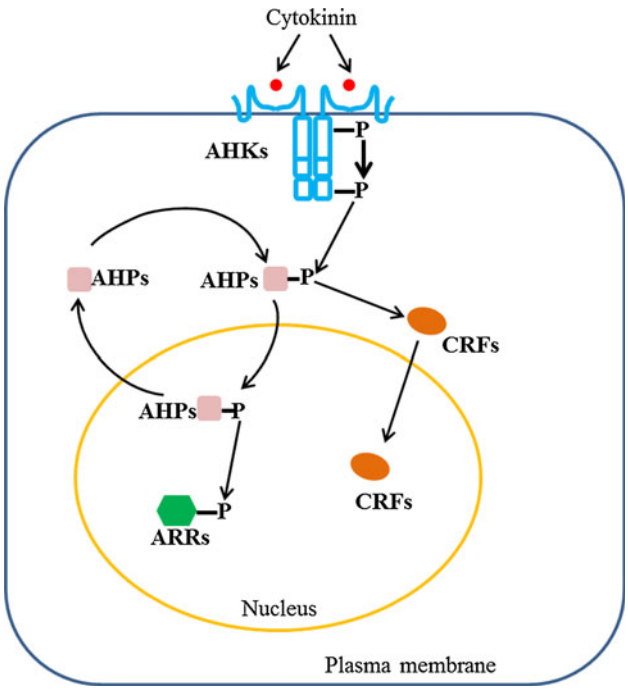

Fig. 1 A schematic diagram of classical cytokinin signal phosphorelay. The perception of cytokinin by one of the AHKs at the plasma membrane results in the autophosphorylation of the receptor, the phosphoryl group is subsequently transferred to an AHP which passes the phosphoryl group to an ARR in the nucleus. AHKs, AHPs, together with CRFs, form a branch pathway of cytokinin signaling

(WOL)/CYTOKININ RESPONSE 1 (CRE1), *ARABIDOPSIS* HISTIDINE KINASE 2 (AHK2) and *ARABIDOPSIS* HISTIDINE KINASE 3 (AHK3) (Mähönen et al. 2000; Inoue et al. 2001; Ueguchi et al. 2001b; Yamada et al. 2001; Suzuki et al. 2001a; Kieber and Schaller 2010). Further studies revealed that these cytokinin receptors play both redundant and specific roles in cytokinin-mediated growth and developmental processes in *Arabidopsis* (Higuchi et al. 2004; Nishimura et al. 2004; Riefler et al. 2006). Recent work on cytokinin receptors has shown that each receptor mediates the specificity of different cytokinins in the signaling pathway (Stolz et al. 2011). Perhaps, more importantly, new experimental works have demonstrated the localization of cytokinin receptors not to the plasma membrane as originally predicted, but to the ER membrane instead (Caesar et al. 2011; Lomin et al. 2011; Wulfetange et al. 2011). Each of these findings is likely to have profound impacts on our understanding of cytokinin signaling.

Structure of the cytokinin receptors: hybrid histidine kinases

The three cytokinin receptors (AHK2, 3, and 4) belong to a small *Arabidopsis* histidine kinase family which comprises 16 known members with diverse roles. These family members of kinases are involved in ethylene signaling [ETR1, ERS1, ETR2, ERS2, and EIN4] (Chang et al. 1993; Hua and Meyerowitz 1998), cytokinin signaling [AHKs] (Ueguchi et al. 2001b; Yamada et al. 2001; Hwang and Sheen 2001), cytokinin-independent activation of two-component signaling pathway [CKI1] (Kakimoto 1996, 1998; Hejátko et al. 2009; Deng et al. 2010), osmotic stress responses [CKI2/AHK5] (Urao et al. 1999; Tran et al. 2007), and light responses [light receptors PhyA-E] (Quail 2002; Schepens et al. 2004; Strasser et al. 2010).

AHK2, AHK3, and AHK4 are hybrid histidine kinases because they contain both the HK domain and the RR domain also known as a receiver domain (West and Stock 2001; Ueguchi et al. 2001a). The HK domain contains a conserved His residue which is autophosphorylated when a stimulus is perceived (West and Stock 2001). The phosphoryl group is transferred to the receiver domain from the HK domain before it is relayed further down the signaling pathway. Amino acid sequence analysis revealed that cytokinin receptors are highly homologous to each other with great similarity in overall predicted protein structure (Ueguchi et al. 2001a). AHKs are all transmembrane proteins that possess: transmembrane segments, a typical ligand-binding domain, a conserved histidine kinase domain (also called transmitter domain), and a receiver-like domain followed by a receiver domain. AHK2 and AHK3 are three pass transmembrane proteins, whereas AHK4 is two pass (Although this has not been specifically examined it would be interesting to explore how different numbers of transmembrane segments might result in functional differences between these proteins). The ligand-binding domain was characterized and named CHASE (cyclases/histidine kinases associated sensing extracellular) domain by two research groups (Anantharaman and Aravind 2001; Mougel and Zhulin 2001). The CHASE domain found in various prokaryotic and eukaryotic receptor-like proteins was shown to directly function as the cytokinin-binding region of the receptor with four specific amino acids crucial for ligand-binding (Heyl et al. 2007). Interestingly, one out of five identified CHASE domain containing proteins in rice contains a Ser/Thr protein kinase domain instead of a His kinase domain (Han et al. 2004; Ito and Kurata 2006; Pareek et al. 2006). This protein was named OsCRL4 (Han et al. 2004) or CHARK (CHASE domain receptor-like serine/threonine kinase) (Ito and Kurata 2006). OsCRL4 was found to be able to complement *cre1* mutation and was suggested to represent a CRE1-like new member of cytokinin receptors in rice (Han et al. 2004). The histidine kinase domain of the *Arabidopsis* cytokinin receptors was found to possess five consensus motifs (H, N, G1, F and G2) as well as a conserved histidine residue. The receiver domain contains three

regions that include the conserved D, D and K amino acids residues (Ueguchi et al. 2001a). Although studies have shown that the conserved histidine residue in the histidine kinase domain and the conserved aspartate residue in the receiver domain are necessary for the signaling capacity, autophosphorylation, and phosphorelay (Hwang and Sheen 2001; Inoue et al. 2001), the specific roles of those motifs remain to be determined. Recently, the crystal structure of the AHK4 CHASE domain (sensor domain) binding to different cytokinins has been determined (Hothorn et al. 2011). The CHASE domain consists of an N-terminal long helix and two PAS-like domains that are connected by a linker helix. It turns out that AHK4 CHASE domains form homodimers in crystals. The membrane-distal PAS domain of AHK4 recognizes cytokinins. The structure of AHK4 CHASE domain in complex with isopentenyladenine (iP) shows that both the adenine moiety of iP and its isopentenyl tail occupy the binding pocket of AHK4. In the lower part of the ligand-binding pocket, the central β-sheet of the PAS domain is lined with small hydrophobic residues, such as Ala and Gly, which are believed to be essential for the receptor activity. Two β-strands in the upper part of the pocket are responsible for hydrophobic interactions as well. Hydrogen bonds formed between Asp262 and the adenine ring within the pocket may be crucial for receptor function. Further examination revealed an additional hydrogen bond between hydroxylated isopentenyl side chain of *trans*-zeatin (*t*Z) with Thr294, the only hydrogen-bond acceptor in the tail-binding pocket, providing the structural basis for the high affinity of AHK4 for *t*Z rather than *cis*-zeatin (*c*Z). This study by Hothorn et al. (2011) also indicates synthetic cytokinins, such as thidiazuron (TDZ) can occupy the binding pocket of AHK4 as natural cytokinins. In summary, by determining the crystal structures of the sensor domain of AHK4 in complex with different cytokinins, the authors revealed how AHK4 interacts with cytokinins and how new useful cytokinins can be potentially designed.

