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The Protein Phosphatases and Protein Kinases of Arabidopsis thaliana

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INTRODUCTION

Protein kinases and protein phosphatases are major post-translational regulators of numerous cellular processes. These enzymes regulate metabolic pathways and are intimately involved in cellular signaling networks. There are over 1000 genes (Wang et al., 2003) in Arabidopsis that encode protein kinases and another 112 genes (Kerk et al., 2002) that encode protein phosphatase catalytic subunits (Table 1). While Arabidopsis contains orthologs of many of the protein kinases found in other eukaryotes, Arabidopsis, and most likely plants in general, also has an unique set of protein kinases. These include the receptor-like protein kinases and related cytoplasmic protein kinases, the calcium-dependent protein kinases and several members of the putative mitogen-activated protein kinase kinase kinases (Wang et al., 2003). The Arabidopsis protein phosphatase catalytic subunits encompass orthologs of the majority of the protein phosphatases found in other eukaryotes. However, the type 2C protein phosphatase family is notably large in number in Arabidopsis (Kerk et al., 2002). The distinct representation of genes encoding protein kinases and phosphatases in the Arabidopsis genome, relative to other eukaryotes, is a reflection of the evolutionary history of plants. The understanding that plants have developed cellular communication systems and basic developmental mechanisms independently from other multicellular eukaryotes (Meyerowitz, 2002) explains why plants have evolved a unique collection of enzymes that regulate protein phopshorylation. Indeed, we pointed out over a decade ago (Stone and Walker, 1995), before the exceptionality of the Arabidopsis kinome was fully appreciated, that plants have an unique repertoire of protein kinases that control the early steps in signaling pathways which is reflective of the unique developmental and environmental responses that govern plant growth and development.

Progress in understanding the role of protein phosphorylation in plant development and environmental responses has made some significant steps in the past few years. While much of the research is focused on Arabidopsis, important insights are also being made in other plant species. Indeed, as genomic and functional data becomes more complete for other plant species, we should be better equipped to answer questions about the fundamental mechanisms plants employ to control their growth, development and responses to environmental stimuli and the role that protein phosphorylation plays in these processes.

This chapter on the protein phosphatases and protein kinases of Arabidopsis takes a gene-centric approach to summarize our current understanding of the functional roles of these important mediators of cellular processes. We have tried to focus on the unique aspects of protein kinases and phosphatases.

RECEPTOR-LIKE PROTEIN KINASES IN ARABIDOPSIS

Receptor-like protein kinases (RLKs) are defined by the presence of a signal peptide, an extracellular domain, a transmembrane domain region that anchors the receptor in a cell membrane, and a carboxy-terminal Serine/Threonine (Ser/Thr) kinase domain. Analysis of the Arabidopsis genome reveals there are at least 610 members in the RLK family (Shiu and Bleecker, 2001), thereby representing more than 2% of the predicted Arabidopsis coding sequences. Due to their large numbers and their diverse functions with roles in development, pathogen resistance, and hormone perception, RLKs have become a target of many investigations. Several reports describing the function of RLKs have been released since the cloning of the Arabidopsis RLK, *ERECTA* (*ER*), in 1996 (Torii et al., 1996).

Table 1. Overview of protein kinases and phosphatases in Arabidopsis.

Class	No. of predicted genes	Reference
A Protein kinases	**>1000**	PlantsP, 2006[a]; Wang et al, 2003
Receptor-like protein kinase	**606**	
Leucine-rich repeat receptor-like kinase (LRR RLK)	232	Shiu and Bleecker, 2001
LRR I RLK	50	
LRR II RLK	14	
LRR III RLK	47	
LRR IV RLK	3	
LRR V RLK	9	
LRR VI RLK	11	
LRR VII RLK	10	
LRR VIII RLK	23	
LRR IX RLK	4	
LRR X RLK	16	
LRR XI RLK	28	
LRR XII RLK	10	
LRR XIII RLK	7	
Receptor-like cytoplasmic kinase (RLCK)	118	Shiu and Bleecker, 2001
Domain of unknown function 26 (DUF26)	45	Shiu and Bleecker, 2001
Lectin receptor kinase (lecRK)	42	Barre et al. 2002
S-locus glycoprotein-like domain (SD)	40	Shiu and Bleecker, 2001
Wall-associated kinase-like (WAKL)	25	Shiu and Bleecker, 2001
Proline extensin-like receptor kinase-like (PERKL)	19	Shiu and Bleecker, 2001
Catharanthus roseus receptor-like kinase-like (CrRLK1L)	17	Shiu and Bleecker, 2001
Wheat LRK10-like 1 (LRK10L-1)	13	Shiu and Bleecker, 2001
TAKL	11	Shiu and Bleecker, 2001
Crinkly4-like (CR4L)	8	Shiu and Bleecker, 2001
Extensin	5	Shiu and Bleecker, 2001
Lysine motif (LysM)	4	Shiu and Bleecker, 2001
Thaumatin	3	Shiu and Bleecker, 2001
RKF3L	2	Shiu and Bleecker, 2001
Unknown receptor kinase 1 (URK 1)	2	Shiu and Bleecker, 2001
C-type lectin (C-lectin)	1	Shiu and Bleecker, 2001
Unclassified	19	Shiu and Bleecker, 2001
Calcium-dependent protein kinase (CDPK)-SNF1-related kinase (SnRK) superfamily	**84**	Hrabak et al., 2003
CDPK	34	
SnRK	38	
CDPK-related kinase (CRK)	8	
Phosphoenolpryruvate carboxylase kinase (PPCK)	2	
PEP carboxylase kinase-related kinase (PEPRK)	2	
Mitogen-activated protein kinase cascade members	**90**	MAPK Group, 2002
Mitogen-activated protein kinase (MAPK)	20	
MAPKK	10	
MAPKKK	60	
GSK3/shaggy-like	**10**	Dornelas et al., 1998
Histidine kinase-like protein	**17**	Schaller et al., 2002
Other protein kinases	**>200**	PlantsP, 2006

Table 1 (*continued)*. Overview of protein kinases and phosphatases in Arabidopsis.

Class	No. of predicted genes	Reference
B Protein phosphatase	**112**	
Protein phosphatase 2C (PP2C)	69	Kerk et al., 2002
Protein tyrosine phosphatase (PTP)	1	Kerk et al., 2002
Protein serine/threonine phosphatase (ST)	23	Kerk et al., 2002
Dual specificity protein phosphatase (DSP)	18	Kerk et al., 2002
Low-Mr protein tyrosine phosphatase (LMW-PTP)	1	Kerk et al., 2002

[a]PlantsP: http://plantsp.genomics.purdue.edu/

Leucine Rich Repeat RLKs

ERECTA

The Arabidopsis Landsberg *erecta* (L*er*) ecotype has been used widely for both molecular and genetic studies. L*er*, which harbors the *er* mutation, originally was isolated from mutagenised seed populations in the 1950s (Redei, 1992). The *er* mutation is responsible for most of the phenotypic differences between the L*er* and Columbia ecotypes; plants mutated for *ER* have compact inflorescences and altered organ elongation (Torii et al., 1996). *ER* encodes a leucine-rich repeat (LRR) RLK that is expressed in the shoot apical meristem and in the young floral organ primordia (Yokoyama et al., 1998). *ER* regulates the inflorescence architecture by affecting the elongation of the internode and pedicels, as well as the shape of lateral organs (Yokoyama et al., 1998, Torii et al., 1996). While these processes also are regulated by auxin (Kepinski and Leyser, 2005a), the identification of a suppressor of a weak *er* allele by activation tagging revealed that *ER* and auxin act independently (Woodward et al., 2005). Nevertheless, *ER* is not the only RLK to control these developmental processes. The overexpression of a dominant-negative form of *ER* showed that *ER* shares functions with other RLKs; in a null *er* mutant, overexpression enhances the *er* phenotype (Shpak et al., 2003).

ER belongs to a small family of seven genes, but only two are closely related to *ER* (Shiu and Bleecker, 2001). Shpak et al. (Shpak et al., 2004) analyzed the functional roles of *ER* and its closest homologs, *ER LIKE1* (*ERL1*) and *ERL2*, using a reverse-genetics approach and a combination of several loss-of-function alleles. Single loss-of-function mutants in either *ERL1* or *ERL2*, or the combination of both mutants, are phenotypically normal. However, the double mutant of *er* with either *erl1* or *erl2* and the *er erl1 erl2* triple mutant display enhanced *er* phenotypes.

In addition to their role during organ development, *ER, ERL1* and *ERL2* play a role in stomatal development (Figure 1 A and B) (Shpak et al., 2005). An *er* single mutant exhibits an increased number of stomatal neighbor cells that fail to differentiate into stomata, which results in a decreased stomatal density. This phenotype is consistent with the identification of *ER* as a regulator of plant transpiration efficiency by a quantitative trait loci approach (Masle et al., 2005). In addition, both *ERL1* and *ERL2* maintain stomata stem cell activity and prevent terminal differentiation of the meristemoid into the guard mother cell since the *erl1, erl2* and *erl1 erl2* mutants have fewer stomatal neighbor cells (Shpak et al., 2005). Furthermore, these three genes control the initial decision by protodermal cells to produce either pavement cells or a stomatal complex by asymmetric division. In Arabidopsis, stomatal patterning follows the "one cell spacing" rule: no two stomata can be directly adjoining each other; there will be at least one pavement cell between two adjacent stomata (Nadeau and Sack, 2002a). The "one cell spacing" rule is disrupted when the three genes are mutated, resulting in clustered stomata (Shpak et al., 2005).

This clustered stomata phenotype is similar to the *too many mouths* (*tmm*) mutant phenotype (Geisler et al., 1998; Yang and Sack, 1995). TMM encodes a receptor-like protein that lacks a cytoplasmic kinase domain (Nadeau and Sack, 2002b). From the genetic interaction between *TMM* and the *ER* genes, *TMM* appears to be epistatic to *ER* and its homologs (Shpak et al., 2005). However, the complex and organ-specific interactions preclude solid conclusions from the current data about the interaction between the *ER* genes and *TMM*. For the *ER* genes, the specificity of function is likely to be due to different *cis*-regulatory elements, since both *ERL1* and *ERL2* can rescue the *ER* phenotype when expressed under the *ER* promoter (Shpak et al., 2004).

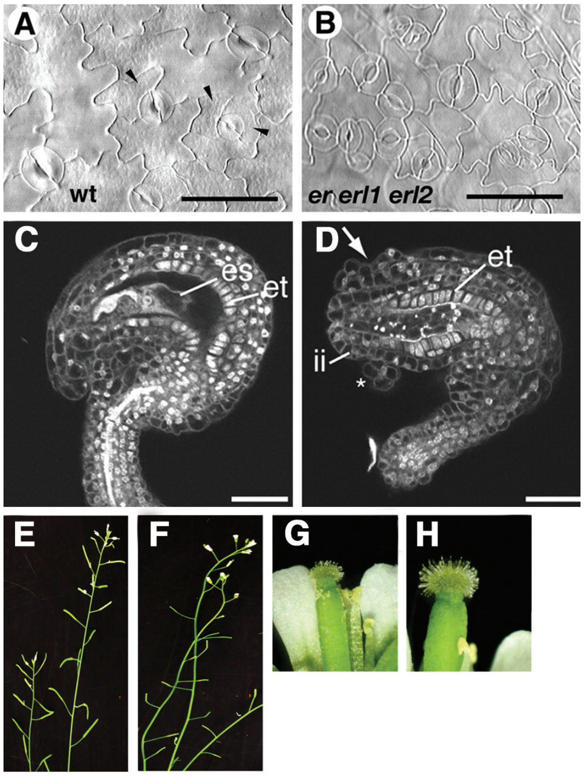

Figure 1. Phenotype of *er erl1 erl2* triple mutant, *sub-4* and *serk1-1 serk2-2* double mutant. **(A and B)** Cleared differential interference contrast images of the abaxial epidermis of a mature rosette leaf of wild type (wt) **(A)** and *er erl1 erl2* triple mutant **(B)** leaves. Arrowheads indicate SLGCs. **(C)** Midoptical section through an early stage-4 wt ovule. **(D)** Midoptical section through a stage-4 ovule from a *sub-4* mutant. Endothelium differentiation has occurred, an outer integument is mostly absent on the adaxial side and the embryo sac cannot be discerned. The arrow marks a spot in the distal abaxial outer integument where irregular cell divisions occurred and the normally two-layered organization is not maintained. Note the aberrant cell shape and the aberrant orientation of the planes of cell division. The group of cells indicated by * are part of an outer integument that is mostly located outside this plane of focus. es, embryo sac; et, endothelium; ii, inner integument. **(E)** Inflorescence of a wt plant showing normal seed pods. **(F)** A wt flower showing pollen grains. **(G)** Inflorescence of the *serk1-1 serk2-2* double mutant with small seedpods and no developing seeds. **(H)** A double *serk1-1 serk2-2* mutant flower with shortened anther filament and no pollen grain. Scale bars in **(A and B)**, 50 mm, **(C and D)** 20 mm. **(A and B)** From Shpak et al., 2005; © 2005 by the American Association for the Advancement of Science, used with permission. **(C-D)** From Chevalier et al., 2005; © 2005 by the National Academy of Sciences of the USA, used with permission. **(E-H)** From Albrecht et al., 2005; © 2005 by the American Society of Plant Biologists, used with permission.

