

Nomenclature for Members of the Two-Component Signaling Pathway of Plants^[W]

Plants make use of “two-component systems” (TCSs) for signal transduction, and these are involved in a number of vital cellular responses, such as responses to cytokinins, ethylene, red light, and osmosensing (Schaller et al., 2011). The TCS as found in plants incorporates three signaling elements: His kinases, His-containing phosphotransfer proteins, and response regulators (Heyl and Schmölling, 2003; Kakimoto, 2003; Schaller et al., 2008). Advances in high-throughput sequencing technologies have led to a dramatic increase in the number of sequenced genomes and ESTs and, concomitantly, an increase in the TCS elements identified in different plant species (Hellmann et al., 2010). As there is no common agreement for the nomenclature of TCS elements in plants, this has led to a confusing and in part contradictory naming of newly identified genes of this pathway. Thus, at the recent cytokinin meeting (Cytokinin Metabolism, Signaling, and Function, July 8–10, 2012, in Berlin), researchers discussed general guidelines that could be applied to the nomenclature of TCS members from all plant species. However, as the gene names for TCS members of the model systems *Arabidopsis thaliana* and rice (*Oryza sativa*) have been grown historically and are well established (Schaller et al., 2007, 2008), the authors suggest applying the following guidelines to all other plant species only.

For the His kinases, the symbol HK should be used followed by a number if more than one gene is detected in a given species (e.g. *HK1*, *HK2*, etc.). If a His kinase also contains a CHASE domain, such as is found in the cytokinin receptors (Anantharaman and Aravind, 2001; Mougél and Zhulin, 2001; Heyl et al., 2007), then the gene should be called *CHASE-domain containing His kinase* and should be designated by the symbol *CHK* followed by a number if more than one gene is detected in a given species (e.g. *CHK1*, *CHK2*, etc.). This nomenclature would not apply to members of the ethylene receptor family (ETR/ERS), which contains both conserved and diverged His kinases and which has its own established nomenclature (Binder et al., 2012). Nor would it apply to members of the phytochrome family (PHY), which are diverged His kinases and likewise have their own established nomenclature (Quail et al., 1994).

For the His-containing phosphotransfer proteins, the symbol HPT should be used followed by a number if more than one gene is detected in a given species (e.g. *HPT1*, *HPT2*, etc.).

The response regulators have been divided in the past into four distinct groups, the type A, type B, type

C, and Clock-related response regulators, with these four types being found throughout land plants (Mizuno, 2004; Schaller et al., 2007, 2008; Pils and Heyl, 2009). Given the prevalence of these four types in many plant species, we propose the use of a consistent set of symbols for their designation. We suggest the use of the symbol RRA referring to the type A response regulators, the symbol RRB referring to the type B response regulators, and the symbol RRC referring to the type C response regulators. The symbol PRR should be used for and restricted to the Clock-related response regulators. It is possible that response regulators may be identified that do not clearly belong to any of these four groups; in such cases, the symbol RR alone should be used. The symbol should be followed by a number if more than one gene of a certain type is detected in a given species (e.g. *RRA1*, *RRA2*, *RRB1*, *RRB2*, etc.).

As noted above, we propose to use the term PRR exclusively for the Clock-related response regulators. This represents a change from how this term has been applied in *Arabidopsis* and rice, where it is used as a symbol for any diverged response regulator. The basis for limiting the use of PRR to the Clock-related response regulators in other plant species is the following. Early in the study of *Arabidopsis* two-component signaling systems, it became apparent that not all identified plant response regulators contained the canonical Asp residue that is necessary for phosphorylation. Therefore, the prefix “pseudo” was added to the respective gene name to indicate the noncanonical nature of these response regulators, the so-called “pseudo-response regulators” (PRRs; Mizuno and Nakamichi, 2005). The founding members of this family were the Clock-related PRRs, which contain two domains: (1) a receiver-like domain in which the canonical Asp residue is substituted, and (2) a Constans/Constans-like/TOC1 domain. Numerous experiments have shown their function in the regulation of the circadian rhythm (for review, see Mizuno and Nakamichi, 2005). Members of the Clock family are widespread throughout land plants but do not always lack the conserved Asp. Significantly, in the moss *Physcomitrella patens*, the Clock-family response regulators are functional but are still designated PRR to indicate their phylogenetic relatedness to the well-characterized Clock-family genes of monocots and dicots (Satbhai et al., 2011). Thus, we propose that the term PRR serve as a specific designation for a gene family that is no longer linked to how they function in a two-component signaling pathway.

To avoid confusion, we suggest not using the prefix pseudo or any other term to highlight noncanonical members of the TCS, except for the Clock-family response regulators. Altered canonical residues (Asp or His) have been reported for other members of TCS

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signaling pathways, such as the pseudo-His phosphotransfer protein, AHP6/APHP1 (Mähönen et al., 2006), and the number of such noncanonical components will undoubtedly increase in the future with continued genomic analysis. However, it is clear that many of these noncanonical members play significant roles and may indeed function in and regulate well-known TCS pathways. Thus, applying the prefix pseudo to them can be misleading, as it implies a lack of functionality. In addition, eliminating the prefix pseudo will avoid confusion with genes that are not expressed (McCouch, 2008). Thus, we propose that the noncanonical members of the TCS should be grouped and labeled only based on their phylogeny (i.e. a noncanonical type B response regulator would still be given the designation RRB).