Ligand–receptor interaction: perception is everything

Elucidating the cytokinin–receptor interaction is an important step to understand the cytokinin signaling pathway. To this end, many assays have been developed to study the cytokinin–receptor interaction including the biological activity of cytokinins, the binding activity and ligand-binding specificity of the receptors. These assays include plant-based bioassays, bacterial assays, and even yeast-based assays. Yamada et al. (2001) revealed that three cytokinins *t*Z, iP, and a synthetic cytokinin TDZ could be perceived by AHK4 receptor expressed in an *E. coli* mutant which lacked the endogenous histidine kinase RcsC. Using live cell hormone-binding assays, Romanov et al. (2005) revealed that AHK4 is highly specific for *t*Z (Table 1), confirming the role of AHK4 as a cytokinin receptor. Examination of the ligand specificity of AHK4 with diverse cytokinin analogues showed the following order of affinity for AHK4: *t*Z > zeatin riboside (ZR) > dihydrozeatin (DZ) > *c*Z > zeatin *O*-glucoside. This affinity order based on the live cell-binding assay is in agreement with that produced with purified bacterial AHK4-expressing membrane preparations, as well as that from a reporter gene assay on the AHK4-expressing *E. coli* clone (Spíchal et al. 2004).

Further hormone-binding assays provided evidence that AHK2 CHASE-TM (TM represents the two transmembrane segments adjacent to the CHASE domain) has similar ligand preferences to AHK4 CHASE-TM (Stolz et al. 2011). Both CHASE-TM domains displayed a much higher affinity to iP and *t*Z than to their riboside forms and a very low affinity to *c*Z. A comparison study on the ligand specificity of AHK3 and AHK4 indicated that AHK3 has a much lower affinity to iP and its riboside form, but a higher affinity to DZ than AHK4 (Table 1; Romanov et al. 2006). In addition, *c*Z could activate AHK3 at 1 μM, but not AHK4 in bacterial assays (Spíchal et al. 2004). What is responsible for these differences in ligand recognition and

Table 1 Cytokinin-binding specificity, tissue expression pattern, and predominant roles of cytokinin receptors in Arabidopsis

Cytokinin receptors	Cytokinin-binding specificity	Tissue expression pattern	Functional roles
AHK2	iP > *t*Z > iPR > *t*ZR > DZ[a]	Leaves, roots, flowers[c]	Root branching[g,h]; abiotic stress responses[i,j]; shoot vascular tissue development[k]
AHK3	*t*Z > *t*ZR > DZ > iP > *c*Z[b]	Leaves, stems, roots and flowers[c,d]	Retardation of leaf senescence and Root branching[g,h]; abiotic stress responses[i,j]; shoot development[h]; shoot vascular tissue development[k]
AHK4	*t*Z > iP > *t*ZR > iPR[b]	Roots[c–f]	Vascular morphogenesis[e]; responses to exogenous cytokinins[h,l]

[a] Stolz et al. (2011), [b] Romanov et al. (2006), [c] Ueguchi et al. (2001a), [d] Higuchi et al. (2004), [e] Mähönen et al. (2000), [f] Mähönen et al. (2006), [g] Kim et al. (2006), [h] Riefler et al. (2006), [i] Jeon et al. (2010), [j] Tran et al. (2007), [k] Hejátko et al. (2009), [l] Nishimura et al. (2004)

signaling ability, however, remains unclear. An in-depth study on the structural difference of the receptor-binding regions as well as the space-filling models of various cytokinins might provide some insights into this intriguing question.

Expression patterns of the cytokinin receptors: both unique and overlapping

All three *Arabidopsis* cytokinin receptors have been detected in different organs, such as roots, leaves, stems, and flowers at varying levels, although individual specificity can be detected (Ueguchi et al. 2001a). *AHK4* generally seems to be root specific detected in this tissue at high levels by RT-PCR, Northern, and with *AHK4* promoter::GUS fusion expression (Ueguchi et al. 2001a; Mähönen et al. 2000; Higuchi et al. 2004). This root-specific expression of *AHK4* was confirmed by in situ hybridization which also further revealed it to be localized to the vascular cylinder and pericycle in primary roots: a pattern specified fairly early during embryogenesis (Mähönen et al. 2000). Similar vascular expression patterns of *AHK4* have also been observed in sections of roots expressing an *AHK4* promoter::GUS fusion (Mähönen et al. 2006). *AHK2* transcript was found to be the most abundant in leaf tissue and least abundant in stems as shown in RT-PCR (Ueguchi et al. 2001a). *AHK3* also shows its highest expression in rosette leaves although it is also strongly expressed among roots, stems, and flowers (Ueguchi et al. 2001a; Higuchi et al. 2004). Overall, *AHK2*, *AHK3*, and *AHK4* receptors have been shown to have overlapping expression patterns with each other, although specificity, such as *AHK4* localization to the root has been reported (Table 1). For a more detailed discussion of these expression patterns, see papers by Higuchi et al. (2004), Nishimura et al. (2004), and Mähönen et al. (2006).

Subcellular localization of the cytokinin receptors: plasma membrane or ER

Cytokinin receptors are predicted to be plasma membrane localized based on the domain analysis, sequence similarity to two-component hybrid molecules, and some experimental evidence obtained with receptor–GFP fusion proteins (Inoue et al. 2001; Kim et al. 2006; Mähönen et al. 2000). Several very recent studies, however, have presented evidence supporting the localization of the AHKs to a different membrane: the endoplasmic reticulum (ER) (Caesar et al. 2011; Lomin et al. 2011; Wulfetange et al. 2011). Analysis of separated plasma membrane and endomembrane fractions indicated a predominant endomembrane location of cytokinin-binding sites; examination of AHK–GFP fusion proteins expressed in tobacco leaf epidermal cells revealed ER localization of these proteins (Wulfetange et al. 2011). Immunoblots with Myc-tagged cytokinin receptors following fractionation of cell membranes lent further support to the ER localization of the receptors (Wulfetange et al. 2011). Similarly, another study has demonstrated the ER localization of fluorophor-tagged AHK3 and AHK4 proteins (Caesar et al. 2011). In addition, the maize ZmHK1 cytokinin receptor has recently also been shown to localize to ER (Lomin et al. 2011). Although the above-experimental evidence strongly indicates that the cytokinin receptors are mainly localized to ER, partial plasma membrane localization cannot be ruled out (Wulfetange et al. 2011). If both localizations exist, the question arises as to when cytokinin signaling occurs from the plasma membrane and when it occurs from the ER membrane. In addition, how the targeting of cytokinin receptors to these different membrane locations in the cell remains to be elucidated. Given that the ER localization of cytokinin receptors is experimentally supported, a revised model of cytokinin signal phosphorelay is proposed here (Fig. 2). This recent finding is critically important as it presents a general shift in thinking about how cytokinin signaling functions in the cell. Previously, it was most important to have cytokinin outside or at the edge of the cell for proper

