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PERCEPTION AND SIGNAL TRANSDUCTION OF CYTOKININS

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■ **Abstract** Cytokinins are plant hormones implicated in diverse and essential processes in plant growth and development, and key genes for the metabolism and actions of cytokinins have recently been identified. Cytokinins are perceived by three histidine kinases—CRE1/WOL/AHK4, AHK2, and AHK3—which initiate intracellular phosphotransfer. The final destination of the transferred phosphoryl groups is response regulators. The type-B *Arabidopsis* response regulators (ARRs) are DNA-binding transcriptional activators that are required for cytokinin responses. On the other hand, the type-A ARRs act as repressors of cytokinin-activated transcription. How phosphorelay regulate response regulators and how response regulators control downstream events are open questions and discussed in this review.

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INTRODUCTION

Cytokinins are N^6-substituted adenine derivatives that have diverse effects on important physiological functions in plants (60). For example, they induce cell division and de novo shoot formation (56, 87), delay senescence (17, 70), activate dormant lateral buds (64), and increase sink strength (73). Widespread, unmodified cytokinin bases are isopentenyladenine and *trans*-zeatin. Ribose or ribose-5′-phosphate may be attached at the N^9 atom of the adenine ring to form cytokinin ribosides or ribotides (Figure 1), and these also generally show cytokinin activity when applied to plants. However, because applied cytokinins undergo interconversion, the actual active forms were not known until recently. Recent receptor-binding assays have shown that the active forms are the free-base cytokinins isopentenyladenine and *trans*-zeatin (115). Cytokinins are inactivated by *O*-glycosylation at the terminal hydroxyl group of the zeatin-type cytokinins or by *N*-glycosylation at the N^3 or N^7 positions of the adenine ring. *O*-glycosylation is reversible and *O*-glycosylated cytokinins are regarded as a storage form. The cytokinin ribosides and *cis*-zeatin, sometimes found in abundance in plants, may also be important as stored or transportable forms. Because cytokinins exist in the apoplasm as well as in the cytoplasm, specific transmembrane transporters for cytokinins may exist. Cytokinin oxidase/dehydrogenase degrades cytokinins by cleaving the side chain (3, 23, 62). Cytokinin metabolism has been reviewed in detail (59).

Until recently, cytokinins were the least understood plant hormones in regard to biosynthesis, metabolism, perception, and signal transduction. However, there were very rapid progresses in cytokinin research including identification of enzymes for the rate-limiting step of cytokinin biosynthesis (35, 98), *O*-glycosylation enzymes (52–54), cytokinin oxidases/dehydrogenases (23, 62), cytokinin receptors (32, 95, 104), and downstream signaling elements (28, 76). This review emphasizes perception and signal transduction of cytokinins. Because it is now evident that cytokinins are perceived by histidine kinases and transduced by a

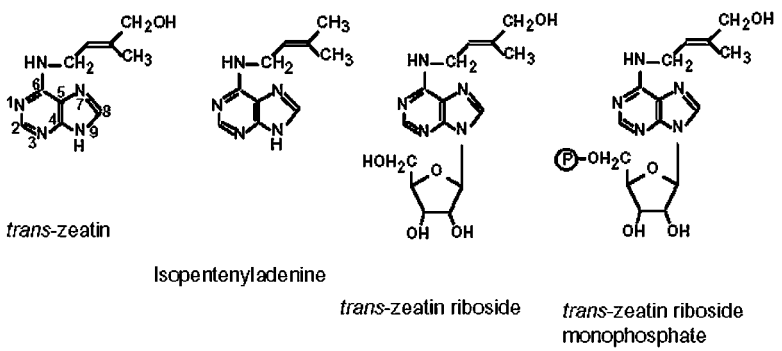

Figure 1 Structures of representative cytokinins.

two-component signaling system, I first outline the general features of the two-component system.

THE TWO-COMPONENT SYSTEM

Signal-induced phosphorylation of proteins is an often-used regulatory mechanism to transduce intracellular or extracellular signals. In animals, phosphorylation on a hydroxyl group of serine (Ser), threonine (Thr), or tyrosine (Tyr) residues is predominantly used. By contrast, in bacteria, phosphorylation on a nitrogen atom of a histidine (His) residue and on an acyl group of an aspartate (Asp) residue is predominantly used (41). The mode of signaling that uses the latter type of phosphorylation has been referred to as the two-component system. The two-component system is prevalent in most bacteria. For instance, in *Escherichia coli* about 1% of the genes code for two-component proteins (58). Until 1993, it was thought that the two-component system was present only in prokaryotes; then two histidine kinases, the ethylene receptor ETR1 of *Arabidopsis* (7) and the osmosensor Sln1 of the budding yeast *Saccharomyces cerevisiae* (48, 66), were found. It is now known that eubacteria, archaea, fungi, and plants also have the two-component system, but no homologous proteins for this system are known in animals, including *Drosophila melangaster*, *Caenorhabditis elegans*, and *Homo sapiens*, for which entire genomes have been sequenced.

The architecture of the two-component systems is modular, and component proteins are made up of various combinations of conserved domains and variable domains (Figure 2). As the name indicates, prototypical two-component systems consist of two proteins, the histidine kinase and the response regulator (RR). Most histidine kinases are transmembrane receptors with a signal-sensing domain (input domain) positioned in the extracellular space and a signal-transducing domain (transmitter domain) positioned in the cytoplasm. RRs are characterized by the presence of receiver domains. Most RRs in prokaryotes additionally have an output (effector) domain that regulates downstream events, but some RRs consist of a receiver domain only [e.g., CheY (90)]. Most output domains in bacteria are DNA-binding transcriptional regulators, but some output domains have other functions, such as acting as enzymes. When an histidine kinase senses a signal, the conserved His residue in the transmitter domain is phosphorylated. Histidine kinases are dimers, and one subunit of an histidine kinase phosphorylates a conserved His residue of the other subunit by using ATP as the phospho-donor. The phosphoryl group is then transferred to the conserved Asp residue of the receiver domain. Phosphorylation of the receiver domain modulates the activity of the attached output domain, or a separate protein if the response regulator consists solely of the receiver domain.

Some bacterial and all eukaryotic two-component systems use more complicated multistep phosphorelay systems (Figure 2*B*). In these systems, the sensor is usually a hybrid histidine kinase that additionally carries a receiver domain. Another domain, HPt, which contains a phosphorylatable His residue, also participates

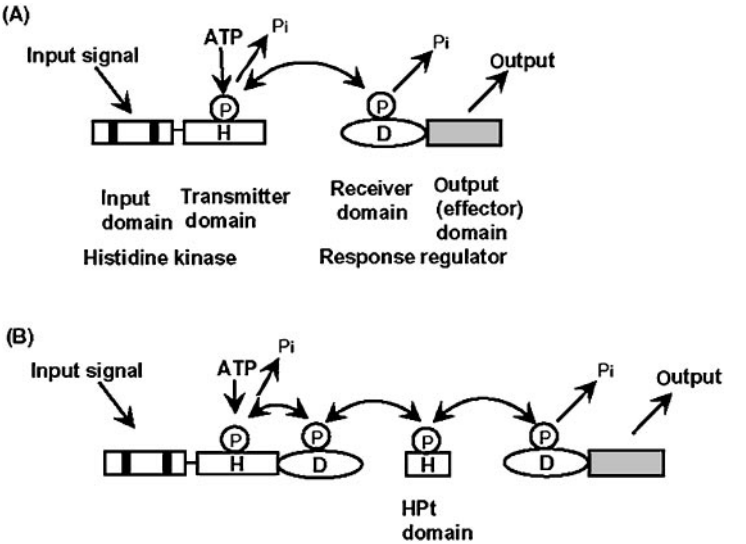

Figure 2 General models of two-component systems. (*A*) The prototypical two-component system. When a histidine kinase perceives the corresponding signal, the conserved histidine residue is phosphorylated. The phosphoryl group is then transferred to the response regulator, which in turn regulates the attached output domain. The phosphotransfer is bidirectional. (*B*) Multistep phosphorelay system. Another domain, the HPt domain, participates in the phosphoryl group. Black vertical bars, transmembrane segments. H and D are the histidine and aspartate residues, respectively, of the phosphorylation sites. The encircled P denotes a phosphoryl group. Pi is the inorganic phosphate.

in the phosphotransfer. The primary sequence of the HPt domain weakly resembles that of a part of the transmitter domain encompassing the phosphorylate His residue, but their three-dimensional structures are strikingly similar (37). The phosphoryl group is relayed alternately between conserved His residues and conserved Asp residues (Figure 2*B*). The phosphoryl group is linked by a high-energy bond, and the phosphotransfer is bidirectional. Many histidine kinases have the phosphatase activity also and the kinase/phosphatase ratio is regulated by an input signal. At the phosphatase-dominated state, the histidine kinase may serve as a drain of the phosphoryl group. The response regulator also has inherent phosphatase activity, which affects the half-life of phosphorylated state (Figure 2*A*,*B*). For more information please refer to recent reviews (90, 91, 110, 113).