In addition to its role in development, *ER* appears to be involved in resistance to pathogens (Godiard et al., 2003; Llorente et al., 2005), suggesting multiple functions for *ER*.

BRASSINOSTEROID INSENSITVE 1

Brassinosteroids (BRs) share similar structures with animal steroid hormones that bind to nuclear receptors (e.g., testosterone and progesterone) (Beato et al., 1995). When applied exogenously, BRs cause several developmental effects, including the promotion of cell elongation and cell division and the inhibition of root growth.

Genetic screens for mutants that do not respond to the application of exogenous BRs identified the *brassinosteroid insensitve1* (*bri1*) mutant (Clouse et al., 1996) and the *brassinosteroid insensitive 2* (*bin2*) mutant (Li and Nam, 2002). *BRI1* encodes a LRR-RLK (Li and Chory, 1997). Treatment of Arabidopsis seedlings with brassinolide (BL) results in the autophosphorylation of BRI1 (Friedrichsen et al., 2000; Oh et al., 2000; Wang et al., 2001). Several in vivo phosphorylation sites have been identified (Wang et al., 2005).

A functional assay was used to study the role of BRs in the BR signaling pathway (He et al., 2000). In rice, a chimeric protein that contains the BRI1 extracellular domain, the transmembrane domain, and a short stretch of the intracellular domain fused to the XA21 kinase domain (Song et al., 1995) elicits a pathogen response when BL is applied. No response is obtained when the chimeric protein is mutated in the island domain of BRI1 or in the kinase domain of XA21. These results suggest that BRI1 is directly involved in the BR signaling pathway.

The direct binding of BL to BRI1 was demonstrated with native and recombinant BRI1 proteins (Kinoshita et al., 2005). BR analogs can be cross-linked to BRI1 in microsomal preparations and in pull-down fractions highly enriched for BRI1-GFP, indicating that BRs and BRI1 directly interact. In addition, recombinant proteins that consist of the island domain and the neighboring C-terminal LRR are sufficient to bind radioactive BL with an affinity comparable to that observed for full-length BRI1 from plants, which suggests that BRI1 is one of the key factors in the perception of BRs in Arabidopsis.

Two of the three *BRI1* homologs (*BRL1* and *BRL3*) also bind BRs. In contrast to the ubiquitous expression of *BRI1*, the expression of both *BRL1* and *BRL3* is restricted to vascular tissue (Cano-Delgado et al., 2004). Both can complement *bri1* when expressed under the *BRI1* promoter, suggesting that BRL1 and BRL3 play a restricted and a partially redundant role in BR signaling (Zhou et al., 2004a; Cano-Delgado et al., 2004). Both the *bri1 brl1* double mutants and the *bri1 brl1 brl3* triple mutants enhance the vascular defect of the *bri1* single mutant. In addition, an activation tagging screen identified *BRL1* as a suppressor of a weak *bri1* allele (Zhou et al., 2004a). The same screen also identified *BRI1 ASSOCIATE KINASE* (*BAK1*), a different LRR RLK, as a suppressor.

VASCULAR HIGHWAY1 (*VH1*, also *BRL2*), the third BRI1 homolog, is unable to bind BRs (Cano-Delgado et al., 2004). *BRL2* is specifically expressed in provascular cells and during the differentiation of the provascular and procambial cells (Clay and Nelson, 2002). Overexpression of *VH1* results in premature leaf cell differentiation, while loss of function displays a phloem-specific defect that results in the blockage of vascular transport and premature senescence of leaves. Loss of function mutations in the other two *BRI1* homologs also leads to defects in the vascular strands.

BRI1 ASSOCIATE KINASE

BAK1, a LRR RLK in the same class as *SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASES1* (*SERK1*) and *2* (discussed below), is BRI1's coreceptor. It was independently identified as an interacting partner with BRI1 in a yeast-two hybrid screen and as a gain-of-function suppressor of a weak *bri1* allele in an activation tagging screen (Li et al., 2002; Nam and Li, 2002). In addition, *elongated* (*elg*), an unique allele of BAK1, displays an enhancement of high-light phototropism and increased sensitivity to BRs (Whippo and Hangartner, 2005). However, it is unclear how the *elg* allele confers hypersensitivity to BRs.

Consistent with the role of BAK1 as an interacting partner of BRI1, the *BAK1* loss-of-function mutant displays a BR-insensitive phenotype (Li et al., 2002; Nam and Li, 2002). However, this phenotype is weaker than *bri1* mutants, suggesting that other members of the *SERK/BAK* family also may be functional in BR preception, perhaps by forming heterodimeres with BRI1. Indeed, BRI1 and BAK1 interact in yeast in the absence of a ligand, suggesting that they may form a pre-existing heterodimer (Li et al., 2002). Interestingly, neither overexpression nor loss of function of *BAK1* influences ligand binding to BRI1 (Kinoshita et al., 2005; Wang et al., 2005). In cowpea protoplasts, the co-overexpression of BRI1and BAK1 results in a shift of BRI1 localization toward endosomal compartments. Furthermore, FRET between BRI1/BAK1 preferentially occurs in endosomes (Russinova et al., 2004). The demonstration of BRI1-BAK1 as a receptor of BRs suggests that other RLKs may serve as receptors for phytohormones.

RECEPTOR LIKE KINASE 1

Abscisic acid (ABA) is involved in stress responses (cold, drought, high salt), seed maturation and dormancy, and stomatal closure (Finkelstein and Rock, 2002). While the ABA signaling pathway is beginning to be elucidated (Himmelbach et al., 2003; Verslues and Zhu, 2005), the receptor and the proteins involved in the perception of ABA are still being identified. *RECEPTOR LIKE KINASE I* (*RPK1)* is a candidate receptor for ABA signaling.

The expression of RPK1 is specifically and rapidly induced by ABA, dehydration, high salt, and cold treatments (Hong et al., 1997). Both a T-DNA knock-out and an antisense line display decreased sensitivity to ABA and down-regulation of ABA-induced genes, while overexpression results in increased ABA sensitivity (Osakabe et al., 2005). Taken together, these results strongly suggest that *RPK1* is involved in an early step of ABA signaling. Future studies are needed to determine if *RPK1* is an ABA receptor and to elucidate the functional mechanism of action.

HAESA

HAESA (formerly *RLK5)* (Walker, 1993) was one of the first Arabidopsis protein kinases to be characterized biochemically. *HAESA* was isolated by screening an Arabidopsis cDNA library with the catalytic domain of *ZmPK1*, a maize receptor protein kinase. The protein kinase domain autophosphorylates on Ser/Thr residues in vitro (primarily by an intermolecular mechanism), and the protein kinase activity is lost by mutation of Lys-711, which corresponds to an invariant Lys found in many protein kinases (Horn and Walker, 1994). In vivo immunoprecipitation shows that HAESA is an active protein kinase *in planta*. In addition, immunoprecipitation identified phosphoproteins with apparent molecular masses of 65 and 85 kDa, which may correspond either to the proteolytic products of HAESA during the immunoprecipitation, or to endogenous substrates interacting with HAESA.

Using a promoter:glucuronidase (GUS) fusion construct and in situ hybridization, HAESA expression can be observed in the floral abscission zones. Expression also can be detected in vegetative tissues at the base of the petioles. Antisense experiments showed that a reduction in the level of HAESA protein is inversely correlated with the degree of defective floral abscission. The GUS expression of *HAESA* is not altered in crosses with *etr1-1* (the ethylene-insensitive mutation), suggesting that HAESA acts in an ethylene-independent pathway of floral abscission (Jinn et al., 2000).

CLAVATA 1

In plants, the shoot apical meristem produces lateral organs and axillary meristems throughout the life cycle. During the vegetative phase, it gives rise to leaves, and secondary meristems and, upon floral induction, produces flowers, bracts, and secondary meristems. The continuous production of lateral organs is possible due to the maintenance of a population of undifferentiated cells, called stem cells, at the tip of the meristem. These stem cells divide slowly, and their daughter cells are either used for their self-perpetuation, or they become the periphery cells and eventually form new lateral organs and the stem. Therefore, the coordination between stem cell accumulation and organ initiation is crucial for the maintenance of a functional meristem.

Three *clavata* (*clv*) mutations in independent loci (*clv1, clv2,* and *clv3*) result in a similar phenotype: over time the inflorescence and floral meristems become progressively larger and the numbers of flowers and floral organs in each whorl increases (Clark et al., 1993; Clark et al., 1995; Kayes and Clark, 1998). CLV1 is a LRR-RLK, CLV2 a LRR receptor-like protein lacking a kinase domain, and CLV3 is a small, secreted protein (Jeong et al., 1999; Fletcher et al., 1999; Clark et al., 1997). Based on the nature of the proteins and comparisons with animal and plant receptors, it has been proposed that CLV1, CLV2, and CLV3 form a receptor complex. The CLV signaling pathway limits the proliferation and/or promotes the differentiation of stem cells in the shoot apical meristem (Laufs et al., 1998; Trotochaud et al., 1999; Jeong et al., 1999; Rojo et al., 2002).

An intriguing characteristic of the *clv1* mutants is that different mutations result in unexpected phenotypes. For example, strong and intermediate alleles contain a missense mutation within the LRR or kinase domains, whereas the weakest alleles lack most of the kinase domain (Clark et al, 1997). A *clv1* null allele displays a weak phenotype. This suggests that strong and intermediate alleles may function as dominant negative alleles and that additional receptors function in overlapping roles with *CLV1* (Dievart et al., 2003). While the obvious candidates for such receptors are the CLV1 homologs- BARELY ANY MERISTEM 1 (BAM1), BAM2, and BAM3- loss of function of these genes results in a phenotype that is opposite of the *clv1* mutant phenotype (Deyoung et al., 2006). The *BAMs* appear to maintain the stem cell population in the shoot apical and floral meristems, and they also regulate vascular strand and male gametophyte development (Deyoung et al., 2006; Hord et al., 2006). The broader function of the *BAM* genes correlates with their wider expression patterns. The different expression patterns seems to explain the different roles of *CLV1* and *BAMs* during meristem development. Indeed, when expressed under the *ER* promoter, *CLV1* can fully rescue the *bam1 bam2* double mutant, while the *BAM1* and *BAM2* genes can partially rescue the *clv1* phenotype. Taken together, these data suggest that the *BAM* genes most likely do not functionally overlap with *CLV1*. However, *STRUBBELIG* (*SUB*), another LRR RLK, has been shown to be important for meristem development.

STRUBBELIG/SCRAMBLED

The *SUB/SCRAMBLED* (*SCR*) gene encodes a LRR RLK. The *sub* mutant was first reported as an ovule-development mutant (Schneitz et al., 1997) because *sub* displays altered development of the outer integument of the ovules that results in semi-sterility (Fig. 1 C and D). However, it now appears that *SUB* has a wider function throughout Arabidopsis development.

sub is allelic to *scr* (Kwak et al., 2005). The overall morphology of the root in the *sub/scr* mutant is indistinguishable from the wild-type. Roots from the *sub/scr* alleles differ from wild-type roots only by the mis-expression of some root epidermal-specific markers. Therefore, *SUB/SCM* appears to regulate the establishment of positional cues that allow the correct specification of the epidermis cells. But, its mechanism of action is still unknown. One possible explanation is that SUB/SCM affects the formation of organs by influencing cell morphogenesis, the orientation of the division plane, and cell proliferation (Chevalier et al., 2005). Indeed, the original *sub* alleles display several other defects besides the ovule phenotype; the number and shape of floral organs are affected, as well as the morphology and cell number of the inflorescence stem. In addition, in both floral and apical meristems, *sub* displays an irregular L2 layer and occasional periclinal divisions, suggesting a role of *SUB* in controlling cell division in the meristems.