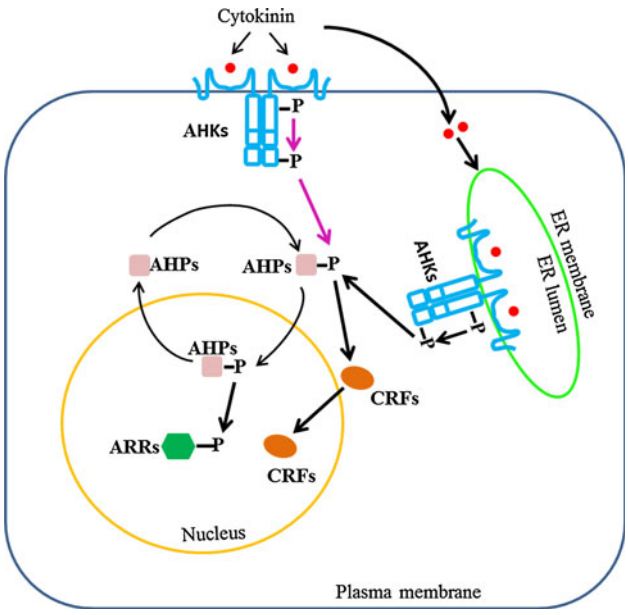

Fig. 2 A modified cytokinin signal phosphorelay model. Cytokinin receptors are mainly localized at the ER membrane but might also be localized at the plasma membrane. Cytokinin binding to one of the AHKs in the ER lumen or at the plasma membrane results in the autophosphorylation of the receptor and the phosphoryl group is subsequently transferred to an AHP which passes the phosphoryl group to an ARR in the nucleus. AHKs, AHPs, together with CRFs, form a branch pathway of cytokinin signaling

perception and then signaling. Now that the receptor is internal, likely requiring cytokinin to be perceived within the lumen of the ER, movement of cytokinin into the cell and cellular organelles could be much more crucial to normal signaling than once thought. Because neither of these transport processes have been well studied their characterization is likely to have profound effects on the field of cytokinin signaling.

Functions of cytokinin receptors in physiological processes: an actor in many roles

Ongoing research has provided evidence that cytokinin receptors play multiple roles in cytokinin-mediated physiological processes (Table 1), such as regulation of root vascular morphogenesis (Mähönen et al. 2000), retardation of leaf senescence (Kim et al. 2006), regulation of shoot vascular development (Hejátko et al. 2009), and mediation of abiotic stress responses (Merchan et al. 2007; Tran et al. 2007; Coba de la Peña et al. 2008; Jeon et al. 2010).

Cytokinin receptors play roles in both root- and shoot-related processes: three coworkers in roots versus two coworkers in shoots

Initial studies on *wol* (AHK4) mutants indicated an important role of the *WOL* allele in vascular cell divisions as mutants had fewer vascular initial cells resulting in a root vascular system composed only of protoxylem (Mähönen et al. 2000; Cano-Delgado et al. 2000). The *wol* mutation is an amino acid substitution in the CHASE domain (T278I) that eliminates the cytokinin binding activity of the WOL protein (Mähönen et al. 2000; Heyl et al. 2007; Yamada et al. 2001). Unlike *wol* mutants, AHK4 T-DNA insertion null mutations *cre1-2* and *cre1-11* did not show visible defects in root morphology under standard conditions, although they exhibited reduced sensitivity to cytokinin inhibition of root growth and adventitious root formation (Higuchi et al. 2004; Inoue et al. 2001; Mähönen et al. 2006). Further experiments demonstrated that the mutated WOL protein serves as a constitutive phosphatase in the absence of cytokinin to inhibit cytokinin signaling mediated by AHK2 and AHK3, which results in the *wol* phenotypes (Mähönen et al. 2006). Notably, single mutants of *AHK2* or *AHK3* responded normally or slightly less to cytokinin in root elongation assay (Higuchi et al. 2004), whereas *cre1-2*, *ahk4-1*, and combinations of *cre1-2* with other *ahk* mutants were resistant to cytokinin, indicating a predominant role of CRE1/AHK4 in responses to exogenous cytokinins (Nishimura et al. 2004; Riefler et al. 2006). Surprisingly, *ahk2ahk3* double mutants displayed a much more branched root system compared with that of wild type, indicating a negative role of AHK2 and AHK3 in regulating root branching (Riefler et al. 2006). Taken together, *AHK2*, *AHK3*, and *WOL/CRE1/AHK4* exert overlapping roles in roots with *WOL/CRE1/AHK4* functioning as a predominant root regulator. This functional overlap of these three receptors is consistent with their overlapping expression patterns (Mähönen et al. 2006).

Unlike the root-specific expression of *AHK4*, both *AHK2* and *AHK3* transcripts are abundant in leaves or stems, raising the possibility that these two receptors play roles in leaf- or shoot-related developmental processes (Ueguchi et al. 2001a; Mähönen et al. 2000; Higuchi et al. 2004; Nishimura et al. 2004). The greatly reduced rosette size in the *ahk2ahk3* double mutant indicates the involvement of these two receptors in shoot development (Riefler et al. 2006). A recent study has implicated *AHK2* and *AHK3* in regulating procambium development, as seen from a reduced number of procambial cell layers found in the mutants of these genes (Hejátko et al. 2009). An additive effect was seen in the *ahk2 ahk3* double mutant that exhibited a stronger phenotype than either single mutant as manifested by a reduction in procambium and vascular bundle size as well as the lack of an interfascicular cambium. In addition, a depletion of endogenous cytokinin in either *CKX1* or *CKX3* overexpressors resulted in similar defects in vascular bundle development, confirming the role of AHK2 and AHK3 mediated cytokinin signaling in vascular bundle development (Hejátko et al. 2009).

Senescence and leaf longevity: receptors for going green

Leaf senescence is a programmed natural process influenced by various internal and external factors and phytohormones such as cytokinins, auxin, ethylene, and ABA are important internal factors involved in that regulation (Lee et al. 2001; Quirino et al. 2000; Smart 1994; Lim et al. 2010). Particularly cytokinins are known to delay leaf senescence as shown in experiments that prolonged the life span of leaves when the cytokinin biosynthetic *IPT* gene was expressed under the control of a senescence-specific promoter *SAG12* (Gan and Amasino 1995) or a heat-shock promoter *HSP18.2* (Merewitz et al. 2010). Identification of an *AHK3* gain-of-function mutant, *ore12-1*, revealed that AHK3, not AHK2 or AHK4, is the primary cytokinin receptor functionally regulating leaf senescence via an ARR2-specific phosphorelay cascade (Kim et al. 2006). This is consistent with the finding that AHK3 is the major contributor to cytokinin-dependent chlorophyll retention in leaves (Riefler et al. 2006). Interestingly, it is currently unknown which AHP might specifically be responsible for transferring the phosporyl group from AHK3 to ARR2 to delay leaf senescence.

A cytokinin-induced delay in leaf senescence has notably been accompanied by a large increase in extracellular invertase activity, suggesting that changed source-sink relations might also be linked to these processes (Lara et al. 2004). This idea is supported by two works in which the invertase expression was manipulated in different ways. One expressing an invertase during senescence in tobacco using a *SAG12*:*Cin1* (an invertase from *Chenopodium rubrum* driven by a senescence-induced promoter). A second approach blocked extracellular invertase activity through the expression of a tobacco invertase inhibitor under control of a cytokinin-inducible promoter, thus rendering cytokinin ineffective in delaying leaf senescence (Lara et al. 2004). Together, these results suggest that extracellular invertase is involved in the suppression of leaf senescence by cytokinins. Surprisingly, not much work had been done in *Arabidopsis* to elucidate how cytokinin signaling and the members of this pathway are linked to senescence and connected to extracellular invertases in this process.

