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REVIEW

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Plant two-component systems: principles, functions, complexity and cross talk

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Abstract Two-component systems have emerged as important sensing/response mechanisms in higher plants. They are composed of hybrid histidine kinases, histidine-containing phosphotransfer domain proteins and response regulators that are biochemically linked by His-to-Asp phosphorelay. In plants two-component systems play a major role in cytokinin perception and signalling and contribute to ethylene signal transduction and osmosensing. Furthermore, developmental processes like megagametogenesis in *Arabidopsis thaliana* and flowering promotion in rice (*Oryza sativa*) involve elements of two-component systems. Two-component-like elements also function as components of the *Arabidopsis* circadian clock. Because of the molecular mode of signalling, plant two-component systems also appear to serve as intensive cross talk and signal integration machinery. In this review we summarize the present knowledge about the principles and functions of two-component systems in higher plants and address several critical points with respect to cross talk, signal integration and specificity.

Keywords Two-component system · Histidine kinase · Phosphotransfer protein · Response regulator · Osmoregulation · Signalling (cytokinin, ethylene)

Abbreviations AHK: *Arabidopsis* histidine kinase · AHP: *Arabidopsis* histidine-containing phosphotransfer domain protein · APRR: *Arabidopsis* pseudo response regulator · ARR: *Arabidopsis* response regulator · CCT: CONSTANS CONSTANS-like TOC1 · CKI: Cytokinin insensitive · CRE: Cytokinin response · CTR: Constitutive triple response · Ehd: Early heading date · EIN: Ethylene insensitive · ERS: Ethylene response sensor · ETR: Ethylene resistant · GARP-motif: Found in Golden2 of maize, *Arabidopsis* B-type response regulators and *Chlamydomonas* Psr1 · HPt: Histidine-containing phosphotransfer domain · NLS: Nuclear localization signal · phyB: Phytochrome B · TCS: Two-component signalling · TOC: Timing of CAB (chlorophyll *a*/*b*-binding protein) expression · WOL: Wooden leg

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Introduction

As plants are not able to escape from changing environmental conditions their survival depends mainly on their skills to react quickly, efficiently and most of all unmistakably. To meet all these requirements they have developed and optimized many different signal perception and transduction systems. A very sophisticated method within signalling cascades is the reversible phosphorylation-dependent regulation of protein activity. Transducers of the phosphoryl residue on such target proteins are protein kinases. These catalyze the phosphate transfer from ATP onto histidine (His), serine, threonine or tyrosine in the protein backbone. A great variety of kinases have evolved within the different species including two-component histidine kinases. Until recently, signal transduction via the two-component system (TCS) was thought to be an exclusively prokaryotic phenomenon. But it has now been proven that this mechanism participates in the perception and integration of various endogenous and exogenous stimuli, especially in higher plants (Lohrmann and Harter 2002; Oka et al. 2002; Hass et al. 2004a). In this review we will summarize the present knowledge about TCS signal transduction and discuss the role of TCS in establishing a signalling network in higher plants.

Principles of TCS signalling

The two-component signalling system serves as a sensing/responding mechanism. In reaction to exoge-

nous or endogenous stimuli, autophosphorylation of the histidine kinase at a conserved histidine residue within the catalytic core of its transmitter domain is induced and, thereby, signalling is initiated. As far as is known from prokaryotic examples, autophosphorylation of histidine kinases is a bimolecular reaction between homodimers, in which one monomer catalyzes the phosphorylation of the conserved histidine residue in the second monomer (Stock et al. 2000; West and Stock 2001; Hass et al. 2004a). However, whether trans-phosphorylation of histidine kinase monomers plays a crucial role in the initiation of TCS signalling in higher plants is not known. In single-step two-component systems, the phosphoryl group is then transferred to an invariant aspartate (Asp) residue within the receiver domain of the response regulator that results in modulation of its activity (Fig. 1a). In prokaryotes, but especially in eukaryotes like yeast and plants, a more complex type of two-component signalling system has been found, the multistep system, with additional phosphorylation steps (Fig. 1b). In the multistep two-component system, the histidine kinase is called a hybrid histidine kinase and carries an additional receiver domain usually at the COOH-terminal end. Instead of transferring the phosphoryl group directly to the response regulator, it is first transferred to a conserved Asp residue of its own receiver domain (Fig. 1b). Additional proteins with a histidine-containing phosphotransmitter (HPt) domain perceive the phosphoryl group from the receiver domain of the hybrid histidine kinase and transfer it to the receiver of the response regulator (Fig. 1b). Phosphorylation of response regulators induces a conformational change in the output domain that alters its biological activity (Stock et al. 2000; West and Stock 2001; Hass et al. 2004a).

The chemistry of multistep TCS signal transduction is a His-to-Asp phosphorelay, which incorporates four phosphoprotein intermediates mechanistically linked by five phosphoryl transfer events. It is the flux of phosphoryl groups rather than stoichiometric phosphorylation that is relevant for the function of TCSs (Stock et al. 2000; West and Stock 2001).