CYTOKININ RECEPTORS ARE HISTIDINE KINASES

The first indication that cytokinins might be perceived by a two-component system came from the identification of the histidine kinase CKI1 (34). *CKI1* was identified as a responsible gene in the dominant *cki1* mutant, which was identified by

screening a large number of *Arabidopsis* calli that had been transformed with a strong transcriptional enhancer for those calli that constitutively exhibited cytokinin responses. When overexpressed in plants, *CKI1* induced typical cytokinin responses independently of cytokinins (28, 34). CKI1 is a candidate cytokinin receptor because it is a histidine kinase and its overexpression caused cytokinin responses. However, no additional data supports CKI1's being a cytokinin receptor. Rather, CKI1 is constitutively active as a histidine kinase when expressed in *E. coli* (115) or in *S. cerevisiae* (T. Kakimoto, unpublished data), which contrasts with the activation of CRE1/WOL/AHK3 by cytokinins in these organisms, as described below. *CKI1* is normally expressed only in the female gametophytes and the endosperm of immature seeds, and *CKI1*-disruptants of *Arabidopsis* are lethal to female gametophytes (66a). It is possible that *CKI1*-mediated constitutive activation of cytokinin-signaling pathway is required for the development of the female gametophytes. It is also possible that the role of CKI1 is unrelated to cytokinins, but that overexpression of *CKI1* caused unexpected crosstalk with the cytokinin signaling pathway, eliciting cytokinin responses.

Genuine cytokinin receptors were later found during several lines of research using forward and reverse genetics. Inoue et al. screened *Arabidopsis* calli for those that were resistant to cytokinins in tissue culture and identified a mutant named *cytokinin response 1-1* (*cre1-1*) (32). The responsible gene *CRE1* (32) is identical to *WOL* (49) and *AHK4* (103), and codes for a histidine kinase. T-DNA insertion mutants for *CRE1/WOL/AHK4* were also cytokinin resistant (32, 104). To determine the molecular function of *CRE1/WOL/AHK4*, a mutant of *S. cerevisiae* was used, in which the only histidine kinase gene, *Sln1*, was disrupted (48, 68). Disruption of *Sln1* is lethal to yeast, owing to the lack of phosphotransfer. When introduced into an *sln1* mutant, *CRE1/WOL/AHK4* rescued the lethality only in the presence of cytokinins (32, 90, 104). Similarly, CRE1/WOL/AHK4 functioned, in a cytokinin-dependent manner, as a histidine kinase in mutants of *Schizosaccharomyces pombe* or *E. coli*, in which a particular histidine kinase gene had been disrupted (95). These results, coupled with the insensitivity to cytokinins of the *Arabidopsis* mutants of *cre1*, provided evidence that CRE1/WOL/AHK4 is a cytokinin receptor. In the yeast system, the conserved His and Asp residues of CRE1/WOL/AHK4 and the HPt domain protein YPD1 of yeast were indispensable for complementation, suggesting that cytokinins activate a phosphorelay (32). Similar cytokinin-dependent activity was also observed for two close relatives of *CRE1/WOL/AHK4*, *AHK2* (M. Higuchi & T. Kakimoto, unpublished data) and *AHK3* (115), indicating that the three proteins (CRE1/WOL/AHK4, AHK2, and AHK3) are cytokinin receptors in *Arabidopsis*. The cytokinin-binding activity of CRE1/WOL/AHK4 was demonstrated by using a membrane fraction obtained from *S. pombe* expressing *CRE1* (115). The membrane fraction bound isopentenyladenine (K_d = 4.6 nM), *trans*-zeatin, benzyladenine, and phenylurea-type cytokinin thidiazuron, but not isopentenyladenosine. These results not only confirmed that CRE1/WOL/AHK4 is a cytokinin receptor, but also showed that it is the cytokinin bases, not ribosides, that are active. More comprehensive analysis,

using diverse cytokinin species, of the binding spectra for these three cytokinin receptors (CRE1/WOL/AHK4, AHK2, and AHK3) would be informative. Interestingly, the extracellular domains at the N-terminal regions of the cytokinin receptors are the Cyclase/Histidine kinase-Associated Sensing Extracellular (CHASE) domain, which is found in diverse receptors of prokaryotes, plants, and the amoeba *Dictyostelium discoideum* (1, 63). The *wooden leg* (*wol*) mutant (see below) carries a mutation in the CHASE domain and is cytokinin resistant, in addition to showing the root phenotype (T. Inoue & T. Kakimoto, unpublished data). The *wol* mutation disrupts the cytokinin-binding activity (115) of CRE1/WOL/AHK4 and disrupts the ability of CRE1/WOL/AHK4 to confer cytokinin dependency on the *sln1* mutant yeast (M. Higuchi & T. Kakimoto, unpublished data), suggesting that the CHASE domain senses cytokinins. Consistent with the notion that *CRE1/WOL/AHK4*, *AHK2*, and *AHK3* code for cytokinin receptors, overexpression of any of these genes increased cytokinin-responsiveness in a protoplast transient assay (28).

Before CRE1/WOL/AHK4 was identified as a cytokinin receptor, the corresponding gene was reported as a responsible gene for the *wol* mutant (49), which was initially identified as a mutant with abnormal roots. The vasculature of a *wol* mutant root consists of a reduced number of cell files, and is composed exclusively of primary xylem—there are no phloem and no parenchyma cells that normally lie between the phloem and the xylem. The *cre1-1* mutant has a similar phenotype (Y. Helariutta, personal communication). Cell divisions that produce root vascular initial cells during embryogenesis and periclinal cell divisions that increase the vascular cell files after germination are both impaired in the *wol* mutant, and these are considered the primary defects in this mutant. Abnormal tissue formation is probably a secondary defect; it is hypothesized that specification of the xylem occurs earlier than specification of other cell types, and the reduced number of cells in the mutants is used up during the specification of xylem. In accordance with this, all cell types in the *wol* mutant were restored when the *fass* mutation, which increases the number of vascular cells, was further introgressed into the mutant (83).

The phenotype in the root was consistent with the expression pattern of the *CRE1* gene. The *CRE1* message was first detected, by in situ RNA hybridization, in the four innermost cells of the globular-stage embryo. From the heart stage onward, it is expressed in the procambium in the cotyledon shoulders, in prospective hypocotyls, and in embryonic roots. After germination, the message is abundant in the procambium and pericycle in the root (49). Also, it appears to be expressed at a high level in the shoot apical meristem, as inferred from the expression pattern of a reporter gene linked under the *CRE1* promoter (K. Miyawaki & T. Kakimoto, unpublished data). The strong expression of *CRE1* in the shoot apical meristem raises the question of why the corresponding mutants have no phenotypes related to the functions of the shoot apical meristem. With regard to this, *AHK3* promoter is active in the shoot apical meristem (K. Miyawaki & T. Kakimoto, unpublished data). Perhaps the three histidine kinases have specific yet overlapping functions.

ROLES OF OTHER HISTIDINE KINASES IN *ARABIDOPSIS*

All two-component regulators and related proteins in *Arabidopsis* have been compiled (27, 82). There are three cytokinin receptors (CRE1/WOL/AHK4, AHK2, and AHK3), five ethylene receptors (ETR1, ERS, ETR2, EIN4, ERS2), five phytochromes (phytochrome A to E), one putative osmosensor (AtHK1), and two other histidine kinases (CKI1 and AHK5/CKI2) of unknown molecular function. Canonical histidine kinases possess five conserved blocks—H, N, G1, F, and G2 (90, 113)—which are necessary for histidine kinase activity (Figure 3). All the conserved blocks are present in the three cytokinin receptors, two of the ethylene receptors (ETR1 and ERS) (4, 7, 24), AtHK1 (107), CKI1 (34), and AHK5/CKI2; however, in three of the ethylene receptors and all of the phytochromes not all