Mediating abiotic response: receptors to keep from stressing out

Cold-induced expression of a subset of type-A ARR genes, including *ARR5*, *ARR6*, *ARR7*, and *ARR15* was shown to be mediated by the receptors AHK2 and AHK3 (Jeon et al. 2010). In fact, the *ahk2 ahk3* double mutant displayed significantly greater freezing tolerance compared with wild type as did *arr5*, *arr6*, and *arr7* loss-of-function mutants, while an ARR7 overexpressing line was hypersensitive to freezing temperatures (Jeon et al. 2010). These results directly link the receptors to cold stress response and suggest a negative role of AHK2 and AHK3 in cold stress tolerance. A similar negative regulatory role has also been suggested for AHK2 and AHK3 in osmotic stress response as evidenced by the increased tolerance of *ahk2*, *ahk3*, and *ahk2 ahk3* double mutants to drought and salt stress (Tran et al., 2007). In addition, *ahk4* mutants also exhibited enhanced salt tolerance when compared with wild type in the presence of cytokinin, suggesting a similar role for AHK4 as well (Tran et al. 2007).

Additional evidence of cytokinin receptors linked to salt stress regulation has been seen in work in other species, such as *Medicago sativa* where *MsHK1*, an *AHK3* homolog showed induction by salt (Coba de la Peña et al. 2008). This has also been seen in *Medicago truncatula* where two of its cytokinin receptors, *MtHK2* and *MtCRE1* were induced in roots both by salt stress and during recovery from this stress (Merchan et al. 2007). These results indicate the conserved roles of cytokinin receptors in mediating stress responses in different plant species likely as negative regulators of abiotic stress signaling (Tran et al. 2007).

Positive regulation of nodulation in legumes

Homologs of AHK4 have been identified in two legumes: MtCRE1 in *Medicago truncatula* (Gonzalez-Rizzo et al. 2006) and LHK1 in *Lotus japonicas* (Tirichine et al. 2007). Both cytokinin receptors were found to play a role in the regulation of root nodulation. *MtCRE1*RNAi roots were insensitive to cytokinin and displayed strongly impaired nodulation when compared with wide type, *MtHK2* RNAi, and *MtHK3* RNAi roots, indicating that MtCRE1-mediated cytokinin signaling is required for normal nodulation in *Medicago* (Gonzalez-Rizzo et al. 2006). A gain-of-function mutation in CHASE domain of LHK1 in lotus resulted in spontaneous formation of root nodules, and conferred cytokinin independent activity on LHK1 (Tirichine et al. 2007). Further examination revealed that cytokinin perception occurs downstream of Nod factor signal transduction, but upstream of cortical cell activation. Together, these results indicate that cytokinin signaling is necessary in root nodulation in legumes.

Regulation of the specificity of cytokinin signaling: location matters

Response to cytokinin across plant tissues is regulated by a number of factors including both the ligand-binding affinity and the spatial expression pattern of specific cytokinin receptors (Stolz et al. 2011). The sensitivity of AHKs to iP and *t*Z was examined by quantifying cytokinin primary response gene (ARR5, 6) transcripts and reporter gene (pARR5:GUS) expression in receptor double mutants containing only a single functional receptor (Stolz et al. 2011). *ahk2 ahk4* mutants were less sensitive to iP compared with *ahk2 ahk3* mutants (Stolz et al. 2011), consistent with the observed higher affinity of AHK4 and weaker affinity of AHK3 to iP (Romanov et al. 2006). However, *ahk2 ahk4* mutants showed a higher sensitivity to *t*Z compared with *ahk2 ahk3* mutants (Stolz et al. 2011). Furthermore, AHK2 and AHK4 were able to activate pARR5:GUS reporter gene to a similar extent in the presence of iP or *t*Z (Stolz et al. 2011).

Overlapping yet distinct expression patterns of the three AHK receptors was seen using the pARR5:GUS reporter gene as a general proxy for cytokinin mediated expression in receptor double mutants (Stolz et al. 2011). All three receptors are strongly active in both the shoot and root tips. AHK2 and AHK3 are actively expressed in parenchyma cells, while AHK3 also stimulates reporter activity in stomata (Stolz et al. 2011). Interestingly, cytokinin has been linked to regulation of stomata opening in concert with other hormones (Acharya and Assmann 2009; Tanaka et al. 2006). Therefore, further examination is needed to determine whether the stomata-specific activity of AHK3 is

responsible for cytokinin-mediated stomata opening. Overall, AHK2 and AHK3 display a broader activity domain in the shoot apex than AHK4 and they lead to increased expression of pARR5:GUS in leaves in the presence of cytokinin that is also lacking for AHK4 (Stolz et al. 2011). This indicates a predominant role of AHK2 and AHK3 in leaves and shoots similar to that seen in Higuchi et al. (2004). Strong pARR5:GUS reporter staining of root vasculature after a 5-h incubation in an *ahk2 ahk3* mutant, but not *ahk2 cre1* and *ahk3 cre1* mutants (Stolz et al. 2011) confirms root vascular specific activity of AHK4 (Mähönen et al. 2000).

The analysis of receptor activity by way of a promoter-swap experiment shows that AHK4, when expressed under the control of an *AHK2* promoter can complement the *ahk2 ahk3* mutant consistent with the similar ligand-binding spectrums of AHK2 and AHK4 (Stolz et al. 2011). Interestingly, *ahk2 ahk3* mutant can be partially complemented by the expression of CHASE-TM of AHK3 fused with the cytoplasmic domain of AHK4 directed by the AHK3 promoter, suggesting an essential role of CHASE domain in specifying AHK3 function (Stolz et al. 2011). Taken together, these results indicate that the differing ligand-binding affinities and expression patterns of AHKs, possibly along with other yet unknown factors, contribute to the specification of cytokinin signaling in *Arabidopsis* plants.

Interestingly, both AHK4 and AHK3 seem to be able to mediate cytokinin specificity in root tissue. AHK3-mediated cytokinin signaling was shown to regulate root meristem size as evidenced by enlarged root meristem in *ahk3* mutants, while AHK4 and AHK2 had little to no effect in this aspect (Dello Ioio et al. 2007). On the other hand, AHK4 is the predominant cytokinin receptor which regulates root vascular development (Mähönen et al. 2000, 2006). This specific activity of AHK3- and AHK4-mediated cytokinin signaling in roots is possibly achieved through differing receptor ligand affinity (Romanov et al. 2006; Bishopp et al. 2011) and expression patterns (Mähönen et al. 2006).

Histidine phosphotransfer proteins: the next step in cytokinin signaling

Structure of HPt proteins

HPt proteins are essential players in the His-Asp-His-Asp phosphorelay which transfer the phosphoryl group from hybrid kinase receptors to downstream RRs (West and Stock 2001). In *Arabidopsis*, there are five HPts (AHP1-5) which carry the conserved phospho-accepting His residue (Heyl and Schmulling 2003; Hutchison et al. 2006). AHP6, also known as APHP1, is a pseudo HPt which does not contain the conserved His residue necessary for phosphotransfer activity (Suzuki et al. 2000; Mähönen et al. 2006). Because AHPs contain only a short HPt domain, their structure is much simpler when compared with that of AHKs (Suzuki et al. 2000). Each of the AHPs is about 150 amino acids long except AHP4 which contains 127 amino acids (Suzuki et al. 2000). An EST database search identified HPts in a variety of plant species which show great similarity in amino acid sequence to AHPs, indicating the conserved nature of these HPt proteins (Suzuki et al. 2000).