One interesting aspect of *SUB* is a possible new biochemical mechanism of signaling. In the SUB kinase domain, the change of two strictly conserved amino acids, which are necessary for kinase activity, results in an inactive kinase in vitro. However, the *sub* phenotype can be rescued by different *SUB* mutants, including ones that were shown to abolish all kinase activity. This suggests that a functional kinase domain is not necessary for SUB function. However, the *sub-4* allele carries a misssense mutation at a conserved position in the kinase subdomain VIa, which suggests that the kinase domain is necessary for SUB function. It also is possible that SUB interacts with an active RLK to form a signaling complex.

While *SUB* emphasizes the importance of signaling during ovule development, the anther and pollen also require functional RLKs for their development. *EXCESS MICROSPOROCYTES1 (EMS1)/EXTRA SPOROGENOUS CELLS* (*EXS*) and *SERK1* and *2* are involved in anther development.

EXCESS MICROSPOROCYTES 1/EXTRA SPOROGENOUS CELLS

In Arabidopsis, the development of the anther is tightly regulated with precise cell division patterns (Sanders et al., 1999). For example, the formation of the sporogenous cells (which undergo a meiotic division to give rise to the male gametophyte) results from the initial division of the archeporial initials. This initial division establishes two cell lineages: one cell differentiates into the sporogenous cells, while the other cell undergoes an additional round of cell division to form the endothecium, middle layer, and tapetum. However, only a limited number of archeporial initials are initiated.

The *ems1* and *exs* allelic mutants highlight the importance of signaling during anther development (Zhao et al., 2002a; Canales et al., 2002). *EMS1/EXS* encodes a LRR RLK that belongs to the same class of LRR RLKs as BRI1. While both mutants display the same male-sterile phenotype, the authors suggest a different function for EMS1/EXS. The mutant exhibits an increased number of sporogenous cells that do not undergo cytokinesis. In addition, the tapetal cells are missing, and the middle layer appears to be either missing or abnormal. This phenotype appears to be due either to an increased number of archeporial initials resulting in an increased number of sporogenous cells (Canales et al., 2002), or a mis-specification of the tapetal cells into sporogenous cells (Zhao et al., 2002a).

The isolation of the *tapetum determinant1* (*tpd1*) mutant favors the second hypothesis. *tpd1* exhibits the same mutant phenotype as *ems1/exs*, and *TPD1* encodes a predicted small protein with a putative signal peptide, sug-

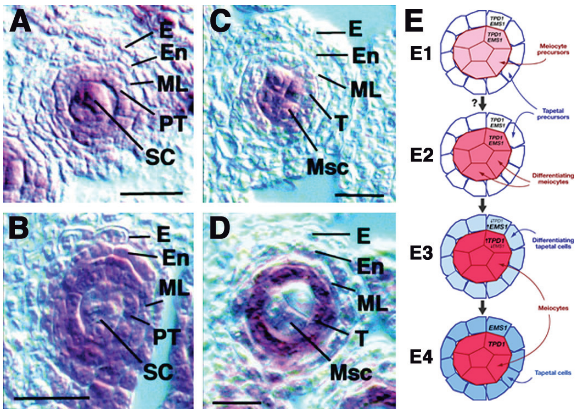

Figure 2. Expression patterns of *TPD1* and *EMS1/EXS* in anthers by in situ hybridization and a model for *EMS1/EXS* and *TPD1* function. (**A)** and **(B)** wt anthers at late stage 4. **(A)** Predominant *TPD1* expression in sporogenous cells. (**B)** Predominant *EMS1/EXS* expression in the primary tapetum. **(C)** and **(D)** wt anthers at early stage 5. **(C)** *TPD1* RNA is detected predominantly in microsporocytes. (**D)** *EMS1/EXS* RNA was found mostly in tapetal cells. E, epidermis; En, endothecium; ML, middle layer; Msc, microsporocyte; PT, primary tapetum; SC, sporogenous cell; T, tapetum. Scale bars in **(A-D)** 20 mm. **(E)** Model for *EMS1/EXS* and *TPD1* function. **(E1)** Initially, *TPD1* and *EMS1* are expressed in the precursors of both meiocytes (the central group) and tapetal cells (the outer ring). (**E2)** An unknown trigger (?) activates the differentiation of meiocytes, as indicated by shading. (**E3)** In the differentiating meiocytes, the expression of *TPD1* increases and that of *EMS1* decreases. The TPD1 protein is secreted and binds to the EMS1 receptors on the neighboring cells, causing an elevation of *EMS1* expression in these cells and a drop in *TPD1* levels. EMS1 then activates a pathway for tapetal differentiation. (**E4)** Further reduction of *EMS1* and *TPD1* in the meiocytes and tapetal cells, respectively, stabilizes the differentiation of tapetal cells. (A-D) From Yang et al., 2003; © 2003 by the American Society of Plant Biologists, used with permission. (E) From Ma, 2005; © 2005 by the Annual Reviews, used with permission.

gesting that it may function as a ligand (Yang et al., 2003). In addition, *EMS1/EXS* and *TPD1* are expressed initially in the precursors of the tapetal and sporogenous cells. (Figure 2 A-D). In the differentiating male sporocytes, the expression of *EMS1/EXS* decreases, whereas there is an increase in the expression of *TPD1*. In contrast, in the differentiated tapetal cells, *TPD1* expression decreases and *EMS1/EXC* expression increases. These observations lead to a model in which an unknown factor triggers the differentiation of male sporocytes, resulting in a decrease in the expression of *EMS1* and an increase in the expression of *TPD1* (Fig. 2 E) (Ma, 2005). In this model, the secreted *TPD1* binds to EMS1/EXS on the surface of the cells that surround the newly differentiated male sporocytes. In these cells, the interaction of TPD1 with EMS1 triggers the activation of a pathway that promotes tapetum differentiation and causes a decrease of *TPD1* expression. The differentiation of the tapetum occurs at the expense of the potential male sporocytes. This model is supported by the observation that the increased number of cells in carpels ectopically expressing *TPD1* is dependent on *EMS1/EXS* (Yang et al., 2005). Interestingly, mutations in two other LRR RLKs from a different class display the similar phenotype as *ems1/exs*.

SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASES

SERK1 and *2* belong to the class same class of LRR RLKs as *BAK1* (Shiu and Bleecker, 2001). *DcSERK1* (from *Daucus carota*) was the first family member to be isolated and is a marker for competence of cells to form somatic embryos (Schmidt et al., 1997). The Arabidopsis ortholog of *DcSERK1* (*SERK1*) also appears to enhance embryogenic competence in culture and is expressed in developing embryos and ovules (Hecht et al., 2001). However, this potential function is only based on the overexpression phenotype. Only recently has the loss of function mutant of *SERK1* been reported (Colcombet et al., 2005; Albrecht et al., 2005). The *serk1* and *serk2* single mutants show no obvious phenotype, but the *serk1 serk2* double mutants are male sterile (Fig. 1 E-H) and exhibit the same phenotype as the *ems1/exs* mutants (i.e., missing tapetal layer and extra sporogenous cells). While both reports proposed that the tapetal cells are miss-specified into sporogenous cells, the function of SERK1 and 2 is still unclear. In addition, it is unknown whether SERK1 and 2 act in the same signaling pathway as EMS1/EXS and TPD1.

The biochemical properties of SERK1 have been extensively studied. SERK1 is a dual Ser/Thr and tyrosine kinase, featuring an inter-molecular mechanism of phosphorylation (Shah et al., 2001). FRET has shown that SERK1 localizes to the plasma membrane and forms a homodimer (Shah et al., 2001). Additional interactions have been found in a yeast two-hybrid experiment with AtCDC48 (an AAA-ATPase), GF14*k* (a 14-3-3 protein), and KAPP (a PP2C kinase associated protein phosphatase) interacting with SERK1 (Rienties et al., 2005). KAPP is also proposed to control the internalization of SERK1 (Shah et al., 2002).

SUB, SERK1 and *2*, and *EMS1/EXC* highlight the importance of LRR RLKs and signaling pathways to proper development of the reproductive organs. However, fertilization and seed development also require RLKs. Since fertilization and seed development also depend on the interaction between the gametophytic and sporophytic tissues (Berleth and Chatfield, 2002), one would expect RLKs to be involved in these processes.

HAIKU 2

Seed development is an agronomically important trait and, therefore, has been the focus of much research. The *haiku2* (iku2) and *miniseeds3* (*mini3*) mutants display reduced seed size associated with reduced growth and early cellularization of the endosperm (Luo et al., 2005). This is a similar phenotype to the previously characterized *iku1* mutant (Garcia et al., 2003). IKU2 encodes a LRR RLK, and MINI3 encodes a WRKY class transcription factor (Luo et al., 2005); IKU1 has not been cloned yet. No mechanism of function for IKU2 has been proposed. However, decreased expression of the *IKU2::GUS* reporter line both in the *iku1* and *mini3* mutants was found. In addition, the *MINI3::GUS* reporter line was only expressed in the *iku1* mutant suggesting the successive action of these three genes (*IKU1, IKU2, MINI3*) in the same pathway of seed development.

FLAGELLIN SENSING 2

FLagellin Sensing (*FLS2*) is a RLK with 28 LRRs. *FLS2* was isolated through map-based cloning as a mutant that is insensitive to flagellin treatment. *FLS2* represents an example of conservation of the innate-immunity response among animals, insects, and plants (Gomez-Gomez and Boller, 2002). Flagellin, a building block of eubacterial flagella, is an elicitor that induces a defense response through a non-host-specific mechanism. Synthetic flagellin peptides with 15 or 22 amino acid residues (flg15 or flg22) are functional elicitors in Arabidopsis of such responses as callose deposition, induction of pathogen-related (PR) genes, and growth inhibition of seedlings (Gomez-Gomez et al., 1999).

The expression of FLS is ubiquitous in different organs and is not altered by flg22 treatment. Two point mutations-one in the LRR domain (*fls2-24*) and the other in the protein kinase domain (*fls2-17*) show an alteration of the *FLS2*-mediated signaling pathway with a blocked flagellin response (Gomez-Gomez and Boller, 2000). These missense mutations demonstrate the importance of both extracellular and cytoplasmic protein kinase domains for the flagellin-mediated defense response. Flg22 treatment of wild-type plants confers resistance to bacterial infection through the enhanced expression of many defense-related genes. From microarray studies between wild-type plants and the *fls2-17* mutant the expression of 966 genes were identified as up regulated by flg22 treatment. Flg22-induced resistance seems to be independent of salicylic acid, jasmonic acid, and ethylene-mediated signal pathways, which are based on the experiments using knockout mutants in transgenic plants of the previously reported signaling components (*npr1, eds1, sgt1, rar1, etr1, ein2, jar1, pad2*, or *pad4*) or overexpression of *NahG* (Zipfel et al., 2004). The FLS2 protein kinase recognizes flg22 and transfers the signal via the following MAPK cascade proteins: AtMEKK1 (MAPKKK), AtMKK4a/AtMKK5a (MAPKKs), and MPK3/MPK6 (MAPKs). The MAPKs are known to activate their target transcription factors (e.g., WRKY29 and FRK1) that control the expression of defense-related genes (Asai et al., 2002).

Other RLKS

CRINKLY-like

The *crinkly4* (*cr4*) mutant was first isolated in maize (Becraft et al., 1996). The mutant plants are shorter, have crinkled leaves, and, due to a wrong cell fate specification of the endosperm, the peripheral endosperm cells develop as starchy endosperm instead of aleurone (Becraft et al., 1996; Becraft and Asuncion-Crabb, 2000). *CR4* encodes an RLK with two different domains in its ectodomain. One domain is similar to the binding domain of the mammalian tumor necrosis factor receptor. The other domain consists of seven repeats, called crinkly repeats. Both domains are thought to participate in protein-protein interactions.