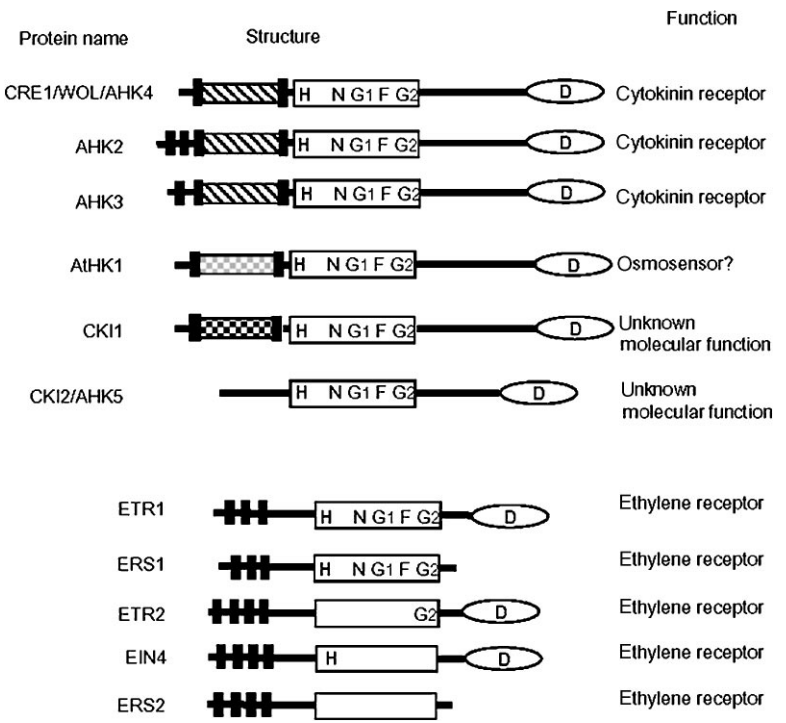

Figure 3 Structural characteristics of histidine kinases and ethylene receptors. *Black rectangle*, transmembrane segment; *striped rectangle*, the extracellular CHASE domain; *checker boxes* between transmembrane segments in CKI1 or AtHK1, extracellular domain; *open rectangle*, the transmitter domain; *H*, *N*, *G1*, *F*, *G2* conserved blocks characteristic of functional histidine kinases are indicated by the letters; *oval*, the receiver domain with the letter *D* indicating the presence of phosphorylatable aspartate residue.

blocks are conserved. Interestingly, ETR1 and ERS can be autophosphorylated at the conserved histidine residue in vitro (16), but other ethylene receptors lacking some of the conserved blocks are also functional as ethylene receptors in plants (25). Bacteriophytochromes, phytochrome-related proteins in bacteria, are canonical histidine kinases (61), but plant phytochromes have significantly diverged and no histidine kinase activity is known. Instead, plant phytochromes have Ser/Thr kinase activity (116). All the cytokinin receptors, CKI1, AtHK1, and all the ethylene receptors each have putative membrane-spanning segments, but CKI2 (AHK5) is predicted to reside in the cytoplasm.

As discussed above, CKI1 is essential for developing female gametophytes, but its molecular function is unclear. *CKI2* was also identified through activation tagging, and overexpression of the cloned *CKI2* induces cytokinin-independent callus growth. However, the molecular function of *CKI2* is also unknown. AtHK1 could function as an osmosensor in yeast (107), and its expression is increased by salt (107) and by cytokinins (Y. Hashimoto & T. Kakimoto, unpublished data).

RESPONSE REGULATORS IN *ARABIDOPSIS*

Arabidopsis has 22 genes for response regulators, which are grouped into two classes, type-A Arabidopsis Response Regulator (ARR) and type-B ARR, on the basis of their domain structures and amino acid sequence similarities (9–11, 21, 26, 27, 29, 40, 82, 86, 109). Products of 11 genes are type-A ARRs, each of which consists of a receiver domain with a short variable extension (less than 90 amino acids) at its carboxyl (C)-terminus (Figure 4*A*); products of the other 11 are type-B ARRs, each of which possesses a DNA-binding domain called GARP (see below) and a variable extension (Figure *4B*). In addition, there are other proteins [*Arabidopsis* pseudo-response regulators (APRRs)] that carry a receiver-like domain without a conserved Asp residue for phosphorylation (Figure 4*C*).

Type-A ARRs

Response regulators in plants were first identified in screenings for cytokinin-inducible genes in *Arabidopsis* (6) and maize (78), as well as in a search of the expressed sequence tag (EST) database (30). Nine type-A ARR genes have been examined for cytokinin inducibility; all were found to be induced by cytokinins. *ARR4* and *ARR5* are also increased by drought, salt, and cold (6, 108). They are all cytokinin primary response genes; cytokinin induction is rapid and resistant to protein synthesis inhibitors (6, 9, 101). The increase in ARR transcript levels in response to cytokinins is due, at least in part, to increased transcription (9). ARR5 transcript was abundant in the shoot and root apical meristems, as shown by in situ RNA hybridization. Also, the activity of the product of the *beta-glucuronidase* (*GUS*) gene expressed under the control of the *ARR5*-promoter was high in the shoot and root apical meristems, and was also detected in the root vascular

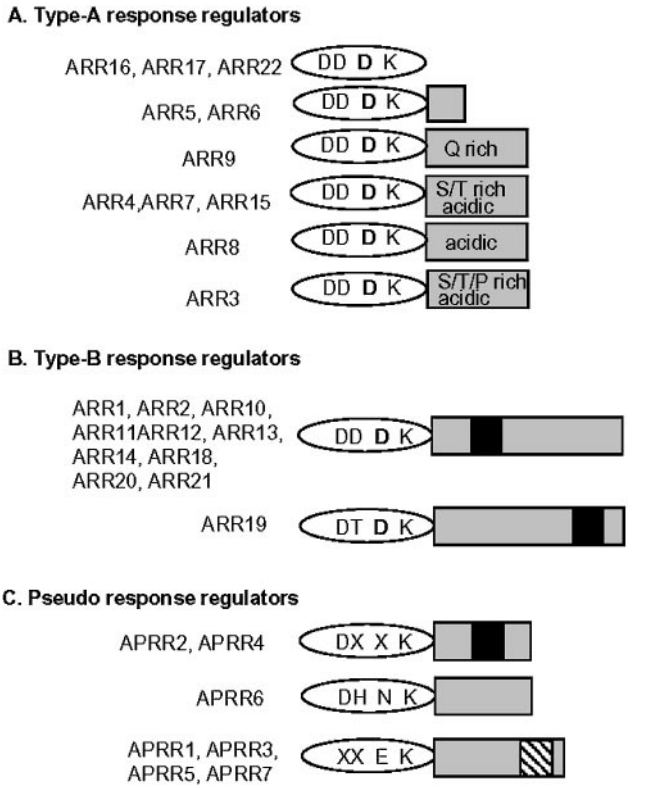

Figure 4 Schematic representation of response regulators and related proteins. (*A*) type-A ARRs, (*B*) type-B ARRs, (*C*) APRRs, (*D*) the site of the aspartate residue for phosphorylation; (*X*) amino acid residues that are different for each of the group members shown on the left. *Ovals*, receiver domains; *filled boxes*, the GARP motif; *hatched box*, the CCT motif; *gray boxes*, variable region.

system and abscission zones (9, 12, 14, 33). Treatment with cytokinins ubiquitously induced the *ARR5* gene (9), indicating that all organs can perceive cytokinins. Transcripts for the type-A response regulators of maize, *ZmRR1* and *ZmRR2*, are also responsive to cytokinins. Unlike with *Arabidopsis ARR4* and *ARR5*, cytokinins induce *ZmRR1* in leaves but not in roots. The protein level of *ZmRR1* was also increased by cytokinins within 1 h (77, 78, 80).

All type-A ARRs tested are also upregulated by mineral nitrogen, such as NO_3^- or NH_4^+, when applied to nitrogen-starved plants. Because a shortage of nitrogen decreases cytokinins, and nitrogen resupply increases cytokinins (81, 99, 100), the up-regulation of the type-A ARRs by nitrogen can be explained by an increased amount of cytokinins (39, 77, 78).

Type-B ARRs

Unlike type-A ARRs, type-B ARRs are not induced by cytokinins (29, 39). Type-B ARRs consist of a receiver domain and a large C-terminal extension, which carries a GARP domain and a C-terminal variable region (Figure *4B*). The GARP domain was identified as a domain common to a class of plant-specific transcription factors and is so named because it is found in GOLDEN2 of maize, ARRs of *Arabidopsis*, and Psr1 of *Chlamydomonas* (71). The C-terminal variable regions of some of these ARRs are rich in glutamine and proline, a feature often observed in transcriptional activators (102). ARR1, ARR2, ARR10, and ARR11 are nuclear-localized when fused to a reporter protein (28, 31, 46, 47, 75). As predicted from their sequences, ARR1 and ARR2 function as transcription factors. In yeast, C-terminal extensions of ARR1, ARR2, and ARR11 function as transcriptional activators when fused to the DNA-binding-domain of GAL4 (46, 75). The activation activities of ARR1 and ARR2 are confined to their C-terminal variable regions, which are rich in glutamine (75). The GARP domain binds to DNA in a sequence-specific manner. Sakai et al. (75) screened random DNA oligomers for sequences that preferentially bound to the GARP domain and identified 5′-AGATT-3′ as the optimal binding sequence. In tobacco cells, ARR1 and ARR2 both activate transcription from a hexamer of the optimal sequence fused to a minimum promoter. Deleting the receiver domains increases transactivation activity, indicating that the receiver domains of ARR1 and ARR2 act negatively on transcriptional activation (75). Lohrmann et al. (47) reported that ARR2 could bind to the promoter sequence of the *PSST* gene of the mitochondrial respiratory complex I (nCI) from *Arabidopsis* in vitro; however, it is an open question as to whether *PSST* genes are regulated by ARR2 in vivo.