In maize, the crystal structure of one HPt protein, ZmHP2, has been determined (Sugawara et al. 2005). ZmHP2 contains four C-terminal helices which form an antiparallel bundle connected to two N-terminal helices by a β-turn. The residue Arg102, close to the phospho-accepting His80 residue, was predicted to promote the formation of interaction complex between ZmHP2 and receiver domains based on the role of corresponding residues in bacterial and yeast HPt proteins. The His80 phosphorylation site is surrounded by conserved residues of ZmHP2 which are localized on three helices of the four-helix bundle and one N-terminal helix. Notably, the protrusion of the imidazole ring of His80 from ZmHP2 molecule surface is believed to be important for phosphate transfer. The conserved residues surrounding His80 possibly act as a docking interface for receiver domains, while the non-conserved residues seem to be responsible for specific activities of different HPt proteins.

Expression patterns and transcriptional regulation of AHPs by cytokinin

AHP1 is predominantly expressed in the roots; *AHP2* and *AHP3* are detectable across the plant including roots, stems, leaves, flowers, and siliques with the highest expression of *AHP2* in roots/flowers and that of *AHP3* in roots/leaves (Suzuki et al. 1998; Hradilová and Brzobohatý 2007). *AHP5*, similar to *AHP2* and *AHP3*, is expressed in various organs (Hradilová and Brzobohatý 2007), while *AHP4* is hardly detectable in leaves and in roots (Suzuki et al. 2000). *AHP6* is expressed in developing protoxylem and pericycle cells, shoot apex, and young leaves (Mähönen et al. 2006). In general, these observations are consistent with those of another study on *AHP* expression profiles through Northern blot hybridization (Tanaka et al. 2004). However, the latter was able to detect *AHP4* transcripts with varying size in aerial parts of plants, indicating the possibility of alternative splicing and/or alternative polyadenylation (Tanaka et al. 2004). The expression of *AHP4* has also been examined by another group that reported *AHP4* expression predominantly in young flowers (Jung et al. 2008). Interestingly, alternative splicing also seems to occur during *AHP5 RNA* processing as shown by

two RT-PCR products of different size specific for *AHP5* transcripts. Sequence analysis revealed the presence of a longer PCR product AHP5L containing the second intron which is absent in the shorter PCR product AHP5, confirming the alternative splicing of AHP5 transcript (Hradilová and Brzobohatý 2007).

Previous data based on the Northern blot and microarray analysis indicate that *AHPs* are not transcriptionally regulated by cytokinin (Suzuki et al. 2000; Rashotte et al. 2003; Brenner et al. 2005), although *AHP5* has been seen to be regulated under specific conditions (Hoth et al. 2003). Hradilová and Brzobohatý (2007), however, demonstrated that AHP1 to 4 transcripts increased in response to both a short-term cytokinin treatment and an increased level of endogenous cytokinin through inducible *IPT* gene expression as shown by qRT-PCR analysis. It is not clear what factors are responsible for these differences; the sensitivity of detection methods or growth-stage specific induction by cytokinin might be reasonable explanations. As such, it is currently unclear whether AHPs are transcriptionally affected by cytokinin and if so what that would mean for signaling processes.

Cellular localization of AHPs

Initial studies on cellular localization of AHPs with AHP–GFP fusion proteins showed that most AHPs were generally localized in the cytosol, but were then translocated to the nucleus upon exposure to or treatment by cytokinin (Hwang and Sheen 2001; Tanaka et al. 2004). A recent paper revealed that AHP2 and AHP5 show both nuclear and cytoplasmic localization in plants and that contrary to what was previously seen, cytokinin treatment has no effect on their subcellular localization (Punwani et al. 2010). Regardless of the role of cytokinin on AHP localization, it is still believed that AHPs function in relaying the phosphate signal from the membrane localized receptor to the response regulators in the nucleus. As such AHPs are thought to move in a cyclic pattern between the cytoplasm and nucleus and back again and thus can be found distributed between these parts of the cell (Figs. 1, 2).

The role of HPt proteins in cytokinin-mediated processes

It is known that HPt proteins are part of the cytokinin signaling pathway, but how do HPts regulate cytokinin-mediated processes? Initial findings using *Arabidopsis* protoplast and a pARR6:LUC reporter assay indicated that the overexpression of AHPs (AHP1, AHP2, or AHP5) had little effect on cytokinin signaling (Hwang and Sheen 2001). However, *Arabidopsis* AHP2 overexpressors showed hypersensitivity to cytokinin in a root elongation assay, although no other morphological changes were observed (Suzuki et al. 2002). An opposite effect was found by eliminating AHPs in loss-of-function T-DNA insertion mutants. This was most easily seen in *ahp* multiple mutants that have reduced cytokinin sensitivity in various cytokinin bioassays and in greatly reduced induction of type-A *ARR* transcripts by cytokinin in an *ahp1,2,3,4,5* mutant (Hutchison et al. 2006). Although single and double AHP mutants responded to cytokinin normally in root elongation assays, presumably due to functional redundancy, the *ahp1,2,3* triple mutant was less responsive to cytokinin than the wild type. In addition, multiple *ahp* mutants which include *ahp2*, *ahp3*, and *ahp5* mutations (*ahp2,3,5, ahp1,2,3,5*, *ahp2,3,4,5*, and *ahp1,2,3,4,5*) displayed a short primary root phenotype which could be rescued by a wild-type *AHP5* gene (Hutchison et al. 2006). Further examination of the root of the quintuple mutant *ahp1,2,3,4,5* showed that exclusive protoxylem development occurred in the vascular cylinder reminiscent of *wol* (Mähönen et al. 2000) and the *ahk2,3,4* triple mutant (Mähönen et al. 2006). In fact, the multiple mutant lines lacking either all receptors (*ahk2,3,4*) or all phosphotransfer proteins (*ahp1,2,3,4,5*) are initially very similar in physiological appearance as small dwarf-like plants with little to no root, indicating a positive role of AHPs in cytokinin signaling. *ahp1,2,3,4,5* mutants, however, can recover through the generation of an adventitious root, that substitutes as a primary root, although plants still produced shorter siliques with fewer, but larger seeds per silique compared with the wild type (Hutchison et al. 2006). Examination of the *ahp1,2,3,4,5* quintuple mutant by another research group also revealed a decrease both in cell size and cell number in leaves (Deng et al. 2010). The greatly reduced cell number in the *ahp1,2,3,4,5* mutant resembles that seen in leaves of *ahk2,3,4* mutant (Nishimura et al. 2004). Taken together, AHPs, as mediators between AHKs and ARRs, are positive regulators with overlapping functions of cytokinin signaling which is essential for various developmental processes.

Although most HPts in *Arabidopsis* function in a similar manner, two of them AHP4 and AHP6 are slightly different. AHP4 is often placed by phylogenetic analysis in the same clade with rice pseudo-HPts (Hutchison et al. 2006), and shows very low transcript levels in both RT-PCR (Suzuki et al. 2000) and transcriptome analysis (Zimmermann et al. 2004). The analysis of *ahp* loss-of-function mutants suggests that AHP4 may play no role, a slightly positive role, or even a negative role in cytokinin signaling, depending on the growth processes examined and the genetic background (Hutchison et al. 2006). Interestingly, a recent study indicates that AHP4 may function in regulating fertility (Jung et al. 2008). However, more work remains to be done to clarify whether the *ahp4* mutant and

the AHP4 overexpressor used in this study have altered cytokinin responses and whether AHP4 regulates fertility via cytokinin signaling.

AHP6 is a pseudo-HPt protein in that it does not contain the conserved His residue necessary for phosphotransfer activity and its transcript expression is down-regulated by cytokinin treatment (Mähönen et al. 2006). It was also found that AHP6 was unable to accept a phosphoryl group from the yeast SLN1 histidine kinase in the in vitro phosphotransfer assay, indicating that AHP6 is not functional in phosphotransfer (Mähönen et al. 2006). Further examination indicates that protoxylem differentiation is negatively regulated by cytokinin but promoted by AHP6 which acts by inhibiting cytokinin signaling (Mähönen et al. 2006). Thus, the balance of the actions of cytokinin and AHP6 is important for the proper specification of protoxylem in the root system.