Arabidopsis contains a family of five RLKs related to *CR4* (Shiu and Bleecker, 2001; Cao et al., 2005): *ACR4* (the Arabidopsis ortholog of *CR4*) (Gifford et al., 2003; Tanaka et al., 2002), *AtCRR1, AtCRR2, AtCRR3,* and *AtCRK1*. *AtCRK1* is the ortholog of *CRK1* from tobacco, which is negatively regulated at the transcriptional level in cell cultures by exogenous cytokinin (Schafer and Schmulling, 2002). *ACR4* is expressed in protodermal cells of the embryo and the shoot (Gifford et al., 2003; Tanaka et al., 2002). While the antisense *ACR4* displays only moderate defects in seed formation and in embryo morphogenesis (Tanaka et al., 2002), the *ACR4* T-DNA insertion alleles exhibit altered integuments and seed coat development but no defects in embryo morphology (Gifford et al., 2003). Surprisingly, no leaf phenotype is observed, which suggests that *ACR4* shares a redundant function with other genes. To check for overlapping functions with other ACR4 homologs, expression patterns were checked and all the members were found to have specific but overlapping expression patterns (Cao et al., 2005). All the possible combinations of the double mutants with acr4 displayed no additional phenotypes. However, the acr4 mutation has been shown to affect the differentiation of leaf epidermal cells, suggesting a similar role for ACR4 and CR4 in the differentiation of leaf epidermis (Watanabe et al., 2004). ACR4 and ABNORMAL LEAF SHAPE1 (ALE1), which encodes a putative subtilisin-like serine protease, genetically interact since the double mutant exhibits a synergistic phenotype (Watanabe et al., 2004). However, the mechanism of that interaction is unknown.

The biochemical activities of ACR4 and its homologs are different. AtCRR1 and AtCRR2 do not have kinase activity in vitro, most likely due to a deletion in the kinase subdomain VIII, but AtCRR2 can be phosphorylat in vitro by the active ACR4 (Cao et al., 2005). However, a mutant of ACR4 that has a mutation abolishing kinase activity can rescue the *acr4* phenotype, suggesting that a functional kinase domain is not necessary for the function of ACR4 (Gifford et al., 2005).

NUCLEAR SHUTTLE PROTEIN (NSP)-INTERACTING KINASES

Nuclear Shuttle Protein (NSP) of geminivirus interacts with an NSP-interacting kinase (NIK, a transmembrane receptor) from tomato (LeNIK) and from soybean (GmNIK). Based on the sequence alignments of *GmNIK* with the Arabidopsis genome, three genes (At5g16000, At3g25560, and At1g60800) show high similarities to *GmNIK* (76%, 70%, and 61% identities, respectively). The gene products are localized at the plasma membrane and autophosphorylate the cytoplasmic Ser/Thr protein kinase domain.

The NSP of geminivirus inhibits the activities of NIK1 and NIK2, but it does not inhibit the activity of AtSERK1, which has a high-sequence similarity. Loss-of-function mutants of NIKs through T-DNA insertion are highly susceptible to the viral infection, suggesting that NIKs may be the targets of the NSP for escaping the antiviral mechanism of the host (Fontes et al., 2004).

CYSTEINE-RICH REPEAT RLKs

AtRLK3 has 12 cysteines in the extracellular domain, 8 of which reside in a tandem repeat of two 25 amino acids motifs in the following pattern: C-X8-C-X2-C-X11-C (DUF26). The expression of *AtRLK3* is induced by reactive oxygen species (ROS), pathogen infection (Czernic et al., 1999), and exogenous application of salicylic acid (Ohtake et al., 2000).

The Cys-rich repeat (CRR) is a novel motif that shares a distinct structure with the Cys-rich region of the S-locus glycoproteins and the SRKs. Forty-one RLKs have the novel CRR motif in their extracellular domains. Twenty RLKs of this group are located on chromosome IV as tandem repeats, suggesting that tandem duplication may be the key mechanism of CRKs' expansion. However, the overall sequence homologies among the genes is not high, even among the 20 tandem-arrayed RLKs (Chen, 2001; Chen et al., 2004).

The overexpression of *CRK5* (At4g23130) by a CaMV35S promoter triggers rapid induction of the disease-resistance gene *PR1* and, therefore, enhances resistance to bacterial infection. An induced expression, using a steroid-inducible promoter, also results in hypersensitive reaction (HR)-like cell death, even in the *eds1, ndr1,* and *npr1* mutants and in the *NahG* transgenic plants (Chen et al., 2003). A yeast two-hybrid screen showed that CRK-interacting proteins (CRKIP1, 2, and 3) interact with CRK5. Phylogenetic analysis showed that *CRK4* (At3g45860), *CRK19* (At4g23270), and *CRK20* (At4g23280) are closely related to *CRK5*. These genes' expression patterns are similar to the expression pattern of *CRK5* and include induction by salicylic acid treatment and pathogen infection and HR-like cell death under the steroid-inducible promoter (Chen et al., 2004).

PROLINE-RICH, EXTENSIN-LIKE RECEPTOR KINASE-1

PROLINE-RICH, EXTENSIN-LIKE RECEPTOR KINASE-1 (*PERK1*) was isolated from *Brassica napus*. It is comprised of a proline-rich extracellular domain (which shows high similarity with extensins), a single transmembrane domain, and a Ser/Thr kinase domain with catalytic activity. Gene expression is induced rapidly by wounding and by fungal infection.

There are 15 genes in the Arabidopsis PERK family (*At*PERKs). The similarities of extracellular, transmembrane, and juxtamembrane domains among the PERKs are less than 70%. However, the similarities of protein kinase domains are high. For example, there is over 80% identity between PERK1 and *At*PERK1. The expressions of the *At*PERK family are both ubiquitous and tissue-specific. Although a single T-DNA insertion mutant of each *At*PERK does not show any phenotypic changes, the simultaneous suppression of several *At*PERKs, using antisense, results in various growth defects, suggesting a functional redundancy (Silva and Goring, 2002; Nakhamchik et al., 2004).

WALL-ASSOCIATED KINASES

Wall-associated kinases (*WAK*) and WAK-like kinases (*WAKL*) have unique properties among the RLKs. They physically link the extracellular matrix and the cytoplasm and, it is proposed, serve as a signaling intermediate between both. In addition, most of the *WAK/WAKL* genes are found in tandem repeats (Verica et al., 2003; Verica and He, 2002).

WAKs/WAKLs carry a cytoplamic Ser/Thr kinase domain and an extracellular domain with some similarity to vertebrate epidermal growth-factor-like domains. The association of WAK/WAKL proteins to the cell wall components was demonstrated by immunohistochemistry for several members (Verica et al., 2003; Wagner and Kohorn, 2001; He et al., 1996). The interaction between WAK1 and the cell wall pectins is dependent on a calcium-induced conformation (Decreux et al., 2005).

The Arabidopsis genome contains 26 WAKs/WAKLs, which are divided into four families (I-IV) (Verica and He, 2002). Functional information is known only about families I and II (Wagner and Kohorn, 2001; Verica et al., 2003). Family I, which is comprised of the five *WAKs*, is expressed predominately in green tissues (Wagner and Kohorn, 2001). Family II, which includes *WAKLs1-7*, is highly expressed in roots and flowers (Verica et al., 2003). Expression of these two families' members is developmentally regulated and wound inducible. In contrast to their well-characterized expression patterns, the function of these genes is still unclear.

The analysis of loss of function in the *WAKs/WAKLs* genes remains a challenge because of the organization of the genes in tandem repeats. To overcome this hurdle, several approaches have been undertaken. Antisense expressions of *WAK2* and *WAK4* showed that both genes are required for cell elongation (Wagner and Kohorn, 2001;

Lally et al., 2001), while the yeast two-hybrid method revealed that WAK1 interacts with a glycine-rich protein (Park et al., 2001). The expressions of WAK1 and WAKL4 are induced by aluminum and several minerals (copper, nickel, and zinc) (Hou et al., 2005; Sivaguru et al., 2003). The overexpression of WAK1 and the loss of function of WAKL4 suggest that both genes are involved in the mineral response. WAK1 may confer resistance to aluminum, while the role of WAKL4 may be more versatile. Indeed, the loss of function of WALK4 results in hypersensitivity to copper and zinc and an increase in tolerance to nickel.

LECTIN DOMAIN PROTEIN KINASES

Legume lectins are carbohydrate-binding glycoproteins (Barre et al., 2002). In Arabidopsis, *Ath.lecRK1* is an RLK that contains a lectin-like domain (Herve et al., 1996). *Ath.lecRK1* was predicted to be a membrane-spanning receptor with an extracellular lectin domain, a membrane-spanning domain, and a cytoplasmic Ser/Thr kinase domain. Subsequent work has identified additional family members; however, not all members are likely to be functional (Herve et al., 1999). Arabidopsis sequence analysis has uncovered three classes of *lecRK* genes, most of which do not appear to contain introns (Barre et al., 2002).

Although the Arabidopsis lectin domains are very similar to the legume lectins, there is little conservation of an Asp and Asn residue that is involved in the binding of two divalent cations (Ca^{2+} and Mn^{2+}) in legume lectins (Barre et al., 2002). Since these two ions are required for monosaccharide binding in legumes, it is unlikely that Arabidopsis lectins are able to bind monosaccharides. However, a conserved hydrophobic cavity found in legume lectins also has been found in all predicted Arabidopsis *lecRKs*, suggesting an ability to bind hydrophobic ligands including some phytohormones (Barre et al., 2002).

Little functional information exists about the *lecRKs*. Expression analysis of *lecRK-a1* showed an increase in promoter activity (as assayed with a GUS reporter gene) in parallel with senescence (Riou et al., 2002). In addition, transcription of *lecRK-a1* is locally activated in response to wounding. Recent work suggests a role for a *lecRK* in protein-protein interactions involved in mediating plasma membrane-cell wall adhesions (Gouget et al., 2006). This *lecRK* contains in its extracellular domain peptide sequences that are similar to peptide sequences that bind a protein from the plant pathogen *Phytophthora infestans*. While this *lecRK* appears to have an active cytoplasmic kinase domain and seven putative glycosylation sites, it is unable to bind simple sugars. However, it is capable of binding peptides containing sequences from the *P. infestans* protein. Binding of these peptides disrupts plasma membrane-cell wall adhesions during plasmolysis, suggesting that *lecRKs* may have structural and signaling roles at the cell surface during plant defense.

S-LOCUS RECEPTOR PROTEIN KINASE

Many Brassica family members are obligate outcrossing plants, with the notable exception of Arabidopsis. Outcrossing in Brassica is maintained by an elaborate signaling mechanism that includes an RLK and a small cysteine-rich ligand (Kachroo et al., 2002; Kemp and Doughty, 2003; Takayama and Isogai, 2003). The *S-LOCUS RECEPTOR PROTEIN KINASE* (*SRK*) is a functional RLK that contains a Ser/Thr cytoplasmic kinase domain, a transmembrane domain, and an extracellular domain that is similar to the S-locus glycoprotein.

Several *SRK*-like genes have been isolated from Arabidopsis, but most have primarily vegetative patterns of expression (Tobias et al., 1992; Walker, 1993; Dwyer et al., 1994). *SRK* and its ligand (*SCR*) have been cloned from *A. lyrata* (Kusaba et al., 2001). Using comparative mapping, a likely candidate *SRK* gene in Arabidopis has been isolated. While this gene initially appeared to be a functional *SRK*, subsequent analysis of its cDNA showed it to be nonfunctional due to a mispredicted intron-exon splice site. Transfer of a functional *SRK-SCR* pair from Lyrata into Arabidopsis, however, restores a self-incompatibility response, indicating that the remaining components of the SRK signaling pathway are found in Arabidopsis (Nasrallah et al., 2002). It will be interesting to determine what role exists for the other *SRK*-like genes that were uncovered earlier.

MAP KINASES (MAPK) IN ARABIDOPSIS

Mitogen-activated protein kinase (MAPK) cascades are three-kinase modules that are evolutionarily conserved (MAPK group, 2002). The basic activation mechanism of MAPKs is conserved across all eukaryotes. A MKKK (MAP kinase kinase kinase) activates a MKK (MAP kinase kinase) by phosphorylating conserved Ser/Thr residues in the activation domain. MKK (a dual-specificity kinase), in turn, activates MAPK by phosphorylating tyrosine and Ser/Thr residues in an activation loop (Thr-Xaa-Tyr, tripeptide motif, Xaa can be Asp, Glu, Gly, or Pro). MAPK cascades can be activated by an array of extracellular stimuli transduced by receptors/sensors in both yeast and in animal cells (Widmann et al., 1999; Morrison and Davis, 2003).