APRRs

Each APRR carries a receiver-like domain without the conserved Asp residue for phosphorylation (Figure *4C*). APRR2 and APRR4 carry the GARP domain. *TOC1/APRR1*, *APRR3*, *APRR5*, *APRR7*, and *APRR9* codes for products that carry the CCT motif [a motif common to CO, COL, and TOC1 proteins (92)] in their C-terminal regions and are photoperiodically expressed (50, 51, 55, 92). TOC1/APRR1 is required for normal circadian rhythms (92).

HPt PROTEINS AND PHOSPHOTRANSFER

In almost all cases, the phosphoryl group is transferred alternately between the side chains of His and Asp residues. In *Arabidopsis*, there are five genes for HPt domain proteins, designated AHP1 to AHP5 (57, 93, 96). The ability of AHPs to participate in the phosphorelay has been demonstrated in heterologous organisms and in vitro. AHP1, but not AHP2 or AHP3, suppressed the lethality of an yeast mutant lacking the HPt protein, Ypd1, suggesting that at least AHP1 can function as a phosphotransfer-mediator in yeast (93). The ability of AHPs to perceive the

phosphoryl group from the cytokinin receptor CRE1/WOL/AHK4 was demonstrated, although indirectly, in *E. coli* (94, 95). AHP2 and AHP4 interfered with the artificial CRE1/WOL/AHK4 → YojN → RcsB phosphorelay system in *E. coli*; this was explained by assuming that the phosphoryl group on CRE1/WOL/AHK4 is acquired more readily by AHP2 and AHP4 than by YojN. The effect of AHP1 and AHP3 was weaker. It has been demonstrated that AHP1 can transfer the phosphoryl group to ARR3 and ARR4 (both type-A) (93), and that AHP2 can transfer the phosphoryl group to ARR6 (type-A) and to ARR10 (type-B) (31). *Arabidopsis* transformants overexpressing *AHP2* were hypersensitive specifically to cytokinins, suggesting that AHP2 may mediate phosphorelay initiated by cytokinin receptors. In the yeast two-hybrid system, AHP1 interacted with AtHK1, ETR1, and CKI1, and AHP2 interacted with ETR1 and CKI1, which raises the possibility of signal crosstalk (106).

ROLES OF RESPONSE REGULATORS IN CYTOKININ SIGNALING

Type-A and Type-B ARRs as Transcriptional Regulators

Two laboratories provided compelling evidence that type-B ARRs are key regulators of cytokinin signaling and directly activate type-A *ARR* genes (28, 76). Hwang & Sheen utilized a transient expression system in *Arabidopsis* mesophyll protoplasts, in which the luciferase (LUC) gene linked under the cytokinin-inducible *ARR6* promoter (*ARR6::LUC*) was used as an indicator of cytokinin response. First, they showed that overexpression of *CRE1*, *AHK2*, or *AHK3* increased LUC activity only in the presence of cytokinins. Unphosphorylatable mutants of CRE1, in which either the conserved His or the Asp residues were mutated, not only did not have such activity, but even imposed dominant negative effects. These results confirmed the notion that the cytokinin signal is transduced by activating phosphotransfer. Next, they showed that AHP1 and AHP2, but not AHP5, were translocated into the nucleus in response to cytokinins, raising the idea that AHPs function as shuttles that carry the phosphoryl group from the cytoplasm to the nucleus. Finally, they investigated the functions of response regulators. The expression of *ARR6::LUC* was increased when either *ARR1*, *ARR2*, or *ARR10*, which code for type-B ARRs, was overexpressed, and it was further activated by cytokinins. Overexpression of type-A *ARR*s (*ARR4* to *ARR7*), on the other hand, inhibited cytokinin-induction of *ARR6-LUC*. These results suggested that type-B ARRs are transcriptional activators and type-A ARRs are transcriptional repressors, and the two types of response regulators form a signaling loop.

Overexpression of *ARR1* or *ARR2* affects general cytokinin responses also; it increases the sensitivity of plants to cytokinins for cytokinin-induced callus growth and shoot formation and inhibits root growth (28, 76). The effect of ARR1 is more pronounced when the N-terminal receiver domain is removed, indicating that the receiver domain negatively regulates the output domain (76). The importance of

ARR1 in cytokinin action was made apparent by the observation that *Arabidopsis* deficient in *ARR1* was less sensitive to cytokinins in a number of diverse physiological processes, including callus growth and induction of type-A *ARR*s (76).

This raises the question of how type-B ARRs activate type-A *ARR* genes. Observing that the type-B binding site, 5′-AGATT-3′, occurs multiple times in the promoter of *ARR6* raised the possibility that type-B ARRs directly activate type-A *ARR*s, and this was proven true. Sakai et al. transformed *Arabidopsis* with a fusion gene that expressed the hormone-binding domain of the glucocorticoid receptor (GR) fused to ARR1, which lacks the receiver domain (76). Proteins linked to the glucocorticoid-binding domain are often inactive but are activated when the domain binds glucocorticoids (2, 45, 74). Without glucocorticoids the transformants were normal, but in the presence of a glucocorticoid dexamethasone, (DEX), they exhibited diverse phenotypes indicative of cytokinin responses, such as disordered cell division around the shoot apical meristem. Treating the transformants with DEX induced *ARR4* to *ARR9*, and this was not inhibited by an inhibitor of translation. The above-mentioned results all indicate that cytokinins activate type-B ARRs, which are required for most or all cytokinin responses, and that type-B ARRs directly activate type-A *ARR* genes. Currently, the only known target genes of type-B ARRs are type-A *ARR*s, which appear to function as negative feedback regulators. In the future, additional target genes may be isolated by screening for genes that are induced when the GR-fusion transformants are treated with DEX in the presence of translation inhibitors.

If cytokinin signaling follows the general principles of the two-component signaling system, then phosphorylation of type-B ARRs modulates the activity of the output domains that govern cytokinin responses. However, in the protoplast transient expression experiment (28), overexpression of an unphosphorylatable mutant ARR2 (a type-B ARR) was as effective as that of the wild-type ARR2 in inducing type-A *ARR*s. Importantly, activation of a type-A ARR promoter by either the wild-type or the mutant ARR2 was further activated by cytokinins. Also, both the wild-type and the mutant type-A ARRs inhibited the cytokinin-induction of type-A *ARR*s. Hwang (28) proposed the presence of a hypothetical factor that inhibits type-B ARRs, and that phosphorelay may liberate type-B ARRs through an unknown mechanism. If this is true, at what point does regulation by means of phosphorylation occur? We discuss this below.

Activation of Receiver Domains—Examples from Prokaryotes

Before further discussing possible mechanisms by which phosphorelay activates cytokinin responses, it is useful to examine some examples of bacterial response regulators with regard to how receiver domains regulate effector proteins or domains. When CheY of *E. coli*, a representative response regulator that does not have an apparent output domain, is phosphorylated, its surface undergoes a conformational change (43, 85, 111). The altered surface then interacts with the target effector protein FliM, which in turn regulates the flagellar motor. Receiver domains

of multidomain response regulators appear to interact with and regulate attached effector domains. For example, the surface of the receiver domain of NtrC that interacts with the attached effector domain undergoes a conformational change when the receiver domain is phosphorylated (38), and the interaction is thought to affect the output activity. Phosphorylation-associated dimerization and oligomerization of response regulators are also known. Examples are NtrC (67) and FixJ (8), whose transcriptional functions are activated after they oligomerize and dimerize, respectively. These and other examples have revealed that different mechanisms are used to regulate the activity of effector domains with the common feature that they rely on phosphorylation-dependent domain-domain interactions (90, 113). Considering the versatility of receiver domains that have evolved to utilize diverse mechanisms for regulation of effector domains and the number of examples of bacterial response regulators that undergo phosphorylation-dependent multimerization, it is also possible that type-A and type-B response regulators form dimers or multimers in diverse combinations, allowing sophisticated output.

Possible Mechanisms of Response Regulator Activation in Plants

As Kieber and colleagues pointed out (11, 21, 26, 40), overall design of cytokinin signaling is strikingly similar to that of auxin signaling, although their components belong to completely different protein families. Auxin signaling involves two types of transcriptional regulators, the AUX/IAA family and the DNA-binding ARF family (44). Most ARFs are transcriptional activators, and AUX/IAAs are transcriptional repressors. While most AUX/IAAs are induced by auxin, ARFs are not. AUX/IAAs and ARFs can form homodimers and heterodimers. A simplified model is that dimers consisting of different combinations of ARFs and AUX/IAA are formed; homodimers and heterodimers consisting of only ARFs are active, while AUX/IAAs act negatively by forming heterodimers with ARFs (44). The auxin signal is transduced by targeting AUX/IAA proteins for 26S proteasome-dependent degradation (20). This architecture is similar to cytokinin signaling in that type-B ARRs are transcriptional activators and their genes are not influenced by cytokinins, while type-A ARRs are transcriptional repressors and their genes are induced by cytokinins.