Interaction of HPt proteins with proteins within and outside the cytokinin signaling pathway-HPts act as a signaling connector

As mediators of a multistep phosphorelay, AHPs have been shown to be able to interact with HKs (Urao et al. 2000; Suzuki et al. 2001a; Dortay et al. 2006), RRs (Urao et al. 2000; Lohrmann et al. 2001; Suzuki et al. 2001b; Tanaka et al. 2004; Dortay et al. 2006), and CRFs (Cutcliffe et al. 2011). An extensive analysis of protein interactions within cytokinin signaling pathway indicated that AHPs act as hubs to interact with members of all other protein families directly associated with cytokinin signaling (Dortay et al. 2006). In addition, AHPs have also been shown to interact with proteins outside cytokinin signaling pathway (Dortay et al. 2008). One example is that AHP1 could interact with the ethylene receptor ETR1 as indicated by yeast two-hybrid assays and fluorescence spectroscopy (Urao et al. 2000; Scharein et al. 2008). It has also been shown that phosphorylation at the conserved His residue is important for the interaction of AHPs with its protein partners (Suzuki et al. 1998; Scharein and Groth 2011). As ETR1 is also a HK receptor protein predicted to be involved in phosphorelay, this may not be very surprising, yet suggests that HPts are involved in signaling role beyond just cytokinin.

Concluding remarks

Cytokinin signaling is a complex pathway that requires coordinated functions of members from multiple gene families. Although functional redundancy was observed with cytokinin receptors and HPt proteins (as well as with RRs), recent work has been revealing specific roles for these players in cytokinin signaling pathway. More importantly, the very recent finding that cytokinin receptors are localized to ER will not only deepen our understanding about cytokinin signaling, but also raise interesting research questions. These recent advances will lead to more profound findings in cytokinin signaling and provide a new perspective in decoding the complex interaction between cytokinin signaling pathway and other signaling pathways.

Acknowledgments We thank all members of the Rashotte laboratory for critical reading of this manuscript.

References

Acharya B, Assmann S (2009) Hormone interactions in stomatal function. Plant Mol Biol 69:451–462

Anantharaman V, Aravind L (2001) The CHASE domain: a predicted ligand-binding module in plant cytokinin receptors and other eukaryotic and bacterial receptors. Trends Biochem Sci 26:579–582

Beier D, Gross R (2006) Regulation of bacterial virulence by two-component systems. Curr Opin Microbiol 9:143–152

Bishopp A, Lehesranta S, Vatén A, Help H, El-Showk S, Scheres B, Helariutta K, Mähönen AP, Sakakibara H, Helariutta Y (2011) Phloem-transported cytokinin regulates polar auxin transport and maintains vascular pattern in the root meristem. Curr Biol 21:927–932

Brenner WG, Romanov GA, Kollmer I, Burkle L, Schmulling T (2005) Immediate-early and delayed cytokinin response genes of *Arabidopsis thaliana* identified by genome-wide expression profiling reveal novel cytokinin-sensitive processes and suggest cytokinin action through transcriptional cascades. Plant J 44:314–333

Caesar K, Thamm AMK, Witthöft J, Elgass K, Huppenberger P, Grefen C, Horak J, Harte K (2011) Evidence for the localization of the *Arabidopsis* cytokinin receptors AHK3 and AHK4 in the endoplasmic reticulum. J Exp Bot 62:5571–5580

Cano-Delgado AI, Metzlaff K, Bevan MW (2000) The eli1 mutation reveals a link between cell expansion and secondary cell wall formation in *Arabidopsis thaliana*. Development 127:3395–3405

Catlett NL, Yoder OC, Turgeon BG (2003) Whole-genome analysis of two-component signal transduction genes in fungal pathogens. Eucaryot Cell 2:1151–1161

Chang C, Kwok SF, Bleecker AB, Meyerowitz EM (1993) *Arabidopsis* ethylene-response gene ETR1: similarity of product to two-component regulators. Science 262:539–544

Coba de la Peña T, Cárcamo CB, Almonacid L, Zaballos A, Lucas MM, Balomenos D, Pueyo JJ (2008) A salt stress-responsive cytokinin receptor homologue isolated from *Medicago sativa* nodules. Planta 227:769–779

Cutcliffe JW, Hellmann E, Heyl A, Rashotte AM (2011) CRFs form protein–protein interactions with each other and with members of the cytokinin signalling pathway in Arabidopsis via the CRF domain. J Exp Bot 62:4995–5002

Dello Ioio R, Linhares FS, Scacchi E, Casamitjana-Martinez E, Heidstra R, Costantino P, Sabatini S (2007) Cytokinins determine Arabidopsis root-meristem size by controlling cell differentiation. Curr Biol 17:678–682

Deng Y, Dong H, Mu J, Ren B, Zheng B, Ji Z, Yang WC, Liang Y, Zuo J (2010) *Arabidopsis* histidine kinase CKI1 acts upstream of histidine phosphotransfer proteins to regulate female gametophyte development and vegetative growth. Plant Cell 22:1232–1248

Dortay H, Mehnert N, Bürkle L, Schmülling T, Heyl A (2006) Analysis of protein interactions within the cytokinin-signaling pathway of *Arabidopsis thaliana*. FEBS J 273:4631–4644

Dortay H, Gruhn N, Pfeifer A, Schwerdtner M, Schmülling T, Heyl A (2008) Toward an interaction map of the two-component signaling pathway of *Arabidopsis thaliana*. J Proteome Res 7:3649–3660

Ferreira FJ, Kieber JJ (2005) Cytokinin signaling. Curr Opin Plant Biol 8:518–525

Gan S, Amasino RM (1995) Inhibition of leaf senescence by autoregulated production of cytokinin. Science 270:1986–1988

Gonzalez-Rizzo S, Crespi M, Frugier F (2006) The *Medicago truncatula* CRE1 cytokinin receptor regulates lateral root development and early symbiotic interaction with *Sinorhizobium meliloti*. Plant Cell 18:2680–2693

Haberer G, Kieber JJ (2002) Cytokinins: new insights into a classic phytohormone. Plant Physiol 128:354–362

Han Q, Jiang H, Qi X, Yu J, Wu P (2004) A CHASE domain containing protein kinase OsCRL4, represents a new AtCRE1-like gene family in rice. J. Zhejiang Univ Sci A 5:629–633

Hejátko J, Ryu H, Kim GT, Dobesova R, Choi S, Choi SM, Soucek P, Horak J, Pekarova B, Palme K et al (2009) The histidine kinases cytokinin-independent1 and *Arabidopsis* histidine kinase 2 and 3 regulate vascular tissue development in *Arabidopsis* shoots. Plant Cell 21:2008–2021

Heyl A, Schmulling T (2003) Cytokinin signal perception and transduction. Curr Opin Plant Biol 6:480–488

Heyl A, Wulfetange K, Pils B, Nielsen N, Romanov GA, Schmulling T (2007) Evolutionary proteomics identifies amino acids essential for ligand-binding of the cytokinin receptor CHASE domain. BMC Evol Biol 7:62–69

Higuchi M, Pischke MS, Mahonen AP, Miyawaki K, Hashimoto Y, Seki M, Kobayashi M, Shinozaki K, Kato T, Tabata S, Helariutta Y, Sussman MR, Kakimoto T (2004) *In planta* functions of the *Arabidopsis* cytokinin receptor family. Proc Natl Acad Sci USA 101:8821–8826