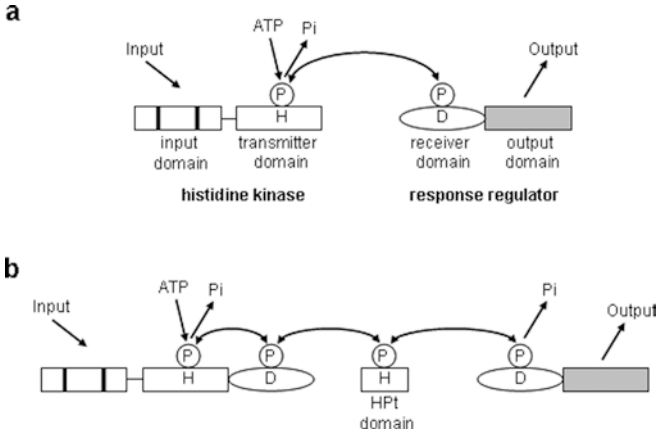

Fig. 1a, b Basic features of the simple (**a**) and the multistep (**b**) two-component signalling system. Signal perception by the histidine kinase induces autophosphorylation of the transmitter domain at a conserved histidine residue (*H*). The phosphoryl group (*P*) is relayed to a conserved Asp residue (*D*) that is localized either in the receiver domain of the cognate response regulator (**a**) or, in the case of hybrid histidine kinases, in an attached receiver domain (**b**). In the multistep two-component signalling system, histidine-containing phosphotransfer (*HPt*) domain proteins function as phosphorhistidine intermediates between the hybrid histidine kinase and the response regulator (**b**). *Black vertical bars* Transmembrane domain (adapted from Kakimoto 2003)

TCS elements of *Arabidopsis thaliana*

Within recent years the composition and function of TCS systems have been intensively studied in several plant species, but in most detail in *Arabidopsis thaliana*. Sequence analysis of the entire *Arabidopsis* genome has revealed that there are 8 canonical histidine kinases (AHKs), 5 HPt proteins (AHPs), 23 response regulators (ARRs) and 9 *pseudo* response regulators (APRRs; Urao et al. 2000a; Hwang et al. 2002).

On the basis of structural and functional properties, the *Arabidopsis* AHKs can be divided into several subfamilies. One subfamily encompasses AHK2, AHK3, and AHK4/CRE1/WOL (histidine kinase 4/cytokinin response/wooden leg), which function as potentially plasmalemma-bound cytokinin receptors (Fig. 2a, Table 1; Kakimoto 2003). The second family is formed by class-I ethylene receptors, ethylene resistant 1 (ETR1) and ethylene response sensor 1 (ERS1), which are associated with the endoplasmic reticulum (Fig. 2a, Table 1; Guo and Ecker 2004). It is noteworthy that ERS1 is the only known plant histidine kinase of the non-hybrid type (Fig. 2a). Interestingly, the ethylene receptor family also comprises three AHK-like proteins, the class-II receptors ETR2, ERS2 and ethylene insensitive 4 (EIN4), which contain a degenerated transmitter domain lacking at least one amino acid crucial for histidine kinase activity (Guo and Ecker 2004). AHK1/AtHK1, which appears to be a membrane-bound osmosensor (Urao et al. 1999), and CKI1 (cytokinin insensitive 1), which participates in female gametophyte development (Pischke et al. 2002; Hejátko et al. 2003), fall into a further subfamily (Fig. 2a, Table 1; Hass et al. 2004a). Due to the lack of any transmembrane domain, AHK5/CKI2 holds an exceptional position and is predicted to be a cytoplasmic hybrid histidine kinase (Fig. 2a, Table 1; Hass et al. 2004a, and references therein).

The *Arabidopsis* HPt domain proteins (AHPs; Fig. 2b) are proposed to act as intermediates in multistep phosphorelays, connecting AHKs with their cognate response regulators (Fig. 1b, Table 1). However, the high structural similarities of AHPs do not allow any prediction of specific functions within a given plant TCS. Studies on intracellular distribution using green fluorescent protein (GFP) fusions indicate that