As discussed above, overexpression of either phosphorylatable or unphosphorylatable type-B ARRs was able to activate transcription of *ARR6::LUC*, which was further activated by cytokinins (28). In other words, an even unusually large amount of unphosphorylated type-B ARR is still controllable by phosphotransfer. On the other hand, both phosphorylatable and unphosphorylatable type-A ARRs repressed transcription of *ARR6::LUC*. Considering the above results, the most plausible point in the response regulator loop for regulation by means of phosphorylation would be type-A ARRs; unphosphorylated type-A ARRs act negatively on type-B-mediated transcription, and phosphorylation of type-A ARRs may relieve their negative action (Figure 5). Because type-A ARRs are increased by

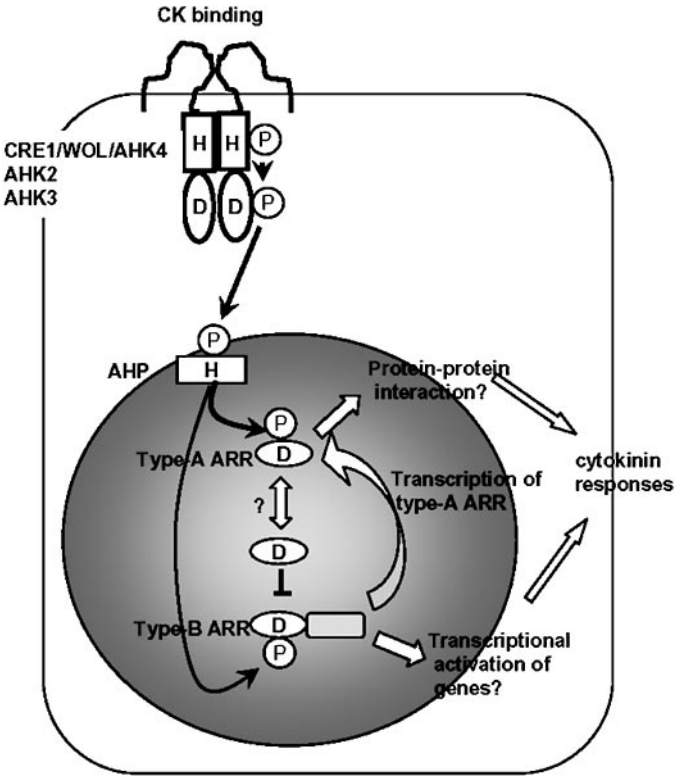

Figure 5 A model of cytokinin signal transduction in *Arabidopsis*. The three cytokinin receptors—CRE1/WOL/AHK4, AHK2, and AHK3—bind to cytokinins and initiate phosphotransfer. The phosphoryl group, indicated by *encircled letter P*, is then transferred to the HPt domain proteins, AHPs. Phosphorylated AHPs are translocated to the nuclei and probably transfer the phosphoryl group to type-A ARRs and/or type-B ARRs. How phosphorylation regulates transctiptional activity of type-B ARRs is an open question. In this model, type-A ARRs function as a molecular switch of type-B ARR activity. Type-A and/or type-B ARRs may have output functions, which is also not well understood.

type-B ARs, even overproduced type-B ARRs could still be controllable by type-A ARRs. If this hypothesis is correct, type-A ARRs not only offer a negative feedback mechanism, but may even be a master switch. This does not exclude the possibility that phosphorylation of some type-B ARRs may have regulatory functions.

Of course, the above hypothesis may be oversimplified. First, no interaction between the ARR members has been reported. Second, individual members in either type of ARR may have overlapping yet distinct functions. Distinct functions between members of the type-A ARR family have been suggested from

Arabidopsis overexpressing type-A *ARR*s. *Arabidopsis* that overexpressed *ARR4* exhibited increased shoot regeneration frequency in tissue culture, whereas those overexpressing *ARR8* exhibited decreased regeneration frequency in response to cytokinins (65). This also parallels auxin signaling: Some ARFs are transcriptional repressors and others are activators (105).

Protein degradation can also play an important role in cytokinin signaling (88). An *Arabidopsis* loss-of-function mutant of the 26S proteasome subunit RPN12 was less responsive to cytokinins, suggesting that cytokinin signaling may involve proteasome-mediated degradation of a negative regulator. Auxin signaling is initiated by ubiquitination of the negative regulators (AUX/IAA proteins) and targeting them to proteasome-mediated degradation pathways (44). Gibberellin also appears to initiate signaling by destroying negative regulators (13). By analogy then, can a proteasome degrade type-A ARR proteins in a cytokinin-dependent manner, in turn liberating type-B ARRs? This scenario may not be plausible because application of cytokinin also increases the protein levels of type-A response regulators, at least in maize (78). The unaffected level of *ARR5* transcript in the *rpn12a* mutant also suggests that it is not very likely because it is expected that the transcript level of type-A *ARR*s would be affected if protein levels of the type-A *ARR*s, which act as negative regulators, were altered in the mutant.

What and Where is the Output?

An important and unanswered question is which type of ARR is linked to downstream events. One plausible scenario is that type-B ARRs regulate unidentified target genes, which in turn bring about cytokinin responses. Another scenario is that type-A ARRs are induced by cytokinins and interact with diverse target proteins, which in turn regulate cytokinin responses. One known protein that interacts with ARR4 (a type-A ARR) is phytochrome B (97). The interaction is specific: ARR4 did not bind phytochrome A, and only ARR4 among several investigated ARRs interacted with phytochrome B. The ARR4 binding stabilizes the active form of phytochrome B and increases the light-sensitivity of the plant. This could be the mechanism behind the cytokinin-regulation of light signaling. Other known type-A ARR interactors are closely related proteins DBP1 and DBP2 with unknown functions (114). DBP1 interacts with ARR4 but not with ARR5. The binding requires the short C-terminal extension of ARR4, suggesting that the C-terminal extensions of type-A ARRs may confer interaction specificity.

THE ROLE OF CYTOKININS

The Role of Cytokinins in Whole Plants

How well do we know the role of cytokinins? Probably not very well. The role of cytokinins has been inferred mostly from the effects of cytokinin application—in other words, from the effects of extra cytokinins added to internal cytokinins. Recently, genes for cytokinin oxidases (CKXs) have been used to decrease

endogenous cytokinin levels in tobacco (84, 112). The constitutively active cauliflower mosaic virus *35S RNA* gene promoter (35S promoter) was used to drive the CKX genes and, depending on the lines, cytokinin levels were decreased to between 30% and 60% of that of the wild type (112). In these lines, growth of aerial parts was severely retarded: The internode length, leaf size, and size of the shoot apical meristem were decreased, whereas the interval of leaf formation (plastochron) was increased. These phenotypes were caused by a reduced rate of cell division: Cell size increased while cell number decreased. By contrast, total rootmass increased, which resulted from the increased size of the cell division zone. It was therefore proposed that cytokinins are positive regulators of cell division in the shoot apical meristem and negative regulators of cell division in the root apical meristem. Interestingly, the transgenics senesce later and exhibit decreased apical dominance, casting doubt on the previous notion that cytokinins are negative regulators of senescence and positive regulators of outgrowth of lateral buds in normal plants. However, we should take into account that local cytokinin levels in these transgenics have not been reported.

Another issue concerning the role of cytokinins is whether they are systemic mediators or local mediators. There are experimental results that support either conclusion. Supporting the role of cytokinins as systemic mediators is the presence of cytokinins in the sap of the xylem (18, 99) and the phloem (18, 22, 36). Cytokinin levels in plants and in the xylem sap are positively correlated with soil minerals (19, 81, 99, 100), especially mineral nitrogen, which suggests that cytokinins mediate information on nutrient status (79, 99, 100). Results against the systemic role were also obtained from transgenic tobacco carrying the *IPT* gene from *Agrobacterium tumefaciens*. In one experiment, reciprocal grafting between transgenic and wild-type plants was made, and phenotypes indicative of increased cytokinins, such as lateral bud outgrowth, were observed only in the transgenic part (15). Böhner & Gatz used a glucocorticoid-inducible and tetracycline-repressible promoter (in which tetracycline acts dominantly) to express *IPT* from *Agrobacterium*, and showed that in a systemically induced plant only buds receiving tetracycline did not exhibit outgrowth (5), further suggesting a paracrine action of cytokinins. Possible reasons for this contradiction are that efficient cytokinin transport may require certain prerequisite conditions; transported cytokinins may be important only when total cytokinin levels are low, for example in nutrient-starved plants; or that cytokinin transport may just be incidental. The role and mechanisms of long-distance, short-distance, and transmembrane transport of cytokinins are important issues yet to be solved.