MAPK cascades function in stress and hormonal responses, as well as in cell proliferation, differentiation, and death (Figure 3). In yeast, animals, and plants, multigene families have been identified that encode each of the three tiers of the MAPK cascades. Diverse combinations of the three components of a MAPK cascade allow diverse input signals to be transduced through the MAPK cascades. The specificity of the MAPK cascades can be maintained by scaffold proteins, substrate specificity, spatiotemporal colocalization, and by interactions between MAPKs (Morrison and Davis, 2003; Elion et al., 2005). In addition, MAPK cascade signals can be attenuated by Ser/Thr phosphatases and dual-specificity tyrosine phosphatases.

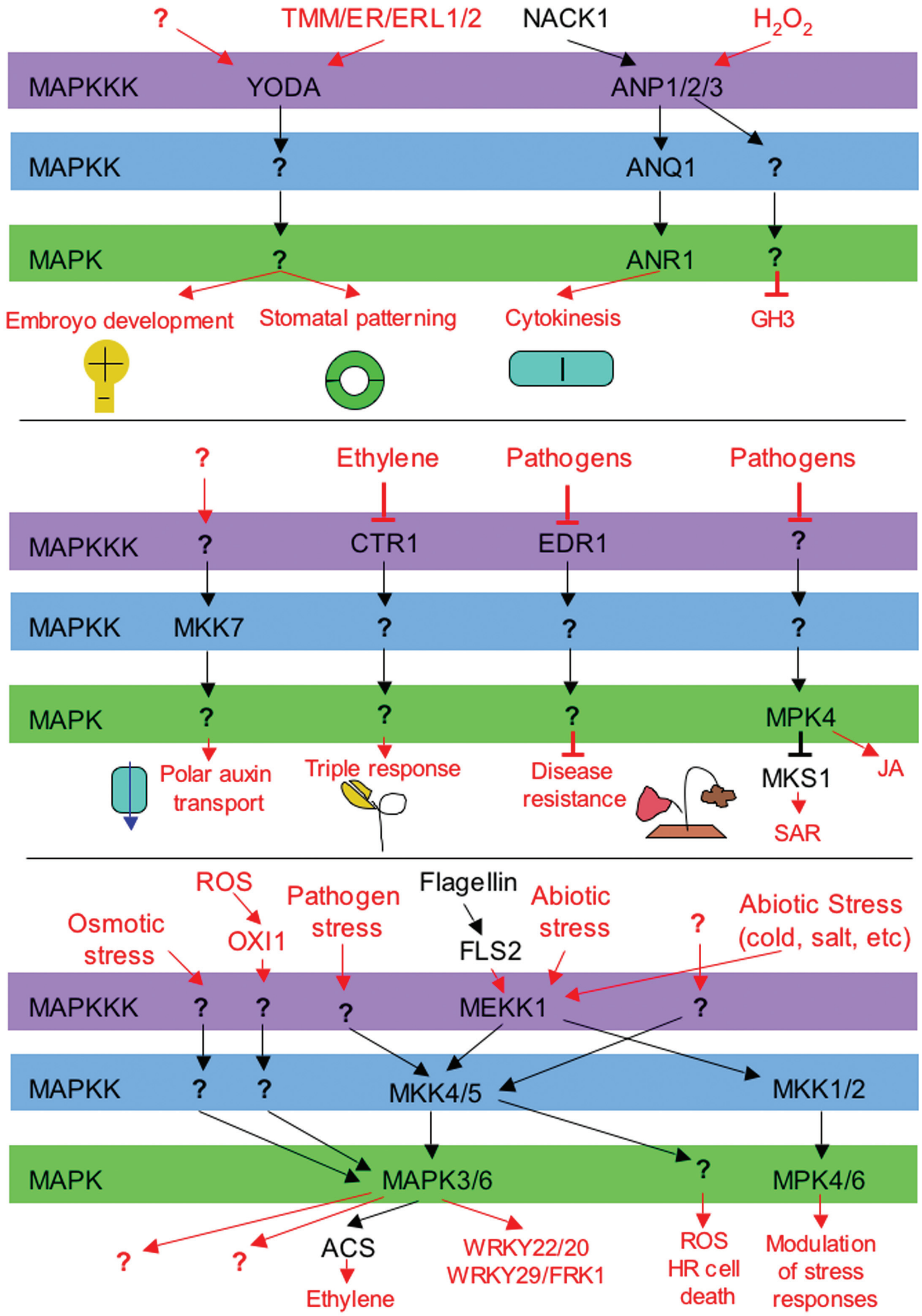

Figure 3. Overview of MAP kinase pathways described in Arabidopsis. Each level of the MAPK cascade is placed in colored boxes. Inputs and any genes that are involved in these inputs, if known, are placed above the colored boxes and the corresponding outputs or targets, if known, are placed below the colored boxes, vertically arranged with the inputs. Arrows and components that are in black indicate a direct connection while those in red indicate an indirect or not entirely known connection. Arrows indicate stimulation while blunted lines indicate inhibition.

In the Arabidopsis genome, about 60 MAPKKKs (MKKK), 10 MAPKKs (MKK), and 20 MAPKs have been identified (MAPK group, 2002). The presence of a diverse group of MKKKs suggests that a wide range of signals may activate downstream components in MAPK cascades. In contrast, the limited number of MKKs suggests that signals from MKKKs could be integrated at the MKK level (MAPK group, 2002).

Arabidopsis MAPKs can be classified into two subtypes: "TEY" MAPKs and "TDY" MAPKs. MAPK activity is controlled by the dual phosphorylation of Thr-Xxx-Tyr in the activation loop. Similar to mammalian ERK1/ERK2, TEY MAPKs have Thr-Glu-Tyr in their activation loops. In contrast, TDY MAPKs have Thr-Asp-Tyr in their activation loops. TDY MAPKs are also distinguished by a long C-terminal tail that is reminiscent of ERK5 (the mammalian MAPK); however, ERK5 has TEY in its activation loop instead of TDY. No plant MAPK has been identified that has TGY or TPY in the activation loop, which are found in the mammalian p38 MAPKs and JNK MAPKs. While the TEY subtype has been extensively studied in many plant species, limited functional information is available about the TDY subtype (He et al., 1999; Schoenbeck et al., 1999; MAPKgroup, 2002; Cheong et al., 2003).

The 10 MKKs encoded in the Arabidopsis genome can be classified into four subgroups (A, B, C, D), according to their sequence (MAPK group, 2002). With the exception of MKK10, the Arabidopsis MKKs have a consensus $S/TX_5S/T$ in their activation domain (in contrast to mammalian MKKs that have a $S/TX_3S/T$ phosphorylation motif). Arabidopsis MKKs also possess the conserved kinase interaction motif ($K/R_{2-3}X_{1-5}L/IXL/I$) in the N-terminal domain, which has been shown to be important for MAPK activation in both animals and plants (MAPK group, 2002; Jin et al., 2003; Tanoue and Nishida, 2003).

In Arabidopsis, the proposed family of MKKKs is more diverse than MKKs and MAPKs. Based on their kinase catalytic domain sequence, MKKKs can be classified into two main categories: 12 MEKK-like protein kinases that are related to animal MEKK/Ste11/Bck1 and 48 Raf-like protein kinases (MAPK group, 2002). It has not been demonstrated that the Raf-like protein kinases in Arabidopsis (represented by CTR1 and EDR1) can function as MKK activators. On the other hand, there is substantial evidence that the MEKK-like MKKKs (YODA, ANP1, ANP2, ANP3, AtMEKK1, NPK1 and MAPKKKα) do function as MKK activators.

MAPK Functions in Arabidopsis

MAPKs in Plant Development

The MKKK YODA has been shown to function in embryo development and in patterning (Lukowitz et al., 2004). In Arabidopsis, embryo development starts with the zygote undergoing elongation and asymmetric division that results in a small apical cell and a larger basal cell. In the *yda* loss-of-function mutant alleles, zygote elongation and asymmetric division are suppressed. The mutant zygote undergoes a nearly symmetrical division, and the resulting apical and basal cells are about equal in size. Like in the wild-type, the mutant apical cell maintains the normal divisions (two rounds of longitudinal divisions followed by one round of transverse division) and differentiates into the proembryo. However, unlike the wild-type, the basal cell of the *yda* mutant undergoes abnormal divisions and no suspensor is established; in the wild-type, the basal cell undergoes a series of transverse divisions that give rise to a file of cells forming the suspensor (Berleth and Chatfield, 2002; Lukowitz et al., 2004).

YODA belongs to the MEKK1/Ste11/Bck1 class of MKKKs. Removal of the N-terminal negative-regulation domain allows YODA to become constitutively active. In the constitutively active YODA mutant, suspensor cells are over-proliferated, which can suppress proper embryo development (Lukowitz et al., 2004).

YODA also functions in stomata development and patterning (Bergmann et al., 2004). Stomata are specialized epidermal structures formed by two guard cells surrounding a pore, through which plants absorb CO_2 and release O_2. Asymmetric cell divisions precede stomata cell specification in Arabidopsis. Perturbation of the frequency of asymmetric cell division, the orientation of the asymmetric division plane, and the polarity of the progeny of the asymmetric division ultimately disrupt stomata development and patterning (Nadeau and Sack, 2002a). As mentioned earlier, in Arabidopsis, stomata patterning follows the "one cell spacing" rule. *yda* mutations disrupt the asymmetric division of stomata precursor cells, which results in stomata excessively clustered together. In contrast, there is no stomata development in *yda* mutants with a deleted N-terminal domain (Bergmann et al., 2004)

Several stomata patterning mutants have been characterized in Arabidopsis. Mutations in genes encoding *TOO MANY MOUTHs* (TMM, a LRR receptor protein) (Nadeau and Sack, 2002b), *STOMATA DENSITY AND DISTRIBUTION 1 (SDD1,* a subtilisin-like serine protease) (Berger and Altmann, 2000), and *ER/ERL1/ERL2* (LRR receptor kinases) (Shpak et al., 2005) disrupt stomata patterning and result in clustered stomata. It is proposed that unknown ligands processed by SDD1 bind to the TMM/ERs' receptors in the target cells, which then triggers the activation of the YODA MAPK cascade and puts stomata development in check. However, the downstream MKK and MAPK cascades need to be identified to uncover the molecular mechanism of YODA function.

MAPKs in Cytokinesis Regulation

Plant cytokinesis is guided by a cytoskeletal microtuble structure, called the phragmoplast. The phragmoplast arises from the spindle midzone following mitotic division. Cytoskeletal microtubles of the phragmoplast direct Golgi-originated vesicles to deposit cell wall building material at the equator of the phragmoplast. The vesicles fuse together to form the cell plate. The cell plate expands radially (by fusion of new vesicles at its periphery) until it reaches the plasma membrane (Smith, 2002; Jurgens, 2005; Kolch, 2005).

The MAPK cascade NPK1-NQK1-NRK1 has been shown to regulate cytokinesis in tobacco BY-2 cells (Takahashi et al., 2004). NPK1 belongs to the Ste11-like MKKK family. It initially was identified because it is expressed specifically during the logarithmic phase of cell division in BY-2 cells. NPK1 localizes at the periphery of the phragmoplast during radial expansion toward the plasma membrane. Overexpression of a dominant negative mutant of NPK1 suppresses cytokinesis, resulting in multi-nucleate cells with cell wall stubs between the nuclei (Nishihama et al., 2001). Virus-induced gene-silencing (VIGS) of NPK1 results in dwarf plants with defective cytokinesis (Jin et al., 2002).

ANP1, ANP2, and ANP3 are the Arabidopsis orthologs of NPK1 and share a similar function in regulating cytokinesis (Krysan et al., 2002). Single loss-of-function mutants of the ANP genes do not have a phenotype. However, the *anp2 anp3* double mutants have defective cell cytokinesis, as evidenced by the presence of multinucleate cells and incomplete cell wall formation. The *anp1 anp2 anp3* triple mutants are gametophyte lethal (Krysan et al., 2002).

Two kinesin-like proteins, NACK1 and NACK2 (NPK1-acitvating kinesin-like protein 1), were identified as upstream activating factors of NPK1 (Nishihama et al., 2002). Biochemical results indicated that NACK1 activates NPK1 through direct protein-protein interactions that are mediated by the coiled-coil domain of NACK1 and NPK1. The motor domain of NACK1 also functions in targeting NPK1 to the equatorial zone of the phragmoplast during anaphase and telophase. NPK1 is mis-localized when the truncated form of NACK1, that lacking the motor domain, is overexpressed. This mislocalization results in incomplete cell plate formation.