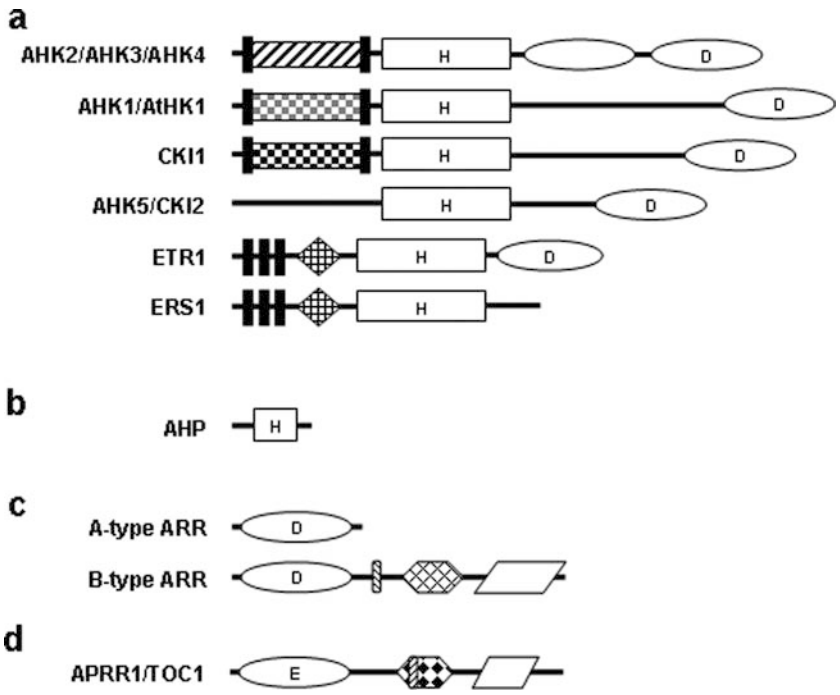

Fig. 2a–d Structural characteristics of canonical histidine kinases (AHK, **a**), HPt domain proteins (AHP, **b**), response regulators (ARR, **c**) and pseudo response regulators (APRR, **d**) from *Arabidopsis thaliana*. **a** *Black vertical bars* Transmembrane domain; *striped rectangle* extracellular CHASE (cyclase/histidine kinase-associated sensory extracellular) domain of AHK2, 3, and 4; *checked rectangles* extracellular domains of CKI1 and AtHK1; *open rectangles* transmitter domains; *open ovals* receiver(-like) domains; *crosshatched quadrate* GAF-domain (named after its existence in cGMP-regulated phosphodiesterases and adenylate cyclases of *Anabaena* and the bacterial transcription factor FhlA) of ETR1 and ERS1. **b** *Open quadrate* Histidine-containing phosphotransfer domain. **c** *Oval* Receiver domain; *striped bar*, NLS; *rhomboid* GARP DNA-binding domain; *open parallelogram* transactivation domain. **d** *Oval* Receiver-like domain; *rhomboid* CCT domain; *striped bar* NLS; *open parallelogram* transactivation domain. *D* Aspartate; *E* glutamic acid; *H* histidine. For further details, see text

AHPs are found in the cytoplasmic as well as in the nuclear compartment, suggesting a shuttling function (Hwang and Sheen 2001; C. Schröder and K. Harter, unpublished).

The final elements of the *Arabidopsis* two-component signalling systems are the response regulators, which are divided into three subfamilies (Table 1).

i. Type-A *Arabidopsis* response regulators (ARR) are relatively small and contain a receiver domain along with short C-terminal extensions (Fig. 2c). They are expressed to slightly different extents in many *Arabidopsis* tissues and up-regulated in response to cytokinin treatment (Kakimoto 2003; To et al. 2004). Although most type-A ARRs do not have an obvious nuclear localization signal (NLS), corresponding GFP fusion proteins are distributed in the cytoplasm as well as in the nucleus (Sweere et al. 2001; Mira-Rodado 2003) or accumulate inside the nucleus (Kiba et al. 2002).

ii. Type-B response regulators also comprise a receiver domain along with an extended C-terminal output domain (Fig. 2c), and their expression is not regulated by any stimulus tested so far. The output domains of B-type ARRs usually contain a GARP DNA-binding motif (so named because it is found in Golden2 of maize, in *Arabidopsis* B-type response regulators and in *Chlamydomonas* Psr1), a C-terminal transactivation domain and at least one NLS (Fig. 2c). Several molecular and cell biological studies have provided strong evidence that B-type response regulators function as nuclear transcription factors binding to target promoters that contain the 5′-W/GAT/W-motif, where W is either A or T (Lohrmann and Harter 2002, and references therein; Imamura et al. 2003).

iii. APRRs have a receiver-like domain but the invariant amino acids including the phosphorylated Asp are substituted by other amino acids (Fig. 2d; Hwang et al. 2002). This is in agreement with the observation that the receiver-like domains of APRR1/TOC1 [timing of chlorophyll *a*/*b*-binding (CAB) expression 1] and APRR2 do not have phospho-accepting activity in vitro (Makino et al. 2000). APRR1 contains a CCT (CONSTANS CONSTANS-like TOC1) motif in the C-terminus—a typical feature of the CONSTANS-like transcription factor family—that is rich in basic residues and contains a putative NLS. Downstream of the CCT motif a region rich in acidic residues is found which may represent a transcriptional activation domain (Strayer et al. 2000). These observations suggest that APRRs may act as nuclear transcription factors. Accumulating evidence indicates a crucial role of APRRs as intrinsic elements of the *Arabidopsis* circadian clock (reviewed in Hayama

Table 1 Summary of characteristic features of selected TCS-related elements. For detailed information and references, see text