Hoth S, Ikeda Y, Morgante M, Wang X, Zuo J, Hanafey MK, Gaasterland T, Tingey SV, Chua NH (2003) Monitoring genome-wide changes in gene expression in response to endogenous cytokinin reveals targets in *Arabidopsis thaliana*. FEBS Lett 554:373–380

Hothorn M, Dabi T, Chory J (2011) Structural basis for cytokinin recognition by *Arabidopsis thaliana* histidine kinase 4. Nat Chem Biol 7:766–768

Hradilová J, Brzobohatý B (2007) Expression pattern of the AHP gene family from *Arabidopsis thaliana* and organ specific alternative splicing in the AHP5 gene. Biol Plant 51:257–267

Hua J, Meyerowitz EM (1998) Ethylene responses are negatively regulated by a receptor gene family in *Arabidopsis thaliana*. Cell 94:261–271

Hutchison CE, Li J, Argueso C, Gonzalez M, Lee E, Lewis MW, Maxwell BB, Perdue TD, Schaller GE, Alonso JM, Ecker JR, Kieber JJ (2006) The *Arabidopsis* histidine phosphotransfer proteins are redundant positive regulators of cytokinin signaling. Plant Cell 18:3073–3087

Hwang I, Sheen J (2001) Two-component circuitry in *Arabidopsis* cytokinin signal transduction. Nature 413:383–389

Inoue T, Higuchi M, Seki M, Hashimoto Y, Kobayashi M, Kato T, Tabata S, Shinozaki K, Kakimoto T (2001) Identification of CRE1 as a cytokinin receptor from *Arabidopsis*. Nature 409:1060–1063

Ito Y, Kurata N (2006) Identification and characterization of cytokinin signaling gene families in rice. Gene 382:57–65

Jeon J, Kim NY, Kim S, Kang NY, Novak O, Ku SJ, Cho C, Lee DJ, Lee EJ, Strnad M, Kim J (2010) A subset of cytokinin two component signalling system plays a role in cold temperature stress response in *Arabidopsis*. J Biol Chem 285:23371–23386

Jung KW, Oh SI, Kim YY, Yoo KS, Cui MH, Shin JS (2008) *Arabidopsis* histidine-containing phosphotransfer factor 4 (AHP4) negatively regulates secondary wall thickening of the anther endothecium during flowering. Mol Cells 25:294–300

Kakimoto T (1996) CKI1, a histidine kinase homolog implicated in cytokinin signal-transduction. Science 274:982–985

Kakimoto T (1998) Genes involved in cytokinin signal transduction. J Plant Res 111:261–265

Kieber JJ, Schaller GE (2010) The perception of cytokinin: a story 50 years in the making. Plant Physiol 154:487–492

Kim HJ, Ryu H, Hong SH, Woo HR, Lim PO, Lee IC, Sheen J, Nam HG, Hwang I (2006) Cytokinin-mediated control of leaf longevity by AHK3 through phosphorylation of ARR2 in *Arabidopsis*. Proc Natl Acad Sci USA 103:814–819

Lara MEB, Garcia MCG, Fatima T, Ehne R, Lee TK, Proels R, Tanner W, Roitsch T (2004) Extracellular invertase is an essential component of cytokinin-mediated delay of senescence. Plant Cell 16:1276–1287

Le DN, Nishiyama R, Watanabe Y, Mochida K, Yamaguchi-Shinozaki K, Shinozaki K, Tran LSP (2011) Genome-wide expression profiling of soybean two-component system genes in soybean root and shoot tissues under dehydration stress. DNA Res 18:17–29

Lee RH, Wang CH, Huang LT, Chen SCG (2001) Leaf senescence in rice plants: cloning and characterization of senescence up-regulated genes. J Exp Bot 52:1117–1121

Lim PO, Lee IC, Kim J, Kim HJ, Ryu JS, Woo HR, Nam HG (2010) Auxin response factor 2 (ARF2) plays a major role in regulating auxin-mediated leaf longevity. J Exp Bot 61:1419–1430

Lohrmann J, Sweere U, Zabaleta E, Baurle I, Keitel C, Kozma-Bognar L, Brennicke A, Schafer E, Kudla J, Harter K (2001) The response regulator ARR2: a pollenspecific transcription factor involved in the expression of nuclear genes for components of mitochondrial complex I in *Arabidopsis*. Mol Genet Genomics 265:2–13

Lomin SN, Yonekura-Sakakibara K, Romanov GA, Sakakibara H (2011) Ligand-binding properties and subcellular localization of maize cytokinin receptors. J Exp Bot 62:5149–5159

Mähönen AP, Bonke M, Kauppinen L, Marjukka R, Benfey PN, Helariutta Y (2000) A novel two-component hybrid molecule regulates vascular morphogenesis of the Arabidopsis root. Genes Dev 14:2938–2943

Mähönen AP, Higuchi M, Tormakangas K, Miyawaki K, Pischke MS, Sussman MR, Helariutta Y, Kakimoto T (2006) Cytokinins regulate a bidirectional phosphorelay network in *Arabidopsis*. Curr Biol 16:1116–1122

Merchan F, De Lorenzo L, González-Rizzo S, Niebel A, Manyani H, Frugier F, Sousa C, Crespi M (2007) Identification of regulatory pathways involved in the reacquisition of root growth alter salt stress in *Medicago truncatula*. Plant J 51:1–17

Merewitz E, Gianfagna T, Huang B (2010) Effects of SAG12-ipt and HSP18.2-ipt expression on cytokinin production, root growth and leaf senescence in creeping bentgrass exposed to drought stress. J Am Soc Hortic Sci 135:230–239

Mok DW, Mok MC (2001) Cytokinin metabolism and action. Ann Rev Plant Phys Plant Mol Bio 89:89–118

Mougel C, Zhulin IB (2001) CHASE: an extracellular sensing domain common to transmembrane receptors from prokaryotes, lower eukaryotes and plants. Trends Biochem Sci 26:582–584

Nishimura C, Ohashi Y, Sato S, Kato T, Tabata S, Ueguchi C (2004) Histidine kinase homologs that act as cytokinin receptors possess overlapping functions in the regulation of shoot and root growth in *Arabidopsis*. Plant Cell 16:1365–1377

Pareek A, Singh A, Kumar M, Kushwaha HR, Lynn AM, Singla-Pareek SL (2006) Whole-genome analysis of *Oryza sativa* reveals similar architecture of two component signaling machinery with *Arabidopsis*. Plant Physiol 142:380–397

Pils B, Heyl A (2009) Unraveling the evolution of cytokinin signaling. Plant Physiol 151:782–791

Punwani JA, Hutchison CE, Schaller GE, Kieber JJ (2010) The subcellular distribution of the *Arabidopsis* histidine phosphotransfer proteins is independent of cytokinin signaling. Plant J 62:473–482

Quail PH (2002) Photosensory perception and signalling in plant cells: new paradigms? Curr Opin Cell Biol 14:180–188

Quirino BF, Noh YS, Himelblau E, Amasino RM (2000) Molecular aspects of leaf senescence. Trends Plant Sci 5:278–282

Rashotte AM, Goertzen LR (2010) The CRF domain defines cytokinin response factor proteins in plants. BMC Plant Biol 10:74–83

Rashotte AM, Carson SD, To JP, Kieber JJ (2003) Expression profiling of cytokinin action in *Arabidopsis*. Plant Physiol 132:1998–2011