The downstream MKK and MPK of NPK1 and ANP1 have been identified through an ingenious use of yeast genetics (Soyano et al., 2003). The phosphorylation relationship within the NPK1 MAPK cascade was established by in vitro and in vivo biochemical analyses. BY-2 cells expressing a dominant negative NQK1 have cell wall formation defects. A loss-of-function mutant of ANQ1 (the Arabidopsis ortholog of NQK1) has large cells with incomplete cell wall formation, while a loss-of-function mutant of ANR1 (the Arabidopsis ortholog of NRK1) shows severe defects in cytokinesis (Soyano et al., 2002; Takahashi et al., 2004). With corroborative evidence from mutant analysis in Arabidopsis, it is clear that NQK1-NRK1 function downstream of NACK1-NPK1 in controlling cytokinesis. AtNACK1-ANP1/ANP2/ANP3-ANQ1-ANR1 is the equivalent orthologous MAPK cascade in Arabidopsis (Soyano et al., 2003). Since cytokinesis is a very dynamic process involving the initiation of the phragmoplast at the midzone spindle, vesicle transporting, vesicle fusion, and phragmoplast radial expansion, it would be interesting to determine what stage is regulated by this MAPK cascade and what is the signaling output.

Besides cytokinesis, the NPK1 (ANP1) MAPK pathway has been demonstrated to function in plant reactive oxygen species (ROS) signaling and auxin responses. Both are discussed below.

MAPKs in Phytohormone Responses

Ethylene. The phytohormone ethylene regulates diverse aspects of plant growth and development including fruit ripening, abscission, senescence, apical hook formation in dark-grown seedlings, as well as abiotic (drought, flooding) and biotic (pathogen attack) stress responses (Ecker, 2004; Guo and Ecker, 2004). In response to ethylene treatment, dark-grown Arabidopsis seedlings exhibit a triple response: an exaggerated apical hook, radial swelling of the hypocotyl and root, and inhibition of hypocotyl elongation (Guzman and Ecker, 1990).

ctr1 was isolated as a mutant with a constitutive triple response in the absence of an exogenous ethylene application (Kieber et al., 1993). *CTR1* encodes a Raf-like protein kinase. Biochemical analysis demonstrated that CTR1 has intrinsic Ser/Thr kinase activity. The N-terminus of CTR1 is unique and may function as an interaction motif. Molecular analysis of a series of *ctr1* mutant alleles suggested that both the kinase activity and N-terminal motif are essential for the function of CTR1 (Huang et al., 2003).

Due to the homology of CTR1 with Raf (a MKKK), it has been proposed that a MAPK cascade functions downstream of CTR1 in the ethylene signal transduction pathway. However, there are no consensus results that support this proposal. It has been shown that ACC (the ethylene precursor) activates MAPK activity of SIMK, MMK3, and MAPKK SIMKK in a *Medicago* cell suspension culture. MPK6 activity also has been reported to be activated by ACC in an Arabidopsis cell suspension culture (Ouaked et al., 2003). However, potential problems with the pharmacological approaches used to find MPK6 activity have been raised (Ecker, 2004; Liu and Zhang, 2004). Recent genetic studies of MPK6 loss-of-function alleles and RNAi transgenic lines of MPK6 do not support ethylene-activation of MPK6, and no discernable effects on ethylene responses have been observed in these mutants (Ecker, 2004; Liu and Zhang, 2004; Menke et al., 2004). Thus, the role of a MAPK cascade in ethylene signaling remains unknown.

In response to various environmental signals, plants increase their ethylene biosynthesis to help cope with various stresses (Zhang and Klessig, 2001; Guo and Ecker, 2004; Chen et al., 2005). Conditional activation of SIPK (the Arabidopsis ortholog of MPK6) through an inducible, constitutively active NtMEK2^{DD} (the MKK upstream of SIPK) in tobacco results in a rapid increase of ethylene production (Kim et al., 2003). The activation of SIPK also coincides with the activation of ACS (ACC synthase) activity, suggesting that a MAPK cascade is involved in post-translational modification or transcriptional activation of ACS (Kim et al., 2003).

Recently, it was demonstrated unequivocally that MPK6 is required for NtMEK2^{DD}-induced ethylene production in Arabidopsis and that MPK6-induced-stress ethylene production is associated with increased ACS activity (Liu and Zhang, 2004). In contrast to ACS activity, ACO (ACC oxidase) activity stays high even without MPK6 activation, which suggests that ACS is the rate-limiting enzyme in stress ethylene biosynthesis (Liu and Zhang, 2004). Based on transcriptional activation by various stresses, *ACS6* was implicated in stress ethylene production (Vahala et al., 1998; Wang et al., 2002). Now, genetic and biochemical evidence demonstrates that MPK6 activation leads to an increase in cellular ACS6 activity as a result of direct phosphorylation of ACS6 by MPK6 (Liu and Zhang, 2004). Mutation of the Ser phosphorylation sites to Ala abolishes MPK6-induced ACS6 accumulation in vivo. Mutation of these Ser residues to Asp, ACS6^{DDD}, which mimics the phosphorylated form of ACS6, results in ACS6 accumulation independent of MPK6 activation. ACS6^{DDD} transgenic plants overproduce ethylene and show an ethylene-induced morphology phenotype (Liu and Zhang, 2004). Thus, the MPK6 signaling cascade functions upstream in regulating stress ethylene biosynthesis. More significantly, the first MAPK in vivo substrate, ACS6, has been identified, which is the beginning of unraveling the complexity of MAPK function in plants.

Auxin. Auxin functions in virtually every aspect of plant growth and development. The mechanism of auxin action has been intensely investigated for decades (Leyser, 2002; Woodward and Bartel, 2005). Recently, it was demonstrated that TIR1 (an F-box protein) is an auxin receptor. Direct interaction of auxin with SCF^{TIR1} triggers SCF^{TIR1} to interact with transcriptional regulators Aux/IAA and to target Aux/IAA for degradation by an ubiquitin-proteasome pathway (Dharmasiri et al., 2005a; Kepinski and Leyser, 2005b). The degradation of Aux/IAA releases ARFs (auxin-response factors), allowing auxin-responsive transcription activation by ARF. However, it remains unknown whether other auxin-signaling mechanisms exist in plants (Dharmasiri et al., 2005b).

Several studies have implicated MAPK cascades in auxin signaling and responses. Protoplast co-transfection experiments demonstrated that NPK1 negatively regulates the induction of GH3, an early auxin response gene (Kovtun et al., 1998). Additional experiments demonstrated that ANP1 shares a similar function in suppressing GH3 expression upon auxin treatment (Kovtun et al., 2000). Hydrogen peroxide (H_2O_2) activates the ANP1 MAPK cascade, and the constitutively active ANP1 induces gene expression response that mimics oxidative stress. H_2O_2 treatment also blocks auxin-inducible GH3 promoter activity. Therefore, it has been proposed that oxidative stress suppresses GH3 activity through the activation of the ANP1 MAPK cascade (Kovtun et al., 2000). However, analyses of loss-of-function alleles of ANP mutants indicate that there is no change in auxin sensitivity. Moreover, a genome-wide gene expression analysis showed no apparent change of auxin-regulated gene expression in the *anp2/anp3* double mutants (Krysan et al., 2002). Thus, additional experiments are needed to address these contradictory results.

Protein degradation and transcriptional regulation do not explain the immediate auxin responses, such as membrane depolarization and calcium spikes (Woodward and Bartel, 2005). Mockaitis and Howell (2000) demonstrated that MAPK activity is activated transiently within 5 minutes of applying auxin to Arabidopsis roots, whereas auxin-inducible MAPK activity disappears in the auxin-resistant mutant (*axr4*), which suggests a correlation of AXR4 function and MAPK activation (Mockaitis and Howell, 2000). While it is possible that MAPK activation could account for the immediate auxin responses, further research is required to test this hypothesis.

Another level of MAPK function in auxin biology is to regulate polar auxin transport (PAT). The Arabidopsis MKK7 overexpression mutant *bushy* and *dwarf1* (*bud1*) develops fewer lateral roots and has less vascular tissue differentiation. While these phenotypes indicate that *bud1* could be involved in auxin synthesis, transport, or signaling, there is no difference between *bud1* and the wild-type with respect to IAA content and root growth inhibition (Dai et al., 2006). This suggests that *bud1* is not involved in either auxin synthesis or in auxin signaling. Direct IAA transport assays indicate that *bud1* negatively regulates PAT, and increased PAT was observed in BUD1 knock-down mutants. A double-mutant analysis of *bud1 axr3-3* indicates that lowered PAT can be rescued partially by elevated auxin sensitivity in an auxin hypersensitive mutant (*axr3-3*). However, when bud1 is crossed with an auxin transport mutant (*doc-1*), the double mutant (*bud1doc-1*) has a more severe PAT defect (Dai et al., 2006). These genetic analyses reinforce the idea that *bud1* is a negative regulatory PAT mutant. Future studies should identify the whole MAPK cascade where MKK7 functions and the downstream effectors, which will help unravel the regulation mechanism of PAT in Arabidopsis.

MAPKs in Stress Responses

Plants are subject to a diverse array of biotic and abiotic stresses. However, most of the stresses are not lethal, as plants have evolved to adapt to environmental stresses. As a first step of adaptation, plants sense the stresses and activate signaling transduction pathways that initiate various defense responses. Accumulating evidence indicates that a subset of plant responses-such as the activation of early defense genes and ROS generation-are shared by responses to abiotic and biotic stresses. MAPK cascades are likely to be one of the converging "hubs" of plant stress response signaling networks.

Pathogen Responses. Plants are constantly under attack by bacteria, fungi, nematodes, and viruses. Plants employ resistance proteins (R proteins) and other receptors/sensors to detect invasion of these pathogens. The detection of pathogen-virulence proteins or elicitors by R proteins/receptors triggers a diverse array of cellular responses, such as ROS generation, synthesis of stress ethylene, ion fluxes, strengthening of cell walls, synthesis of phytoalexins, up-regulation of pathogen-related proteins, and induction of hypersensitive response (HR) cell death (Pedley and Martin, 2005). Often, a local defense response against one pathogen can trigger the induction of a broad-spectrum resistance to different pathogens throughout the plant; this is called systemic acquired resistance (SAR).

Activation of a MAPK cascade in a defense response was proposed initially in tobacco. A salicylic-acid-induced pathogen-related gene expression is mediated by NtSIPK (a tobacco ortholog of MAPK6) (Zhang and Klessig, 1997). Following infection of resistant tobacco leaves with tobacco mosaic virus (TMV), both NtSIPK and NtWIPK (a tobacco ortholog of MAPK3) were activated in an N-resistance gene-dependent manner (Zhang and Klessig, 1998). The tobacco N gene is a member of the TIR-NBS-LRR family of resistance proteins (Whitham et al., 1994). Constitutively active NtMEK2 (an MKK) induces HR cell death, which is preceded by the activation of endogenous NtSIPK and NtWIPK. HR cell death is often associated with plant disease resistance. In addition, the phytoalexin and salicylic acid biosynthesis enzymes HGMR (3-hydroxy-3-methylglutaryl CoA reductase) and PAL (l-phenylalanine ammonia lyase) also can be induced by the MAPK cascade NtMEK2-NtSIPK/NtWIPK, indicating that a MAPK cascade regulates multiple defense responses upon pathogen attack (Yang et al., 2001). Gene silencing of *NtMEK2, NtSIPK,* and *NtWIPK* by VIGS results in a much compromised N gene-mediated TMV resistance, indicating that the NtMEK2-NtSIPK/NtWIPK cascade plays a positive role in TMV resistance (Jin et al., 2003). Similarly, gene silencing of *MEK1* (*NQK1*, Arabidopsis *MKK6* ortholog) and *Ntf6* (*NRK1*, Arabidopsis *MPK13* ortholog) by VIGS results in attenuated N gene-mediated resistance against TMV (Liu et al., 2004). As mentioned before, NQK1-NRK1 coupled with NPK1 (MKKK) regulates the cytokinesis process in tobacco.