Family	Gene	Name (*species*)	Expression	Intracellular localization	Function
Histidine kinases	AHK1/AtHK1	*Arabidopsis thaliana* histidine kinase 1	All tissues, predominantly in roots; osmotic stress inducible	Plasmalemma?	Putative osmosensor
	AHK2	*A. thaliana* histidine kinase 2	All tissues	Plasmalemma?	Cytokinin receptor
	AHK3	*A. thaliana* histidine kinase 3	All tissues	Plasmalemma?	Cytokinin receptor
	AHK4/CRE1/WOL	*A. thaliana* histidine kinase 4, cytokinin receptor, wooden leg	All tissues, predominantly in roots	Plasmalemma?	Cytokinin receptor
	AHK5/CKI2	*A. thaliana* histidine kinase 5, cytokinin insensitive 2	Unknown	Cytoplasm?	Unknown
	CKI1	Cytokinin insensitive 1 (*A. thaliana*)	Female gametophyte	Unknown	Female gametophyte development
	ETR1	Ethylene resistant 1 (*A. thaliana*)	All tissues	Endoplasmic reticulum	Ethylene receptor
	ERS1	Ethylene response sensor 1 (*A. thaliana*)	All tissues	Endoplasmic reticulum?	Ethylene receptor
HPt-proteins	AHPs	*A. thaliana* HPt-proteins	All tissues	Cytoplasm & nucleus	Transfer of phosphoryl residue from HK to RR
A-type response regulators	ARR4	*A. thaliana* response regulator 4	All tissues; cytokinin/light inducible	Cytoplasm & nucleus	Modulation of light and cytokinin response pathways
B-type response regulators	ARR1	*A. thaliana* response regulator 1	All tissues, predominantly in vegetative tissues	Nucleus	Cytokinin signalling
	ARR2	*A. thaliana* response regulator 2	All tissues, predominantly in pollen	Nucleus	Cytokinin/ethylene signalling; hormone signal integration
	ARR21	*A. thaliana* response regulator 21	Siliques	Unknown	Unknown
	Ehd1	*Oryza sativa* early heading date 1	Inducible by short-day conditions	Nucleus?	Flowering promotion
Pseudo response regulator	APRR1/TOC1	*A. thaliana* pseudo response regulator 1, timing of CAB expression	Circadian rhythm inducible	Nucleus	Circadian clock component

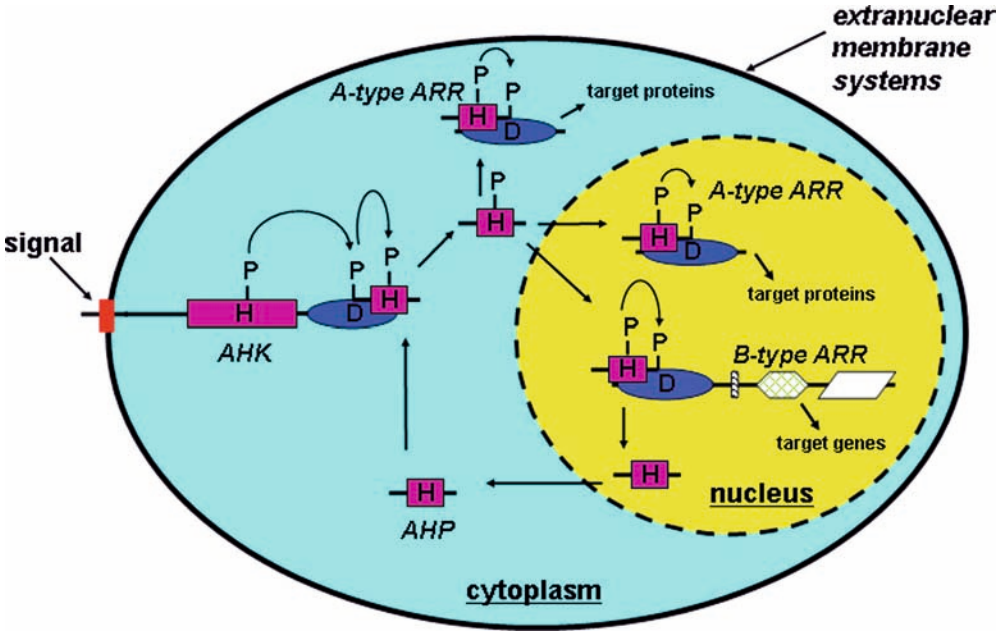

Fig. 3 General model of two-component signal transduction in *Arabidopsis*. Stimulus perception by a membrane-associated and extranuclear histidine kinase induces autophosphorylation. Afterwards the phosphoryl residue is relayed to an AHP. The phosphorylated AHP interacts with the cognate ARR either in the cytoplasm (type-A ARRs) or in the nucleus (type-A and type-B ARRs). By transfer of the phosphoryl group to the receiver domain, the ARRs are activated, which results in regulation of target proteins (type-A ARRs) or target genes (type-B ARRs). *H* Histidine residue, *P* phosphoryl group, *D* aspartate residue. For further details, see text

and Coupland 2003; Eriksson et al. 2003). APRR1/TOC1 functions together with the circadian clock-associated 1 protein in a transcriptional feedback loop that is important for the circadian clock and controls the photoperiodic flowering response as well as the circadian rhythm in the expression of clock-regulated genes in *Arabidopsis* (Strayer et al. 2000). Furthermore, differential APRR function may also account for natural variations between *Arabidopsis* ecotypes in clock-controlled processes (Michael et al. 2003).