The Role of Cytokinins in Cell Division

Cytokinins are involved in the regulation of both G_1–S and G_2–M transitions. Their involvement in G_1–S regulation is supported by the observation that cytokinins increase the G_1 cyclin, cyclin D3 (89), and that constitutive expression of cyclin D3 caused cytokinin-independent growth of *Arabidopsis* calli. Interestingly, the

overexpressed calli were green while the wild-type calli were not, suggesting the surprising possibility that cyclin D3 may also regulate chloroplast development (72).

Cytokinins are also important for the regulation of the G_2–M transition. In tobacco BY-2 cells, which are cytokinin-autonomous, endogenous zeatin-type cytokinins peaked around the S and M phases (69). Application of lovastatin, an inhibitor of mevalonic acid synthesis, inhibits cytokinin biosynthesis and mitosis. Zeatin overrides the lovastatin inhibition of mitosis, indicating that zeatin is indispensable for G_2–M transition (42). The regulation of G_2–M transition is probably mediated by activation of CDK. In tobacco pith explants, application of auxin increases the immunodetectable amount of CDK protein, and additional application of a cytokinin resulted in activation of CDK, possibly through dephosphorylation of CDK (117). The missing link between response regulators and control of cell cycle remains an important and interesting question.

CONCLUSIONS

Recently, our knowledge of the biosynthesis, metabolism, degradation, perception, and early stages of signal transduction of cytokinins has increased considerably. Yet important questions remain unanswered. First, we do not understand the role of cytokinins very well. This shortcoming has recently begun to be addressed through utilizing genes for cytokinin oxidases to lower cytokinin levels; future, more elaborate experiments using tissue-specific and inducible-expression systems will definitely be informative. Future analyses of knockout plants for all genes encoding enzymes of cytokinin biosynthesis, metabolism, degradation, receptors, and signal transduction proteins will further uncover the roles of cytokinins, as well as the roles of individual genes. To understand how cytokinin levels are regulated, we must uncover the regulatory mechanisms of the enzymes that catalyze biosynthesis, interconversion, and degradation of cytokinins. As for the signal transduction, it is yet to be determined how phosphorelay regulates the transcriptional activity of type-B response regulators. Also, the output of the response regulator loop is largely unknown. Profiling genes regulated by type-B ARRs and identifying presumptive ARR interactors is important. Crosstalk is another issue. An inportant question is whether signaling pathway initiated by different histidine kinases are separated or converge in the downstream. Different tissues or cells respond differently to cytokinins, suggesting that there is crosstalk between signaling initiated by cytokinins and other signals, or that signaling elements differ between different cells. Understanding the complex cytokinin-regulated signaling network that governs morphogenesis will be a challenging task.

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LITERATURE CITED

1. 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.* 26:579–82
2. Aoyama T, Chua NH. 1997. A glucocorticoid-mediated transcriptional induction system in transgenic plants. *Plant J.* 11:605–12
3. Bilyeu KD, Cole JL, Laskey JG, Riekhof WR, Esparza TJ, et al. 2001. Molecular and biochemical characterization of a cytokinin oxidase from maize. *Plant Physiol.* 125:378–86
4. Bleecker AB, Kende H. 2000. Ethylene: a gaseous signal molecule in plants. *Annu. Rev. Cell. Dev. Biol.* 16:1–18
5. Bohner S, Gatz C. 2001. Characterisation of novel target promoters for the dexamethasone-inducible/tetracycline-repressible regulator TGV using luciferase and isopentenyl transferase as sensitive reporter genes. *Mol. Gen. Genet.* 264:860–70
6. Brandstatter I, Kieber JJ. 1998. Two genes with similarity to bacterial response regulators are rapidly and specifically induced by cytokinin in Arabidopsis. *Plant Cell* 10:1009–19
7. Chang C, Kwok SF, Bleecker AB, Meyerowitz EM. 1993. Arabidopsis ethylene-response gene ETR1: similarity of product to two-component regulators. *Science* 262:539–44
8. Da Re S, Schumacher J, Rousseau P, Fourment J, Ebel C, Kahn D. 1999. Phosphorylation-induced dimerization of the FixJ receiver domain. *Mol. Microbiol.* 34:504–11
9. D'Agostino IB, Deruere J, Kieber JJ. 2000. Characterization of the response of the Arabidopsis response regulator gene family to cytokinin. *Plant Physiol.* 124:1706–17
10. D'Agostino IB, Kieber JJ. 1999. Phosphorelay signal transduction: the emerging family of plant response regulators. *Trends Biochem. Sci.* 24:452–56
11. Deruere J, Kieber JJ. 2002. Molecular mechanisms of cytokinin signaling. *J. Plant Growth Regul.* 21:32–39
12. Dewitte W, Chiappetta A, Azmi A, Witters E, Strnad M, et al. 1999. Dynamics of cytokinins in apical shoot meristems of a day-neutral tobacco during floral transition and flower formation. *Plant Physiol.* 119:111–22
13. Dill A, Jung HS, Sun TP. 2001. The DELLA motif is essential for gibberellin-induced degradation of RGA. *Proc. Natl. Acad. Sci. USA* 98:14162–67
14. Emery RJ, Ma Q, Atkins CA. 2000. The forms and sources of cytokinins in developing white lupine seeds and fruits. *Plant Physiol.* 123:1593–604
15. Faiss M, Zalubilova J, Strnad M, Schmulling T. 1997. Conditional transgenic expression of the ipt gene indicates a function for cytokinins in paracrine signaling in whole tobacco plants. *Plant J.* 12:401–15
16. Gamble RL, Coonfield ML, Schaller GE. 1998. Histidine kinase activity of the ETR1 ethylene receptor from Arabidopsis. *Proc. Natl. Acad. Sci. USA* 95:7825–29
17. Gan S, Amasino RM. 1995. Inhibition of leaf senescence by autoregulated production of cytokinin. *Science* 270:1986–88
18. Goodwin PB, Gollnow BI, Letham DS. 1978. Phytohormones and growth correlations. In *Phytohormones and related compounds: a comprehensive treatise*, ed. TJV Higgins, pp. 215–49. Amsterdam: Elsevier/North-Holland Biomedical Press
19. Goring H, Mardanov AA. 1976. Influence of nitrogen deficiency on K/Ca ratio and cytokinin content of pumpkin

seedlings. *Biochem. Physiol. Pflanz* 170: 261–64
20. Gray WM, Kepinski S, Rouse D, Leyser O, Estelle M. 2001. Auxin regulates SCF(TIR1)-dependent degradation of AUX/IAA proteins. *Nature* 414:271–76
21. Haberer G, Kieber JJ. 2002. Cytokinins. New insights into a classic phytohormone. *Plant Physiol.* 128:354–62
22. Hall SM, Baker DA. 1972. The chemical composition of *Ricinus* phloem exudate. *Planta* 106:131–40
23. Houba-Herin N, Pethe C, d'Alayer J, Laloue M. 1999. Cytokinin oxidase from Zea mays: purification, cDNA cloning and expression in moss protoplasts. *Plant J.* 17:615–26
24. Hua J, Chang C, Sun Q, Meyerowitz EM. 1995. Ethylene insensitivity conferred by Arabidopsis ERS gene. *Science* 269: 1712–14
25. Hua J, Meyerowitz EM. 1998. Ethylene responses are negatively regulated by a receptor gene family in Arabidopsis thaliana. *Cell* 94:261–71
26. Hutchison CE, Kieber JJ. 2002. Cytokinin signaling in Arabidopsis. *Plant Cell* 14 (Suppl.):S47–59
27. Hwang I, Chen HC, Sheen J. 2002. Two-component signal transduction pathways in Arabidopsis. *Plant Physiol.* 129:500–15
28. Hwang I, Sheen J. 2001. Two-component circuitry in Arabidopsis cytokinin signal transduction. *Nature* 413:383–89
29. Imamura A, Hanaki N, Nakamura A, Suzuki T, Taniguchi M, et al. 1999. Compilation and characterization of Arabidopsis thaliana response regulators implicated in His-Asp phosphorelay signal transduction. *Plant Cell Physiol.* 40:733–42
30. Imamura A, Hanaki N, Umeda H, Nakamura A, Suzuki T, et al. 1998. Response regulators implicated in His-to-Asp phosphotransfer signaling in Arabidopsis. *Proc. Natl. Acad. Sci. USA* 95:2691–96
31. Imamura A, Yoshino Y, Mizuno T. 2001. Cellular localization of the signaling components of Arabidopsis His-to-Asp phosphorelay. *Biosci. Biotechnol. Biochem.* 65:2113–37
32. Inoue T, Higuchi M, Hashimoto Y, Seki M, Kobayashi M, et al. 2001. Identification of CRE1 as a cytokinin receptor from Arabidopsis. *Nature* 409:1060–63
33. Jacqmard A, Detry N, Dewitte W, Van Onckelen H, Bernier G. 2002. In situ localisation of cytokinins in the shoot apical meristem of Sinapis alba at floral transition. *Planta* 214:970–73
34. Kakimoto T. 1996. CKI1, a histidine kinase homolog implicated in cytokinin signal transduction. *Science* 274:982–85
35. Kakimoto T. 2001. Identification of plant cytokinin biosynthetic enzymes as dimethylallyl diphosphate: ATP/ADP isopentenyltransferases. *Plant Cell Physiol.* 42:677–85
36. Kamboj JS, Blake PS, Baker DA. 1998. Cytokinins in the vascular saps of Ricinus communis. *Plant Growth Regul.* 25:123–26
37. Kato M, Mizuno T, Shimizu T, Hakoshima T. 1997. Insights into multistep phosphorelay from the crystal structure of the C-terminal HPt domain of ArcB. *Cell* 88:717–23
38. Kern D, Volkman BF, Luginbuhl P, Nohaile MJ, Kustu S, Wemmer DE. 1999. Structure of a transiently phosphorylated switch in bacterial signal transduction. *Nature* 402:894–98
39. Kiba T, Taniguchi M, Imamura A, Ueguchi C, Mizuno T, Sugiyama T. 1999. Differential expression of genes for response regulators in response to cytokinins and nitrate in Arabidopsis thaliana. *Plant Cell Physiol.* 40:767–71
40. Kieber JJ. 2002. Cytokinins. In *The Arabidopsis Book*, ed. EM Meyerowitz. Rockeville, MD: Am. Soc. Plant Biol.
41. Klumpp S, Krieglstein J. 2002. Phosphorylation and dephosphorylation of