How the same MAPK module can modulate two different cellular outputs remains to be solved. One possible explanation is that different MKKKs employ the same MKK-MPK module and produce different cellular responses. In the Arabidopsis genome, there are 60 MKKKs, 10 MKKs, and 20 MPKs, which suggests the MKK-MPK could be the convergance point. Another explanation is that the same MKKK-MKK-MPK functions in different cellular-response processes through different substrates that have different spatiotemporal expression patterns, which contributes to signaling specificity. It also is possible that the attenuated N gene-mediated resistance against TMV in the *MEK1* and in the *Ntf6* silencing mutants is a non-specific effect; the defective cell wall formation in the silencing mutants may allow TMV to move freely from cell to cell.

Similar to the tobacco orthologs NtSIPK and NtWIPK, the Arabidopsis MAPK6 (AtMPK6) and MAPK3 (AtMPK3) are activated by bacterial and fungal pathogens (Tena et al., 2001; Zhang and Klessig, 2001; Pedley and Martin, 2005). Protoplast transient expression assays demonstrated that AtMPK6 and AtMPK3 are in the MAPK signaling pathway involving FLS2-MEKK1-MKK4/MKK5-MPK3/MPK6-WRKY22/WRKY20. FLS2 activates the MAPK cascade and, eventually, the WRKY transcription factors.

This pathway is the most complete innate immunity signaling cascade to be characterized so far (Asai et al., 2002). In a FLS2-dependent manner, both AtMPK3 and AtMPK6 are activated by flg22, a fungal PAMP (pathogen associated molecular pattern). Constitutively active MKK4/MKK5 activate AtMPK3 and AtMPK6 and, thus, bypass FLS2 in activating promoter activity of the downstream early response genes (*WRKY29* and *FRK1*) in the flg22 signaling pathway. In contrast, the dominant negative MKK4/MKK5 can partially suppress flg22 activation of the downstream early response genes. These results suggest that MKK4/MKK5-MPK3/MPK6 are signaling components downstream of FLS2. Constitutively active MEKK1 activates MKK4/MKK5 (which, again, can bypass flg22 signaling and activate flg22 early response genes *WRKY29* and *FRK1*), indicating that MEKK1 is the upstream MKKK (Asai et al., 2002). However, genetic evidence from whole-plant studies is needed to confirm the assembly of the flg22 signaling cascade and to identify new components.

The *enhance disease resistance 1* (*edr1*) mutant is resistant to *Pseudomonas syringae* and *Erysiphe cichoracearum* (powdery mildew). Disease-resistant proteins (e.g., PR-1 and PR-5) are not constitutively expressed in the *edr1* mutant, which indicates that the disease resistance in *edr1* is not due to SAR (Frye and Innes, 1998). The *EDR1* gene encodes a B3 subgroup MKKK (Frye et al., 2001; MAPK group, 2002). An in vitro kinase assay showed that the EDR1 C-terminal kinase domain has Ser/Thr kinase activity (Tang and Innes, 2002). Overexpression of a kinase-defective EDR1 leads to elevated resistance to *E. cichoracearum*, suggesting that the EDR1 MAPK cascade may function as a negative regulator of pathogen response signaling pathways (Tang and Innes, 2002; Tang et al., 2005). However, further research is needed to clarify if EDR1, in fact, functions as an MKKK.

Oxidative Stress Responses. ROS generated upon stress stimulation can serve as a signaling molecule to help initiate stress responses. This has been proposed to be a general mechanism across all eukaryotes (Hancock et al., 2001; Apel and Hirt, 2004; Wagner et al., 2004). In plants, ROS generation is an early response to pathogen infection and to abiotic stresses (Zhang and Klessig, 2001; Apel and Hirt, 2004; Mittler et al., 2004). Moreover, ROS can up-regulate MAPK activity, suggesting that ROS can serve as a signaling molecule that functions upstream of the MAPK cascade. In Arabidopsis, MPK3 and MPK6 become transiently activated upon ozone (O_3) treatment (Ahlfors et al., 2004). In tobacco, ROS activates NtSIPK and NtWIPK (Kumar and Klessig, 2000; Samuel et al., 2000). These results support the idea that MAPK signaling could be the missing link between ROS generation and downstream resistance gene activation. However, there are examples where a pathogen-induced ROS burst is not required for MAPK activation (Romeis et al., 1999), indicating that MAPK activation and the ROS burst could be parallel events.

Studies have shown that an activated MAPK cascade, in turn, can induce ROS generation, which suggests that there may be a positive feedback loop between ROS generation and MAPK activation. In Arabidopsis, transiently induced, constitutively active MKK4 or MKK5 can initiate ROS and HR cell death (Ren et al., 2002). Independently, it has been shown that HR cell death transiently induced by a constitutively active tobacco MEK2^{DD} was attenuated by VIGS of NbrbohB. NbrhobB is a homolog of a respiratory burst oxidase that is required for H_2O_2 accumulation upon fungal infection (Yoshioka et al., 2003). Together, these results indicate that ROS generation induced by MAPK activity is an important regulator of the HR response.

In Arabidopsis, OXIDATIVE SIGNAL-INDUCIBLE1 (OXI1) may be one of the candidate signaling components between ROS and a MAPK cascade. Expression and protein kinase activity of OXI1 is highly induced upon H_2O_2 treatment and wounding. Loss-of-function *oxi1* mutants are more susceptible to fungal pathogen attack. The *oxi1* mutant has reduced MPK3 and MPK6 activation upon H_2O_2 or elicitor treatment, indicating that OXI1 is required for full activation of ROS-induced MPK3 and MPK6 activity (Rentel et al., 2004).

Salt, Drought, Cold, and Wounding Responses. MAPK cascades respond to a diverse array of abiotic stresses, including salinity, cold, drought, wounding, touching, and other osmotic stresses. AtMEKK1 is transcriptionally activated by touch, cold, and salt stresses (Mizoguchi et al., 1996). MPK4 and MPK6 are activated by cold, drought, touching, and wounding stresses (Ichimura et al., 2000). MPK3 and MPK6 have been induced by osmotic stresses in both Arabidopsis and in tobacco (Hoyos and Zhang, 2000; Droillard et al., 2002). However, genetic evidence from intact plants is needed to confirm MAPKs function in abiotic stresses.

While MPK4 is not activated by hyperosmotic stress, the loss-of-function mutant *mpk4* is more tolerant to hyperosmotic stresses (Droillard et al., 2004). Upon hyperosmotic stress, the drought-inducible gene promoter of *RAB18* is induced in the *mpk4* mutant but not in the wild-type control seedlings, which suggests that MPK4 may play a negative role in regulating hyperosmotic-stress responses (Droillard et al., 2004). AtMEK1s immunoprecipitated from seedlings that have been subjected to drought, high salt, wounding, and cold stresses have higher kinase activity toward recombinant kinase-inactive MPK4 (Matsuoka et al., 2002). This supports that AtMEK1 (AtMKK1) functions upstream of MPK4 in a stress-response pathway. But, again, genetic evidence is needed to support this conclusion.

A yeast two-hybrid interaction analysis indicated that AtMEKK1-AtMKK1/AtMKK2-AtMPK4 may form a MAPK cascade (Ichimura et al., 1998). Yeast osmosensitive MAPK cascade mutant *pbs2Δhog1Δ* complementation results showed that the combination of MKK2-MPK6 specifically complements the yeast osmosensing defect (Teige et al., 2004). Cold- and salt-stress-activated MKK2 specifically phosphorylates MPK4 and MPK6 recombinant proteins. Protoplast transient co-transformation studies showed that cold and salt activation of MPK4 and MPK6 are mediated by MKK2 (Teige et al., 2004). More convincingly, the *mkk2* null mutant and the *MKK2^{EE}* overexpression mutant do not have any observable phenotype under normal growth conditions. However, when subjected to cold or salt stress, the *mkk2* mutants are hypersensitive to cold and salt stresses. Conversely, the *MKK2^{EE}* overexpression mutants are tolerant to freezing and cold stresses. The impairment of the cold-stress activation of MPK4 and MPK6 in the *mkk2* null mutants indicates that MKK2 mediates MPK4/MPK6 activation in cold-stress responses (Teige et al., 2004). Protoplast transient expression experiments showed that MKK2 mediates the activation of MPK4/MPK6 by the gain-of-function AtMEKK1. This indicates that AtMEKK1 could be an upstream MKKK of MKK2 during cold and salt-stress responses (Teige et al., 2004). Again, genetic evidence from null *AtMEKK1* mutants is needed to support this conclusion.

Systemic Acquired Resistance (SAR). Characterization of the *mpk4* mutant indicates a negative regulatory role in SAR (Petersen et al., 2000). While the *mpk4* mutant has dwarf stature, small curly leaves, and reduced fertility, it has a normal response to phytohormones and to abiotic stresses. Upon pathogen challenge, the *mpk4* mutant shows enhanced resistance to pathogens. In the *mpk4* mutant, the disease resistant genes (PR1, PR2, PR5) that are normally induced by SAR are, instead, constitutively induced. Whole-genome expression profiling by microarrays has confirmed the up-regulation of disease resistance gene expression in *mpk4*, providing further evidence of a negative regulatory role of MPK4 in pathogen resistance and SAR (Petersen et al., 2000).

Salicylic acid is necessary for SAR. In the *mpk4* mutant, about 9-fold and 25-fold higher salicylic acid and salicylic acid glucosides are accumulated. Double-mutant analysis showed that *nahG* (a salicylate hydroxylase that converts salicylic acid to catechol) can rescue the *mpk4* mutant phenotype, which indicates *mpk4* is epistatic to salicylic acid in SAR signaling. Suprisingly, however, jasmonate-inducible genes are suppressed in the *mpk4* mutant. Removing salicylic acid by the *nahG mpk4* double mutant is not sufficient to release the suppression of jasmonate-inducible gene expression (Petersen et al., 2000). This indicates that MPK4 positively regulates jasmonate responses independently of its negative role in the regulation of SAR. Future work should identify the MAPK cascade and the target genes of MPK4, which will help in understanding the molecular mechanism of SAR.

Regulation of MAPK Cascade Specificity in Arabidopsis

As discussed, many MAPK components are involved in different cascades and assume different functions. In the Arabidopsis genome, there are more than 60 predicted MKKKs but only 10 MKKs and 20 MPKs, suggesting that there is signaling convergence and divergence on different levels of MAPK cascades. This raises the issue of how MAPK cascade signaling specificity is maintained. Five regulation mechanisms have been proposed: substrate specificity, colocalization (scaffolding), attenuation through phosphatases and inhibitor, interaction between MAPK cascades, and spatiotemporal regulation.

Substrate Specificity

Substrate specificity may be one of the regulation mechanisms. The same MAPK module can activate different substrates (depending on the spatiotemporal expression of individual substrates) and, thus, can determine the ultimate outputs of the MAPK cascade. However, so far, only two MAPK substrates have been identified in Arabidopsis: ACS6 and MKS1. More information on MAPK substrates is needed to substantiate this mode of regulation.

ACS6 was the first MAPK substrate to be identified. ACS6 (an ACC synthase) is phosphorylated and stabilized by MPK6, which is essential for stress ethylene production, as discussed before (Liu and Zhang, 2004). MKS1 was identified as a MPK4 substrate that regulates plant defense responses (Andreasson et al., 2005). A yeast two-hybrid screen identified MKS1 as an interaction partner of MPK4. Analysis of the sequence of MKS1 indicates the existence of a potential MAPK phosphorylation site. Biochemical analysis showed that immunoprecipitated MPK4 from Arabidopsis seedlings can phosphorylate recombinant MKS1 in vitro; in vivo immunoprecipitation experiments confirmed the interaction between MKS1 and MPK4. MKS1 overexpression *35S:MKS1* transgenic plants have a dwarf phenotype (similar to *mpk4* null mutants), whereas the *MKS1* RNAi transgenic plants have a normal growth phenotype, indicating a functional link between these two proteins. Similar to what was observed in the *mpk4* mutant, PR proteins that are normally induced in SAR are up-regulated in the *35S:MKS1* transgenic plants. These transgenic plants are also more resistant to pathogen attack, which reinforces the functional interaction between MKS1 and MPK4. Since the *mpk4* mutant can be rescued partially by reducing MKS1 expression, this indicates that MPK4 negatively regulates MKS1 activity.