Although analysis in plants has just begun, several studies using heterologous yeast or *Escherichia coli* systems indicate that, with the exception of APRRs, most AHKs, AHPs and ARRs in principle function as phosphotransfer proteins and may constitute a multitude of different phosphorelay signalling pathways in plants (Lohrmann and Harter 2002, and references therein). In summary, the presently available data provide evidence for a general framework of TCS signalling in higher plants that follows the scheme depicted in Fig. 3: After perception of the cognate stimulus followed by autophosphorylation the hybrid histidine kinase relays the phosphate residue to an AHP that either stays in the cytoplasm or shuttles into the nucleus and transfers the phosphoryl group to its cognate A-type or B-type ARR. Phosphorylation modulates the activity of A-type ARRs or the transcription factor capacity of the B-type ARRs, which in turn regulates their target proteins or target genes, respectively. Interestingly, *A-type ARR* genes themselves are targets of B-type ARRs (reviewed in Sheen 2001; Haberer and Kieber 2002; Heyl and Schmülling 2003; Kakimoto 2003), implicating a tight functional relationship that will be discussed in detail below.

Function of TCS elements in cytokinin signalling

Cytokinins are adenine derivates implicated in nearly all aspects of plant growth and development, including, for instance, cell division, root and shoot development, apical dominance, responses to light, and leaf senescence (Mok and Mok 2001; Werner et al. 2001). The cytokinin receptor subfamily consists of the three hybrid histidine kinases AHK2, AHK3 and AHK4/CRE1/WOL (Fig. 2a, Table 1). It is noteworthy that all three cytokinin receptors carry an additional receiver-like motif of unknown function embedded between the transmitter and the receiver domain (Fig. 2a). Without going into experimental details it is assumed that the members of this subfamily function as cytokinin-sensing hybrid histidine kinases that transfer the hormone signal from the extracellular space over the plasmalemma to the cytoplasmic side of the plant cell (reviewed in Heyl and Schmülling 2003; Kakimoto 2003; Hass et al. 2004a). However, how this signal transfer is accomplished remains to be elucidated.

Through yeast two-hybrid and ternary protein–protein interaction studies it has been shown that AHK4/CRE1/WOL, like other *Arabidopsis* hybrid histidine kinases, interacts with several AHPs including AHP1 and AHP2 (Urao et al. 2000b; Mira-Rodado 2003; Tanaka et al. 2004; C. Grefen and K. Harter, unpublished). The principle ability of AHPs to perceive a phosphoryl group from AHK4/CRE1/WOL has been suggested by an indirect interference assay in *E. coli*. (Suzuki et al. 2001, 2002). Furthermore, it has been demonstrated in *Arabidopsis* mesophyll protoplasts that cytokinin appears to induce the import of AHP1 and AHP2 into the nucleus (Hwang and Sheen 2001). These observations led to the assumption that the first step

after cytokinin perception and autophosphorylation is the transfer of the phosphoryl group from the receptors to at least one AHP, followed by the translocation of the phosphorylated HPt protein into the nuclear compartment (Fig. 3).

Nuclear type-B response regulators, especially ARR1 and ARR2, have been shown to positively mediate cytokinin responses and directly activate cytokinin-responsive genes (Table 1; Hwang and Sheen 2001; Sakai et al. 2001; Hass et al. 2004b). Because expression of B-type ARRs is not altered by cytokinin (Rashotte et al. 2003), their regulation is very likely controlled through posttranslational modification. As deletion of the entire receiver domain or mutation of the conserved phosphorylatable Asp to glutamate (Glu) creates dominant active versions (Imamura et al. 2003; Hass et al. 2004b; Tajima et al. 2004), activation of B-type ARRs is very likely achieved by TCS-mediated phosphotransfer. As exemplarily shown for ARR2, phosphorylation does not seem to change the intracellular distribution or DNA-binding activity but positively interferes with the transactivation capacity of B-type ARRs (Hass et al. 2004b). These results indicate that the primary cytokinin TCS response pathway is pre-formed in plant cells and awaits activation by the hormone.

The so far best analysed targets of B-type ARRs are the genes encoding the type-A subfamily members. Several recent studies indicate that A-type response regulators act as negative regulators of cytokinin responses having partially overlapping functions. Multiple loss-of-function type-A *arr* alleles result in cytokinin hypersensitivity and an increase in both the amplitude and period of cytokinin induction of cytokinin primary response genes, whereas overexpression of type-A ARRs usually induces cytokinin hyposensitivity on physiological and gene expression levels (Hwang and Sheen 2001; Osakabe et al. 2002; Kiba et al. 2003; To et al. 2004). However, distinct morphological differences among type-A *arr* mutants and type-A ARR overexpressors have been observed, indicating a certain degree of gene-specific and potentially antagonistic functions (Osakabe et al. 2002; Kiba et al. 2002, 2003; To et al. 2004). Interestingly, expression profiling using RNA from 30-day-old, non-hormone-treated *arr2* loss-of-function mutant plants revealed that the low basal expression of *A-type ARR* genes is not significantly altered (Hass et al. 2004b). In combination with the kinetics of their induction by exogenous cytokinin, these data suggest that type-A ARRs are not an intrinsic part of the primary cytokinin TCS signalling pathway but rather function to modulate cytokinin responsiveness (e.g. desensitization) and to maintain cytokinin homeostasis (Hass et al. 2004b; To et al. 2004). This may occur by direct or indirect interference of A-type ARRs with B-type response regulator function. One proposed hypothesis for the molecular mechanism of this interference is that type-A ARRs suppress phosphorelay-mediated activation of B-type ARRs by competing for phosphoryl groups delivered by AHPs (To et al. 2004). A further possibility is that type-A ARRs may act indirectly by increasing the activity of one or more negative regulators of B-type ARRs (To et al. 2004). Despite these findings the molecular identity of those B-type ARR target genes that actually initiate the gene expression cascade eventually resulting in the cytokinin responses of higher plants remains unknown.