As gene names for members of the TCS across different species will be quite similar, it is suggested adding a two-letter designation (e.g. *Pp* for *P. patens*) in front of the gene name to identify the species in question where appropriate. No such prefix is required if only sequences from the same species are used in an analysis.

Prior to assigning symbols to the genes, it is essential that a phylogenetic analysis be performed. This is particularly important for the response regulators if they are to be correctly annotated as members of the type A, type B, type C, or Clock subfamilies. The original distinction between type A and type B RRs was based on the domain structure alone (type A RRs contain only an RR domain, while the type B RRs contain

an additional Myb-like DNA-binding domain; Heyl and Schmülling, 2003; Schaller et al., 2008). However, the type C RRs have a similar domain structure to the type A RRs, so some other criterion than domain structure must be used to distinguish these two types of RRs. Furthermore, recent analysis also indicates that some members of the type B RR clade have substantially diverged DNA-binding domains that can be missed using standard motif searches (Tsai et al., 2012; N. Gruhn, M. Halawa, B. Snel, M.F. Seidl, and A. Heyl, unpublished data). Thus, the only means to clearly determine if a particular RR gene is a member of the type A, type B, type C, or Clock RR family is to perform a phylogenetic analysis. We suggest that such a phylogenetic analysis be performed (1) using the conserved TCS domain of the signaling element (see below) and (2) incorporating the well-established family members from Arabidopsis and rice into the analysis. The most relevant TCS domains for phylogenetic analysis are as follows: (1) for His kinases, the region spanning from the HisKA through the HATPase motifs (or the HATPase motif itself as a minimum); (2) for His phosphotransfer proteins, the HPT motif; and (3) for response regulators, the receiver domain motif (Table I). CHASE domain-containing His kinases will likely group with other known CHASE domain His kinases, but the presence of the CHASE domain should be confirmed. These conserved motifs can be identified through online sequence analysis programs such as SMART (<http://smart>).

Table I. Proposition for a standardized nomenclature for plant TCS elements

For comparative analyses of TCS elements including several plant species, a two-letter code should be used as a prefix (*PpHK1* for *P. patens HK1*). It is recommended to use the domains as given in Supplemental Data Files S1 to S3 for phylogenetic analyses. This table contains both bona fide and diverged TCS signaling elements, such as might be found when performing searches with TCS domains.

TCS Component Name	Standardized Abbreviation	Reference Gene Identifiers from Arabidopsis and Rice
His kinases	HK	At1g67840, At2g17820, At2g47430, At5g10720, Os06g08450, Os06g44410, Os12g19530
	CHK	At1g27320, At2g01830, At5g35750, Os01g69920, Os02g50480, Os03g50860, Os10g21810
	ETR/ERS	At1g04310, At1g66340, At2g40940, At3g04580, At3g23150, Os02g57530, Os03g49500, Os04g08740, Os05g06320, Os07g15540
	PHY	At1g09570, At2g18790, At4g16250, At4g18130, At5g35840, Os03g0719800, Os03g19590, Os03g54084
His phosphotransfer protein	HPT	At1g03430, At3g16360, At3g21510, At3g29350, At5g39340, Os01g54050, Os05g09410, Os05g44570, Os08g44350, Os09g39400
Response regulators	RRA	At1g10470, At1g19050, At1g59940, At1g74890, At2g40670, At2g41310, At3g48100, At3g56380, At3g57040, At5g62920, Os01g72330, Os02g35180, Os02g42060, Os02g58350, Os04g36070, Os04g44280, Os04g57720, Os07g26720, Os08g26990, Os08g28900, Os08g28950, Os11g04720, Os12g04500
	RRB	At1g49190, At1g67710, At1g68210, At2g01760, At2g25180, At2g27070, At3g16857, At3g62670, At4g16110, At4g18020, At4g31920, At5g07210, At5g49240, At5g58080, Os01g67770, Os02g08500, Os02g55320, Os03g12350, Os04g28120, Os04g28130, Os04g28150, Os04g28160, Os05g32880, Os05g32890, Os06g08440, Os06g43910, Os08g17760, Os08g35650, Os08g35670, Os10g32600
	RRC	At3g04280, At5g26594, Os03g53100, Os04g13480
	PRR (exclusively for Clock-related RRs)	At2g46790, At5g02810, At5g24470, At5g60100, Os02g40510, Os03g17570, Os07g49460, Os09g36220, Os11g05930
	RR (for potential new clades)	At4g00760

embl-heidelberg.de/). To facilitate the comparison with known TCS elements, we have included sequences of these domains from the Arabidopsis and rice TCS elements in FASTA format in Supplemental Data Files S1 to S3. Phylogenetic analysis can be performed with a number of online programs, such as the phylogeny.fr pipeline (<http://www.phylogeny.fr/>).

We hope that the nomenclature for the TCS members in plants proposed here will lead to a standardization of the annotation of members of the different protein families and thus facilitate phylogenetic analysis and comparison across different plant species, in order to better understand the different functions that may be associated with each clade.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Data File S1. His kinase domains from Arabidopsis and rice.

Supplemental Data File S2. HPT domains from Arabidopsis and rice.

Supplemental Data File S3. Receiver domains of response regulators from Arabidopsis and rice.

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