histidine residues in proteins. *Eur. J. Biochem.* 269:1067–71
42. Laureys F, Dewitte W, Witters E, Van Montagu M, Inze D, Van Onckelen H. 1998. Zeatin is indispensable for the G2-M transition in tobacco BY-2 cells. *FEBS Lett.* 426:29–32
43. Lee SY, Cho HS, Pelton JG, Yan D, Henderson RK, et al. 2001. Crystal structure of an activated response regulator bound to its target. *Nat. Struct. Biol.* 8:52–56
44. Leyser O. 2002. Molecular Genetics of Auxin Signaling. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 53:377–98
45. Lloyd AM, Schena M, Walbot V, Davis RW. 1994. Epidermal cell fate determination in Arabidopsis: patterns defined by a steroid-inducible regulator. *Science* 266:436–39
46. Lohrmann J, Buchholz G, Keitel C, Sweere U, Kircher S, et al. 1999. Differentil expression and nuclear localization of response regulator-like proteins from *Arabidopsis thaliana*. *Plant Biol.* 1:495–505
47. Lohrmann J, Sweere U, Zabaleta E, Baurle I, Keitel C, et al. 2001. The response regulator ARR2: a pollen-specific transcription factor involved in the expression of nuclear genes for components of mitochondrial complex I in Arabidopsis. *Mol. Genet. Genomics* 265:2–13
48. Maeda T, Wurgler-Murphy SM, Saito H. 1994. A two-component system that regulates an osmosensing MAP kinase cascade in yeast. *Nature* 369:242–45
49. Mahonen AP, Bonke M, Kauppinen L, Riikonen M, Benfey PN, Helariutta Y. 2000. A novel two-component hybrid molecule regulates vascular morphogenesis of the Arabidopsis root. *Genes Dev.* 14:2938–43
50. Makino S, Kiba T, Imamura A, Hanaki N, Nakamura A, et al. 2000. Genes encoding pseudo-response regulators: insight into His-to-Asp phosphorelay and circadian rhythm in Arabidopsis thaliana. *Plant Cell Physiol.* 41:791–803
51. Makino S, Matsushika A, Kojima M, Yamashino T, Mizuno T. 2002. The APRR1/TOC1 quintet implicated in circadian rhythms of Arabidopsis thaliana: I. Characterization with APRR1-overexpressing plants. *Plant Cell Physiol.* 43: 58–69
52. Martin RC, Mok MC, Habben JE, Mok DW. 2001. A maize cytokinin gene encoding an O-glucosyltransferase specific to cis-zeatin. *Proc. Natl. Acad. Sci. USA* 98:5922–26
53. Martin RC, Mok MC, Mok DW. 1999. A gene encoding the cytokinin enzyme zeatin O-xylosyltransferase of Phaseolus vulgaris. *Plant Physiol.* 120:553–58
54. Martin RC, Mok MC, Mok DW. 1999. Isolation of a cytokinin gene, ZOG1, encoding zeatin O-glucosyltransferase from Phaseolus lunatus. *Proc. Natl. Acad. Sci. USA* 96:284–89
55. Matsushika A, Makino S, Kojima M, Yamashino T, Mizuno T. 2002. The APRR1/TOC1 quintet implicated in circadian rhythms of Arabidopsis thaliana: II. Characterization with CCA1-overexpressing plants. *Plant Cell Physiol.* 43:118–22
56. Miller CO, Skoog F, Okumura FS, von Saltza MH, Strong FM. 1956. Isolation, structure and synthesis of kinetin, a cell division factor from deoxyribonucleic acid. *J. Am. Chem. Soc.* 78:1375–80
57. Miyata S, Urao T, Yamaguchi-Shinozaki K, Shinozaki K. 1998. Characterization of genes for two-component phosphorelay mediators with a single HPt domain in Arabidopsis thaliana. *FEBS Lett.* 437:11–14
58. Mizuno T. 1997. Compilation of all genes encoding two-component phosphotransfer signal transducers in the genome of Escherichia coli. *DNA Res* 4: 161–68
59. Mok DW, Mok MC. 2001. Cytokinin Metabolism and Action. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 52:89–118
60. Mok MC. 1994. Cytokinins and plant development–an overview. In *Cytokinins–Chemistry, Activity, and Function*, ed. MC Mok, 155–66. Boca Raton: CRC Press

61. Montgomery B, Lagarias J. 2002. Phytochrome ancestry: sensors of bilins and light. *Trends Plant Sci.* 7:357
62. Morris RO, Bilyeu KD, Laskey JG, Cheikh NN. 1999. Isolation of a gene encoding a glycosylated cytokinin oxidase from maize. *Biochem. Biophys. Res. Commun.* 255:328–33
63. Mougel C, Zhulin IB. 2001. CHASE: an extracellular sensing domain common to transmembrane receptors from prokaryotes, lower eukaryotes and plants. *Trends Biochem. Sci.* 26:582–84
64. Napoli CA, Beveridge CA, Snowden KC. 1999. Reevaluating concepts of apical dominance and the control of axillary bud outgrowth. *Curr. Top. Dev. Biol.* 44:127–69
65. Osakabe Y, Miyata S, Urao T, Seki M, Shinozaki K, Yamaguchi-Shinozaki K. 2002. Overexpression of Arabidopsis response regulators, ARR4/ATRR1/IBC7 and ARR8/ATRR3, alters cytokinin responses differentially in the shoot and in callus formation. *Biochem. Biophys. Res. Commun.* 293:806–15
66. Ota IM, Varshavsky A. 1993. A yeast protein similar to bacterial two-component regulators. *Science* 262:566–69
66a. Pischke MS, Jones LG, Otsuga D, Fernandez DE, Drews GN, Sussman MR. 2002. An Arabidopsis histidine kinase is essential for megagametogenesis. *Proc. Natl. Acad. Sci. USA* 99:15800–805
67. Porter SC, North AK, Wedel AB, Kustu S. 1993. Oligomerization of NTRC at the glnA enhancer is required for transcriptional activation. *Genes Dev.* 7:2258–73
68. Posas F, Wurgler-Murphy SM, Maeda T, Witten EA, Thai TC, Saito H. 1996. Yeast HOG1 MAP kinase cascade is regulated by a multistep phosphorelay mechanism in the SLN1-YPD1-SSK1 "two-component" osmosensor. *Cell* 86:865–75
69. Redig P, Shaul O, Inze D, Van Montagu M, Van Onckelen H. 1996. Levels of endogenous cytokinins, indole-3-acetic acid and abscisic acid during the cell cycle of synchronized tobacco BY-2 cells. *FEBS Lett.* 391:175–80
70. Richmond AE, Lang A. 1969. Effect of kinetin on protein content and survival of detached leaves. *Science* 125:650–51
71. Riechmann JL, Heard J, Martin G, Reuber L, Jiang C, et al. 2000. Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes. *Science* 290:2105–10
72. Riou-Khamlichi C, Huntley R, Jacqmard A, Murray JA. 1999. Cytokinin activation of Arabidopsis cell division through a D-type cyclin. *Science* 283:1541–44
73. Roitsch T, Ehness R. 2000. Regulation of source/sink relations by cytokinins. *Plant Growth Regul.* 32:359–67
74. Sablowski RW, Meyerowitz EM. 1998. A homolog of NO APICAL MERISTEM is an immediate target of the floral homeotic genes APETALA3/PISTILLATA. *Cell* 92:93–103
75. Sakai H, Aoyama T, Oka A. 2000. Arabidopsis ARR1 and ARR2 response regulators operate as transcriptional activators. *Plant J.* 24:703–11
76. Sakai H, Honma T, Aoyama T, Sato S, Kato T, et al. 2001. ARR1, a transcription factor for genes immediately responsive to cytokinins. *Science* 294:1519–21
77. Sakakibara H, Hayakawa A, Deji A, Gawronski SW, Sugiyama T. 1999. His-Asp phosphotransfer possibly involved in the nitrogen signal transduction mediated by cytokinin in maize: molecular cloning of cDNAs for two-component regulatory factors and demonstration of phosphotransfer activity in vitro. *Plant Mol. Biol.* 41:563–73
78. Sakakibara H, Suzuki M, Takei K, Deji A, Taniguchi M, Sugiyama T. 1998. A response-regulator homologue possibly involved in nitrogen signal transduction mediated by cytokinin in maize. *Plant J.* 14:337–44
79. Sakakibara H, Takei K. 2002. Identification of cytokinin biosynthesis genes in arabidopsis: a breakthrough for