Role of TCS elements in ethylene signal transduction

Ethylene, the simplest unsaturated hydrocarbon, controls seed germination, growth of seedlings, vegetative senescence and abscission, and mediates responses to biotic and abiotic stress (Neljubow 1901; Goeschl et al. 1966; Abeles et al. 1992).

Analysis of ethylene signal transduction in *Arabidopsis* revealed the presence of a small family of ethylene receptors. However, from the five family members only ETR1 and ERS1 contain the amino acid residues essential for histidine kinase activity (Fig. 2, Table 1). This observation opened the discussion on whether the transmission of the ethylene signal occurs, at least in part, through a TCS phosphorelay mechanism. Genetic and molecular studies on the triple response of *Arabidopsis*, however, established a different ethylene response pathway that leads from hormone perception at the ER to transcriptional regulation in the nucleus. In this model, ethylene signalling appears to occur without involvement of two-component elements and histidine kinase activity of the receptors (Guo and Ecker 2004). However, there is accumulating evidence that an ethylene receptor-dependent TCS mechanism may contribute to or interfere with the "classical" non-histidine kinase pathway:

i. Quadruple loss-of-function ethylene receptor mutants and *etr1*/*ers1* double mutants have a more severe phenotype than the loss-of-function allele of *constitutive triple response 1* (*CTR1*; Hall and Bleecker 2003; Guo and Ecker 2004). CTR1 physically associates with ETR1 (and ERS1) and acts as an immediate downstream element for all ethylene receptors (Huang et al. 2003). In addition, seedlings carrying a *ctr1* loss-of-function allele are still responsive to ethylene (Larsen and Chang 2001). As shown recently (Guo and Ecker 2003; Potuschak et al. 2003), EIN3—a crucial transcription factor in ethylene signal transduction—is rapidly degraded in the absence and stabilized in the presence of ethylene. Besides that, EIN3 protein turnover remains responsive to ethylene in the *ctr1* loss-of-function mutant (Guo and Ecker 2003). Based on these results the existence of an additional signalling mechanism should be taken into consideration.
ii. Comprehensive genetic studies revealed that ETR1 and ERS1 play unique and dominant roles in the initiation of ethylene responses contingently due to their histidine kinase activity (Gamble et al. 2002; Wang et al. 2003).

iii. In yeast, ETR1 interacts with several *Arabidopsis* HPt proteins including, for instance, AHP2 (Urao et al. 2000b). AHP2 in turn efficiently associates with several response regulators and creates a biochemical bridge between ETR1 and the B-type response regulator ARR2 (Urao et al. 2000b; Lohrmann et al. 2001; C. Grefen and K. Harter, unpublished). It is noteworthy that overexpression of AHP2 caused increased sensitivity to ethylene in at least one of three independent transgenic *Arabidopsis* lines (Suzuki et al. 2002).
iv. *Arabidopsis* plants carrying a loss-of-function allele of *ARR2* display reduced sensitivity not only to cytokinin but also to ethylene in the triple response and in expression of ethylene-responsive genes. The opposite phenotype became evident when a dominant active version of ARR2 was ectopically expressed in *Arabidopsis* (Hass et al. 2004b).
v. In *Arabidopsis* mesophyll protoplasts, ARR2 regulates the activity of the ethylene-responsive *ERF1* (ethylene response factor) gene in an ethylene- and Asp phosphorylation-dependent manner (Hass et al. 2004b).
vi. Asp phosphorylation of ARR2 depends on the presence of ETR1 in a plant cell-free phosphorelay system (Hass et al. 2004b).

Hence, further and detailed physiological, molecular and biochemical analyses are necessary to clarify the functional relationship between the "classical" non-histidine kinase pathway and a TCS phosphotransfer mechanism in ethylene signal transduction.