Rashotte AM, Mason MG, Hutchison CE, Ferreira FJ, Schaller GE, Kieber JJ (2006) A subset of *Arabidopsis* AP2 transcription factors mediates cytokinin responses in concert with a two-component pathway. Proc Natl Acad Sci USA 103:11081–11085

Riefler M, Novak O, Strnad M, Schmulling T (2006) *Arabidopsis* cytokinin receptor mutants reveal functions in shoot growth, leaf senescence, seed size, germination, root development, and cytokinin metabolism. Plant Cell 18:40–54

Romanov GA, Spíchal L, Lomin SN, Strnad M, Schmülling T (2005) A live cell hormone-binding assay on transgenic bacteria expressing a eukaryotic receptor protein. Anal Biochem 347:129–134

Romanov GA, Lomin SN, Schmülling T (2006) Biochemical characteristics and ligand-binding properties of Arabidopsis cytokinin receptor AHK3 compared to CRE1/AHK4 as revealed by a direct binding assay. J Exp Bot 57:4051–4058

Scharein B, Groth G (2011) Phosphorylation alters the interaction of the *Arabidopsis* phosphotransfer protein AHP1 with its sensor kinase ETR1. PLoS ONE 6:e24173

Scharein B, Voet-van-Vormizeele J, Harter K, Groth G (2008) Ethylene signaling: identification of a putative ETR1–AHP1 phosphorelay complex by fluorescence spectroscopy. Anal Biochem 377:72–76

Schepens I, Duek P, Fankhauser C (2004) Phytochrome-mediated light signalling in *Arabidopsis*. Curr Opin Plant Biol 7:564–569

Smart CM (1994) Gene expression during leaf senescence. New Phytol 126:419–448

Spíchal L, Rakova NY, Riefler M, Mizuno T, Romanov GA, Strnad M, Schmulling T (2004) Two cytokinin receptors of *Arabidopsis thaliana*, CRE1/AHK4 and AHK3, differ in their ligand specificity in a bacterial assay. Plant Cell Physiol 45:1299–1305

Stolz A, Riefler M, Lomin SN, Achazi K, Romanov GA, Schmülling T (2011) The specificity of cytokinin signalling in *Arabidopsis thaliana* is mediated by differing ligand affinities and expression profiles of the receptors. Plant J 67:157–168

Strasser B, Sanchez-Lamas M, Yanovsky MJ, Casal JJ, Cerdan PD (2010) *Arabidopsis thaliana* life without phytochromes. Proc Natl Acad Sci USA 107:4776–4781

Sugawara H, Kawano Y, Hatakeyama T, Yamaya T, Kamiya N, Sakakibara H (2005) Crystal structure of the histidine-containing phosphotransfer protein ZmHP2 from maize. Protein Sci 14:202–208

Suzuki T, Imamura A, Ueguchi C, Mizuno T (1998) Histidine containing phosphotransfer (HPt) signal transducers implicated in His-to-Asp phosphorelay in *Arabidopsis*. Plant Cell Physiol 39:1258–1268

Suzuki T, Sakurai K, Imamura A, Nakamura A, Ueguchi C, Mizuno T (2000) Compilation and characterization of histidine-containing phosphotransmitters implicated in His-to-Asp phosphorelay in plants: AHP signal transducers of *Arabidopsis thaliana*. Biosci Biotechnol Biochem 6:2486–2489

Suzuki T, Miwa K, Ishikawa K, Yamada H, Aiba H, Mizuno T (2001a) The *Arabidopsis* sensor His-kinase, AHK4, can respond to cytokinins. Plant Cell Physiol 42:107–113

Suzuki T, Sakurai K, Ueguchi C, Mizuno T (2001b) Two types of putative nuclear factors that physically interact with histidine-containing phosphotransfer (Hpt) domains, signaling mediators in His-to-Asp phosphorelay, in *Arabidopsis thaliana*. Plant Cell Physiol 42:37–45

Suzuki T, Ishikawa K, Yamashino T, Mizuno T (2002) An *Arabidopsis* histidine-containing phosphotransfer (HPt) factor implicated in phosphorelay signal transduction: overexpression of AHP2 in plants results in hypersensitiveness to cytokinin. Plant Cell Physiol 43:123–129

Tanaka Y, Suzuki T, Yamashino T, Mizuno T (2004) Comparative studies of the AHP histidine-containing phosphotransmitters implicated in His-to-Asp phosphorelay in *Arabidopsis thaliana*. Biosci Biotechnol Biochem 68:462–465

Tanaka Y, Sano T, Tamaoki M, Nakajima N, Kondo N, Hasezawa S (2006) Cytokinin and auxin inhibit abscisic acid-induced stomatal closure by enhancing ethylene production in *Arabidopsis*. J Exp Bot 57:2259–2266

Tirichine L, Sandal N, Madsen LH, Radutoiu S, Albrektsen AS, Sato S, Asamizu E, Tabata S, Stougaard J (2007) A gain-of-function mutation in a cytokinin receptor triggers spontaneous root nodule organogenesis. Science 315:104–107

To JP, Kieber JJ (2008) Cytokinin signaling: two-components and more. Trends Plant Sci 13:85–92

Tran LSP, Urao T, Qin F, Maruyama K, Kakimoto T, Shinozaki K, Yamaguchi Shinozaki K (2007) Functional analysis of AHK1/ATHK1 and cytokinin receptor histidine kinases in response to abscisic acid, drought, and salt stress in *Arabidopsis*. Proc Natl Acad Sci USA 104:20623–20628

Ueguchi C, Koizumi H, Suzuki T, Mizuno T (2001a) Novel family of sensor histidine kinase genes in *Arabidopsis thaliana*. Plant Cell Physiol 42:231–235

Ueguchi C, Sato S, Kato T, Tabata S (2001b) The AHK4 gene involved in the cytokinin-signaling pathway as a direct receptor molecule in *Arabidopsis thaliana*. Plant Cell Physiol 42:751–755

Urao T, Yakubov B, Satoh R, Yamaguchi-Shinozaki K, Seki M, Hirayama T, Shinozaki K (1999) A transmembrane hybrid-type histidine kinase in *Arabidopsis* functions as an osmosensor. Plant Cell 11:1743–1754

Urao T, Miyata S, Yamaguchi-Shinozaki K, Shinozaki K (2000) Possible His to Asp phosphorelay signaling in an *Arabidopsis* two-component system. FEBS Lett 478:227–232

Werner T, Schmülling T (2009) Cytokinin action in plant development. Curr Opin Plant Biol 12:527–538

West AH, Stock AM (2001) Histidine kinases and response regulator proteins in two-component signaling systems. Trends Biochem Sci 26:369–376

Wulfetange K, Lomin SN, Romanov GA, Stolz A, Heyl A, Schmülling T (2011) The cytokinin receptors of *Arabidopsis* are located mainly to the endoplasmic reticulum. Plant Physiol 156:1808–1818

Yamada H, Suzuki T, Terada K, Takei K, Ishikawa K, Miwa K, Yamashino T, Mizuno T (2001) The *Arabidopsis* AHK4 histidine kinase is a cytokinin-binding receptor that transduces cytokinin signals across the membrane. Plant Cell Physiol 42:1017–1023

Yonekura-Sakakibara K, Kojima M, Yamaya T, Sakakibara H (2004) Molecular characterization of cytokinin-responsive histidine kinases in maize. Differential ligand preferences and response to *cis*-zeatin. Plant Physiol 134:1654–1661

Zimmermann P, Hirsch-Hoffmann M, Hennig L, Gruissem W (2004) GENEVESTIGATOR. *Arabidopsis* microarray database and analysis toolbox. Plant Physiol 136:2621–2632