understanding the metabolic pathway and the regulation in higher plants. *J. Plant Growth Regul.* 21:17–23

80. Sakakibara H, Taniguchi M, Sugiyama T. 2000. His-Asp phosphorelay signaling: a communication avenue between plants and their environment. *Plant Mol. Biol.* 42:273–78
81. Salama AM, Wareing PF. 1979. Effects of mineral nutrition on endogenous cytokinins in plants of sunflower. *J. Exp. Bot.* 30:971–81
82. Schaller GE, Mathews DE, Gribsov M, Walker JC. 2002. Two-component signaling element and histidyl-aspartyl phosphorelays. In *The Arabidopsis Book*, ed. EM Meyerowitz. Rockville, MD: Am. Soc. Plant Biol.
83. Scheres B, Di Laurenzio L, Willemsen V, Hauser MT, Weisbeek P, Benfey PN. 1995. Mutations affecting the radial organization of the *Arabidopsis* root display specific defects throughout the embryonic axis. *Development* 121:53–62
84. Schmulling T. 2002. New insights into the functions of cytokinins in plant development. *J. Plant Growth Regul.* 21:40–49
85. Schuster M, Silversmith RE, Bourret RB. 2001. Conformational coupling in the chemotaxis response regulator CheY. *Proc. Natl. Acad. Sci. USA* 98:6003–8
86. Sheen J. 2002. Phosphorelay and transcription control in cytokinin signal transduction. *Science* 296:1650–52
87. Skoog F, Miller CO. 1957. Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. *Symp. Soc. Exp. Biol.* 11:118–31
88. Smalle J, Kurepa J, Yang P, Babiychuk E, Kushnir S, et al. 2002. Cytokinin growth responses in Arabidopsis involve the 26S proteasome subunit RPN12. *Plant Cell* 14:17–32
89. Soni R, Carmichael JP, Shah ZH, Murray JA. 1995. A family of cyclin D homologs from plants differentially controlled by growth regulators and containing the conserved retinoblastoma protein interaction motif. *Plant Cell* 7:85–103
90. Stock AM, Robinson VL, Goudreau PN. 2000. Two-component signal transduction. *Annu. Rev. Biochem.* 69:183–215
91. Stock J, Da Re S. 2000. Signal transduction: response regulators on and off. *Curr. Biol.* 10:R420–24
92. Strayer C, Oyama T, Schultz TF, Raman R, Somers DE, et al. 2000. Cloning of the Arabidopsis clock gene TOC1, an autoregulatory response regulator homolog. *Science* 289:768–71
93. 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.* 39:1258–68
94. 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.* 43:123–29
95. Suzuki T, Miwa K, Ishikawa K, Yamada H, Aiba H, Mizuno T. 2001. The Arabidopsis sensor His-kinase, AHk4, can respond to cytokinins. *Plant Cell Physiol.* 42:107–13
96. 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.* 64:2486–89
97. Sweere U, Eichenberg K, Lohrmann J, Mira-Rodado V, Baurle I, et al. 2001. Interaction of the response regulator ARR4 with phytochrome B in modulating red light signaling. *Science* 294:1108–11
98. Takei K, Sakakibara H, Sugiyama T. 2001. Identification of genes encoding adenylate isopentenyltransferase, a cytokinin biosynthesis enzyme, in Arabidopsis thaliana. *J. Biol. Chem.* 276:26405–10

99. Takei K, Sakakibara H, Taniguchi M, Sugiyama T. 2001. Nitrogen-dependent accumulation of cytokinins in root and the translocation to leaf: implication of cytokinin species that induces gene expression of maize response regulator. *Plant Cell Physiol.* 42:85–93
100. Takei K, Takahashi T, Sugiyama T, Yamaya T, Sakakibara H. 2002. Multiple routes communicating nitrogen availability from roots to shoots: a signal transduction pathway mediated by cytokinin. *J. Exp. Bot.* 53:971–77
101. Taniguchi M, Kiba T, Sakakibara H, Ueguchi C, Mizuno T, Sugiyama T. 1998. Expression of Arabidopsis response regulator homologs is induced by cytokinins and nitrate. *FEBS Lett.* 429:259–62
102. Triezenberg SJ. 1995. Structure and function of transcriptional activation domains. *Curr. Opin. Genet. Dev.* 5:190–96
103. Ueguchi C, Koizumi H, Suzuki T, Mizuno T. 2001. Novel family of sensor histidine kinase genes in Arabidopsis thaliana. *Plant Cell Physiol.* 42:231–35
104. Ueguchi C, Sato S, Kato T, Tabata S. 2001. The AHK4 gene involved in the cytokinin-signaling pathway as a direct receptor molecule in Arabidopsis thaliana. *Plant Cell Physiol.* 42:751–55
105. Ulmasov T, Hagen G, Guilfoyle TJ. 1999. Activation and repression of transcription by auxin-response factors. *Proc. Natl. Acad. Sci. USA* 96:5844–49
106. Urao T, Miyata S, Yamaguchi-Shinozaki K, Shinozaki K. 2000. Possible His to Asp phosphorelay signaling in an Arabidopsis two-component system. *FEBS Lett.* 478:227–32
107. Urao T, Yakubov B, Satoh R, Yamaguchi-Shinozaki K, Seki M, et al. 1999. A transmembrane hybrid-type histidine kinase in Arabidopsis functions as an osmosensor. *Plant Cell* 11:1743–54
108. Urao T, Yakubov B, Yamaguchi-Shinozaki K, Shinozaki K. 1998. Stress-responsive expression of genes for two-component response regulator-like proteins in Arabidopsis thaliana. *FEBS Lett.* 427:175–78
109. Urao T, Yamaguchi-Shinozaki K, Shinozaki K. 2001. Plant histidine kinases: an emerging picture of two-component signal transduction in hormone and environmental responses. *Sci STKE* 2001:RE18
110. Varughese KI. 2002. Molecular recognition of bacterial phosphorelay proteins. *Curr. Opin. Microbiol.* 5:142–48
111. Welch M, Oosawa K, Aizawa S, Eisenbach M. 1993. Phosphorylation-dependent binding of a signal molecule to the flagellar switch of bacteria. *Proc. Natl. Acad. Sci. USA* 90:8787–91
112. Werner T, Motyka V, Strnad M, Schmulling T. 2001. Regulation of plant growth by cytokinin. *Proc. Natl. Acad. Sci. USA* 98:10487–92
113. West AH, Stock AM. 2001. Histidine kinases and response regulator proteins in two-component signaling systems. *Trends Biochem. Sci.* 26:369–76
114. Yamada H, Hanaki N, Imamura A, Ueguchi C, Mizuno T. 1998. An Arabidopsis protein that interacts with the cytokinin-inducible response regulator, ARR4, implicated in the His-Asp phosphorylay signal transduction. *FEBS Lett.* 436:76–80
115. Yamada H, Suzuki T, Terada K, Takei K, Ishikawa K, et al. 2001. The Arabidopsis AHK4 histidine kinase is a cytokinin-binding receptor that transduces cytokinin signals across the membrane. *Plant Cell Physiol.* 42:1017–23
116. Yeh KC, Lagarias JC. 1998. Eukaryotic phytochromes: light-regulated serine/threonine protein kinases with histidine kinase ancestry. *Proc. Natl. Acad. Sci. USA* 95:13976–81
117. Zhang K, Letham DS, John PC. 1996. Cytokinin controls the cell cycle at mitosis by stimulating the tyrosine dephosphorylation and activation of p34cdc2-like H1 histone kinase. *Planta* 200:2–12

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