TCS elements in plant osmosensing, megagametogenesis and flowering promotion

Because of high structural similarity with the *Saccharomyces cerevisae* osmosensing histidine kinase SLN1 (synthetic lethal of N-end rule), *Arabidopsis* AHK1/AtHK1 has, for some time, been implicated in plant osmosensing (Urao et al. 1999). Expression of AHK1/AtHK1 in a yeast double mutant lacking its two osmosensors suppressed the lethal growth phenotype on high-salinity media and activated the high-osmolarity glycerol response pathway (Urao et al. 1999). Interestingly, AHK1/AtHK1 seems to be active under normal growth conditions and changes from the active to the inactive state in response to increasing osmolarity. Though not proven for plants yet, these observations suggest that AHK1/AtHK1 senses changes in osmolarity and subsequently transduces the stress signal to the nucleus (Urao et al. 1999). AHK1/AtHK1 interacts with the *Arabidopsis* HPt protein AHP2 in the yeast two-hybrid system (Urao et al. 2000b), suggesting that the transduction of the stress signal could occur via a multistep phosphorelay (Fig. 3). However, the precise molecular action of AHK1/AtHK1 in plants requires further investigation.

The hybrid histidine kinases CKI1 and CKI2/AHK5 were originally identified in a screen for cytokinin-independent growth of *Arabidopsis* calli and, therefore, argued to be prime candidates for cytokinin receptors (reviewed in Heyl and Schmülling 2003; Kakimoto 2003). However, the function of CKI1 (and AHK5/CKI2) as a cytokinin receptor is in question. Based on two recent studies (Pischke et al. 2002; Hejátko et al. 2003), it became clear that CKI1 function is essential for specific processes during female gametophyte development in *Arabidopsis*. Cytokinin is unlikely to play a role in this developmental process and is probably not the ligand for CKI1 since membranes isolated from fission yeast expressing CKI1 do not bind cytokinin (Yamada et al. 2001). It has, therefore, to be proposed that other compounds bind to CKI1 to modulate histidine kinase activity. In yeast two-hybrid experiments, CKI1 interacts with the HPt proteins AHP1 and AHP2 (Urao et al. 2000b). In addition, CKI1 acts as a phosphatase in vitro, when co-incubated with phosphorylated AHP1 and AHP2 (Nakamura et al. 1999). Hence, the molecular mechanism by which CKI1 modulates female gametophyte development could involve a TCS phosphorelay.

Almost nothing is known about the function of AHK5/CKI2, which represents the only plant histidine kinase without a transmembrane domain (Fig. 2a, Table 1). In yeast, AHK5/CKI2 forms a ternary protein complex with AHP1 and ARR4, suggesting a role in regulation of cytokinin responsiveness and/or modulation of light signal transduction (Mira-Rodado 2003).

In a recent study, Doi and colleagues (2004) showed that a canonical B-type response regulator, the gene for which has been named *Early heading date 1* (*Ehd1*), promotes flowering under short-day conditions in rice (*Oryza sativa*; Table 1). Interestingly, although Ehd1 has a classical receiver domain and a functional GARP DNA-binding motif, it very likely has no ortholog in *Arabidopsis*. *Ehd1* transcript accumulates under short-day conditions but, in contrast to APRRs, Ehd1 appears not to be an intrinsic circadian clock component. Ehd1 very likely promotes flowering by direct regulation of certain *MADS box* genes and of *Flowering Locus T group*-like inducer genes which are implicated in the regulation of photoperiodic flowering. These findings indicate that a novel two-component signalling cascade, which is different from the systems of *Arabidopsis*, is integrated into the conserved pathway of photoperiodic control of flowering in rice (Doi et al. 2004).

Cross talk and specificity in plant TCS signalling: conclusion and perspectives.

Overexpression of type-A response regulators or disruption of type-A ARR gene function induces not only alterations of cytokinin responsiveness but, in the case of ARR3, ARR4, ARR5 and ARR6, it also changes the reaction to light (Sweere et al. 2001; Mira-Rodado 2003; To et al. 2004). For instance, *arr4* single, *arr3,4* double

and *arr3,4,5,6* quadruple mutants at the adult stage show a long-petiole phenotype similar to the altered shade-avoidance response described for phytochrome B (phyB) mutants. This suggests that some type-A ARRs function as positive regulators in phytochrome signalling (Lohrmann and Harter 2002; To et al. 2004). This is in agreement with the hypersensitive red-light response of transgenic *Arabidopsis* plants that overexpress ARR4 (Sweere et al. 2001; Mira-Rodado 2003). Combinatorial application of cytokinin and red light restores the wild-type phenotype of ARR4-overexpressing seedlings, suggesting a complex interference between phytochrome and cytokinin signal transduction (Mira-Rodado 2003). The efficiency of interference depends on Asp phosphorylation of ARR4, indicating the involvement of a TCS phosphorelay in this process (Mira-Rodado 2003). The molecular basis of this interference is the physical interaction between ARR4 and phyB that results in the stabilization of the photoreceptor's active, far-red-absorbing, Pfr form in vivo (Sweere et al. 2001). Most intriguingly, the stabilizing activity of ARR4 on phyB–Pfr can be triggered by exogenous application of cytokinin (Mira-Rodado 2003). In combination with the light-hyposensitive phenotype of the *cre1-1* cytokinin receptor mutant, these results support a model that a TCS-dependent cross talk integrates light (phyB) input and hormone (cytokinin)-regulated homeostasis, enabling the plant to react most efficiently to environmental clues.

The interference of two-component signalling systems with other plant signalling pathways also became evident in recent studies on the specific function of several *Arabidopsis* B-type response regulators (Tajima et al. 2004; Hass et al. 2004b). Comparative microarray analysis of 30-day-old *arr2* loss-of-function plants revealed an altered expression of around 600 genes that are related to auxin, ethylene, and biotic and abiotic stress signal transduction (Hass et al. 2004b). When a dominant active, non-phosphorylatable Asp-to-Glu mutated form of ARR2 was ectopically expressed, even more dramatic changes in around 1,600 genes were observed. In addition to genes involved in hormone homeostasis and hormone signal transduction, as well as in responses to biotic and abiotic stress, altered transcript levels were detected for genes contributing to photomorphogenesis, protein folding and degradation, and development (Hass et al. 2004b). Similar results were reported for *Arabidopsis* plants ectopically expressing a receiver-deficient and constitutively active form of ARR21 (Tajima et al. 2004). Thus, the expression of a constitutively active B-type ARR has an enormous influence on the activity of a multitude of different plant signalling pathways. The resulting disturbance of whole-plant homeostasis and physiology is very likely the explanation for the very dramatic phenotype of these plants reflecting many defects in growth and development (Imamura et al. 2003; Tajima et al. 2004; Hass et al. 2004b). In opposite argumentation these data reveal that TCSs may not only represent prevalent mechanisms for signal transduction but may also establish a complex network that is predominantly responsible for integration, fine-tuning and cross talk of many plant signalling cascades (Lohrmann and Harter 2002).

However, the question of how specificity is established or maintained within such a network comes into mind. For instance, TCS elements tend to be notoriously promiscuous in respect to protein–protein interactions and function in heterologous systems (Lohrmann and Harter 2002). Plant TCS elements participate in phosphorelays from biologically irrelevant fungal and prokaryotic species. In addition, the cytokinin receptor AHK4/CRE1/WOL (Fig. 2a) can act as turgor sensor in yeast (Reiser et al. 2003), and CKI1 artificially interferes with cytokinin signalling when overexpressed in *Arabidopsis* calli and protoplasts (Kakimoto 1996; Hwang and Sheen 2001). Therefore, plants have to limit exorbitant "networking" and cross talk to gain specific responses in, for example, cytokinin signal transduction. Several possibilities of how this could be achieved are feasible:

i. Differential expression in space and time of closely related TCS subfamily members appears, in part, to be a possibility in the case of cytokinin receptors (Ueguchi et al. 2001) and B-type ARRs (Lohrmann et al. 1999; Horak et al. 2003; Tajima et al. 2004) but appears to play a minor role for A-type ARRs and AHPs (Miyata et al. 1998; Tanaka et al. 2004; To et al. 2004). The B-type response regulators ARR20 and ARR21, for instance, show predominant expression in reproductive organs and siliques whereas gene activity of other B-type ARRs is more ubiquitous (Horak et al. 2003; Tajima et al. 2004).
ii. However, differential expression incompletely explains the observation that phenotypic anomalies of plants expressing dominant active forms of B-type ARRs under the control of the constitutive viral 35S promoter are different or, in the case of ARR20, restricted to the late reproductive developmental stage (Tajima et al. 2004). Thus, posttranscriptional mechanisms may contribute to TCS element regulation. These could affect TCS protein stability, interaction with tissue-specific co-activators or second site modifications.
iii. As derivable from Fig. 3, the five canonical AHPs play a major role in the distribution of phosphoryl groups from membrane-associated histidine kinases to downstream response regulators. Because the *Arabidopsis* HPt proteins are more or less constitutively expressed in almost every *Arabidopsis* tissue (Miyata et al. 1998), the vital question arises as to how a given AHP "recognizes" an activated histidine kinase and forwards phosphoryl residues predominantly to the cognate response regulator that eventually initiates the signal-specific response. Similar to the regulation of mitogen-activated protein kinase (MAPK) modules (Pawson and Scott 1997) the

existence and participation of scaffold- or chaperone-like proteins that associate the appropriate TCS elements at the membrane/cytoplasm interface and/or guide the phosphorylated AHP into the nucleus or other subcellular compartments should be taken into consideration. Cross talk, fine-tuning and signal integration may occur when the proposed scaffold and guidance function is not completely restricted to one specific TCS but allows a certain degree exchange of phosphoryl groups with other TCS pathways. The output of such a cross-talking network then reflects the weighted integration of several input pathways, allowing the plant to respond and adapt optimally to changes in endogenous and environmental conditions.

Therefore, future research has to address the following challenging but fascinating questions:

i. How are the level and activity of TCS elements regulated and maintained during growth and development and in response to environmental conditions to restrict certain TCS signalling pathways to specific tissues and cells?
ii. Which molecular mechanisms other than transcriptional regulation may contribute to this (e.g. protein degradation and posttranslational modifications besides Asp phosphorylation)?
iii. Can we identify the proposed scaffold- or chaperone-like proteins that may help to form specific TCS signalling complexes and may guide plant HPt proteins from the hybrid histidine kinase to the cognate response regulator?

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