A MAPK that negatively regulates downstream substrates of another MAPK cascade has been demonstrated in other organisms. For example, a yeast MAPK (Fus3) controls the degradation of Tec1, a substrate of an invasive growth MAPK (Kss1) (Ptashne and Gann, 2003; Elion et al., 2005). Since MAPK cascades are extremely conserved signaling modules across all the eukaryotes, information learned from other systems will help to piece together what has been learned in Arabidopsis. The converse also is true: what has been learned about MAPK cascade signaling in Arabidopsis will contribute to the understanding of MAPK cascade signaling mechanisms in other organisms.

Colocalization (Scaffolding)

Scaffolding may be another mechanism to maintain MAPK cascade signaling specificity. Scaffolding has been demonstrated best in yeast and mammalian systems, where there are multiple examples of how scaffold proteins help confer signaling specificity (Elion et al., 2005; Kolch, 2005). So far, however, no MAPK cascade scaffolding protein has been characterized in Arabidopsis. One reason for this is that the MAPK scaffolding proteins in Arabidopsis could have a significantly different identity from their mammalian counterparts. Or, it could be that MAPK cascade components mediate protein-protein interactions themselves and function as scaffolding proteins in Arabidopsis. The yeast osmotic-stress regulator Pbs2 (a MKK and a scaffolding protein) is an example of one such dual-function MAPK component. An oxidative-stress responsive MKKK (MsOMTK1) from alfalfa has been demonstrated to activate, and to interact physically with, a MAPK (MsMMK3) through a protoplast-transient transformation assay, suggesting that MKKK in plants may serve a similar dual function (Nakagami et al., 2004).

Attenuation Through Phosphatases and Inhibitor

Yeast mating and invasive growth MAPK pathways share multiple common components. One mechanism to maintain signaling specificity is to attenuate nonessential MAPKs by means of protein phosphatases. During invasive growth, for example, a yeast MAPK (Fus3) can be selectively repressed by a phosphatase (Msg5) (Elion et al., 2005). In Arabidopsis, several phosphatases targeting specific MAPKs have been identified. AtDsPTP1 (Arabidopsis dual-specificity protein tyrosine phosphatase) dephosphorylates and inactivates AtMPK4 (Gupta et al., 1998). Another tyrosine phosphatase AtMKP1 is a mutant that is hypersensitive to genotoxic stresses (UV-C and methyl methanesulphonate) (Ulm et al., 2001). Yeast two-hybrid experiments showed that AtMKP1 interacts specifically with MPK3, MPK4, and MPK6, indicating that MKP1 may regulate stress responses through the regulation of MAPKs (Ulm et al., 2002). In addition, MP2C (a PP2C phosphatase in alfalfa) has been demonstrated to be a specific negative regulator of SIMK (salt stress-inducible MAPK) (Meskiene et al., 1998; Meskiene et al., 2003). As more MAPK-regulatory phosphatases are thoroughly characterized, more insights into how MAPK cascades are regulated in plants are likely.

Interaction Between MAPK Cascades

Interaction between MAPK cascades may be another level of regulation to maintain signaling specificity. In tobacco,

Table 2. Characteristics of the CDPK-SnRK families in Arabidopsis.

Family	Kinase domain	Ca^{2+}-binding domain	Autoinhibitory domain	Acylation sites (in multiple family members)
CDPK	Yes	Yes	Yes	Yes
SnRK	Yes	No	Variable	No
CRK	Yes	No	No	Yes
PPCK/PEPRK	Yes	No	No	No

silencing SIPK can lead to increased activation of WIPK upon prolonged ozone treatment (Samuel and Ellis, 2002). *WIPK* transcription and protein accumulation is regulated positively by SIPK activation (Liu et al., 2003). Upon wounding, MPK3 (WIPK ortholog in Arabidopsis) activity was 2-3 fold higher in MPK6 (SIPK ortholog in Arabidopsis) gene-silencing lines than that in wild-type controls (Menke et al., 2004). One possible explanation for these results could be that MPK6 and MPK3 have partially redundant functions. The down-regulation of MPK6 could be compensated functionally by the up-regulation of MPK3.

Spatiotemporal Regulation

Signaling specificity also may be spatiotemporally regulated. In mammalian cells, it has been well documented that, upon activation, MAPK can translocate to the nuclei or to other subcelluar organelles, where they activate a specific set of substrates (Widmann et al., 1999). Plant cells may have adopted a similar mechanism. Upon peptide elicitor Pep-13 treatment, parsley MAPKs (PcMPK3a/b, and PcMPK6) rapidly translocate and accumulate in nuclei, but the putative upstream MKK (PcMKK5) show constitutive cytosolic localization (Ligterink et al., 1997; Kroj et al., 2003; Lee et al., 2004). In Arabidopsis, immunolocalization *in planta* also has demonstrated that, upon ozone treatment, MPK3 and MPK6 rapidly translocate to nuclei (Ahlfors et al., 2004). Future research should address the mechanism by which MAPK translocation is regulated in plants.

ADDITIONAL PROTEIN KINASES IN ARABIDOPSIS

CDPK-SnRK Super-Family in Arabidopsis

The CDPK-SnRK (calcium-dependent protein kinase-SNF1 related protein kinase) super-family of protein kinases contains a predicted 84 members in the Arabidopsis genome (Hrabak et al., 2003). All of the members contain a Ser/Thr kinase domain but also have various other domains in the remainder of the protein, including a highly variable N-terminal domain and a C-terminal region that often includes regulatory domains. This super-family is sub-divided into smaller families including the CDPKs, the SnRKs, and a few families containing limited members in the Arabidopsis genome (Table 2).

CDPKs

The predicated 34 CDPKs in Arabidopsis contain four characteristic domains: a variable N-terminal domain, the kinase domain, an autoinhibitory domain, and a calmodulin-like domain (Harmon et al., 2000; Cheng et al., 2002; Hrabak et al., 2003). The autoinhibitory domain has a pseudosubstrate site that may act to inhibit the activity of the protein kinase (Harmon et al., 1994). This domain is located immediately C-terminal to the kinase domain and is sometimes called the junction domain. At the C-terminal end of the protein is a calmodulin-like domain. This domain contains at least one Ca^{2+}-binding elongation factor (EF) hand, and most members contain four EF hands (Cheng et al., 2002; Hrabak et al., 2003).

Although they do not contain transmembrane domains, many CDPKs are predicted to be membrane associated because the N-terminal region often contains putative myristoylation and palmitoylation sites (Hrabak et al., 2003). Indeed, one CDPK has been found to be associated with the endoplasmic reticulum (ER) membrane, and the first 10 amino acids containing these acylation sites were sufficient for this targeting (Lu and Hrabak, 2002). A more extensive study found several family members that are targeted to the plasma membrane and a few that are both cytoplasmic- and nuclear-localized or associated with the peroxisome (Dammann et al., 2003).

Regulation of the CDPKs is not completely clear. It is predicted that Ca^{2+} binding in the calmodulin-like domain induces a conformational change that results in the release of the autoinhibitory domain, which allows the kinase to be active (Harmon et al., 2000; Cheng et al., 2002). Additional work has shown different Ca^{2+}-binding affinities at different sites within the calmodulin-like domain (Christodoulou et al., 2004). The higher affinity sites may bind Ca^{2+}, but the kinase remains inhibited. Only when the lower affinity sites are filled is the inhibition released. In addition to Ca^{2+}, there is some evidence that phospholipids and 14-3-3 proteins also may play a regulatory role (Binder et al., 1994; Camoni et al., 1998). Autophosphorylation also may play a regulatory role as well (Cheng et al., 2002). Future work is needed to determine how the numerous CDPKs are regulated to allow the proper signals to be transmitted in their respective pathways.

The *in planta* functions for the CDPKs are quite varied and not yet entirely understood. Studies in Arabidopsis and in other plants have shown that CDPKs are involved in hormone signaling, in various aspects of growth and development, in water relations, in metabolic functions, and in responses to abiotic and biotic stresses (Cheng et al., 2002; Lee and Rudd, 2002; Ludwig et al., 2004). Since Ca^{2+} fluxes are involved in many signaling events, future research into how specificity is maintained and into the role of CDPKs as signaling nodes will be of particular interest. As would be expected from the diverse signaling pathways in which CDPKs are active, the substrates identified in Arabidopsis and other species are also quite diverse (Cheng et al., 2002). For example, in Arabidopsis, CDPKs have been found to phosphorylate phenylalanine ammonia-lyase, an enzyme involved in pathogen defense, and the Ca^{2+} pump (ACA2) (Hwang et al., 2000; Cheng et al., 2001).

SnRKs

With a predicted 38 members, the SnRK (SNF1-related protein kinases) family is slightly larger than the CDPK family in Arabidopsis (Hrabak et al., 2003). This family is most closely related to SNF1 from yeast and AMP-activated protein kinases from animals. As in yeast and in animals, many of these family members are predicted to play important roles in metabolic regulation in Arabadopis (Halford and Hardie, 1998; Halford et al., 2003; Halford et al., 2004). Unlike the CDPKs, the SnRKs do not have C-terminal Ca^{2+}-binding domains; however, Ca^{2+} does play a role in the regulation of some members. The SnRK family, for example, includes the calcineurin B-like (CBL)-interacting protein kinases (CIPKs). CBLs are a family of 10 Ca^{2+}-binding proteins in Arabidopsis that physically interact with CIPKs (Shi et al., 1999; Kim et al., 2000; Luan et al., 2002; Kolukisaoglu et al., 2004). Details for one CIPK (SOS2) and one CBL (SOS3) are discussed later in this chapter.

In yeast and in animals, the SnRK protein kinase forms a heterotrimeric complex. Homologs to these interacting partners have been isolated in Arabidopsis and have been shown to physically interact with SnRKs (Halford et al., 2003) and with members of the ubiquitin-mediated protein degradation pathway (Farras et al., 2001). Several metabolic substrates have been identified for the SnRKs (Halford et al., 2003). In addition, a transcription factor that mediates ABA-responsive gene expression also was identified recently as a substrate (Choi et al., 2005). Several stress-related functions have been reported for SnRK family members, including responses to hyperosmotic or salt stress and ABA-mediated responses to water stress (Yoshida et al., 2002; Boudsocq et al., 2004).

To date, the best-described SnRK is *salt overly sensitive2* (*sos2*). *sos2* is a member of the *SnRK* family and was identified as one of the *sos* mutants that hyperaccumulate Na+ during salt stress (Liu et al., 2000). Unlike most CDPK-SnRK family members, SOS2 contains a Thr in the activation loop (Hrabak et al., 2003). A Thr to Asp mutation, as well as a Ser to Asp mutation or a Tyr to Asp mutation, in the activation loop results in a constitutively active SOS2, as does the removal of an interaction domain to which SOS3 binds (Guo et al., 2001; Gong et al., 2002). SOS3 is a Ca^{2+}-binding protein that contains an EF hand. SOS3 physically interacts with SOS2 and and binding is required for kinase activity (Halfter et al., 2000). The SOS3-binding site is an autoinhibitory domain that blocks substrate access to the SOS2 catalytic site in the absence of active SOS3 (Guo et al., 2001).

SOS2 and SOS3 are regulators of SOS1, a plasma membrane Na^+/H^+ exchanger (Qiu et al., 2002). SOS3 recruits SOS2 to the plasma membrane where SOS1 is localized (Quintero et al., 2002). This suggests a model where the SOS3 Ca^{2+}-sensor recruits and activates the SOS2 protein kinase to the plasma membrane where the SOS1 Na^+ transporter is phosphorylated and activated during salt stress (Quintero et al., 2002; Guo et al., 2004). In addition to SOS1, SOS2 also regulates the vacuolar H^+/Ca^{2+} antiporter CAX1 independently of SOS3 (Cheng et al., 2004). Future work into this dual regulation should prove interesting.

Other CDPK-SnRKs

The remaining CDPK-SnRK families contain fewer predicted members in the Arabidopsis genome. There are eight predicted CRK (CDPK-related protein kinase) family members (Hrabak et al., 2003). These are similar to the CDPKs, except that the Ca^{2+}-binding EF hands are degenerated and appear to be nonfunctional. Two PPCKs (phospho*enol*pyruvate carboxylase protein kinases), which are calcium-independent protein kinases that phosphorylate PEP carboxylase, also are found in the Arabidopsis genome, as well as two PEPRKs (PEP carboxylase protein kinase-related protein kinases) (Hrabak et al., 2003). Most recently, a calmodulin-binding protein kinase, named CRCK1 (Calmodulin-Binding Receptor-Like Cytoplasmic Kinase1), that is involved in stress signaling pathways was isolated in Arabidopsis (Yang et al., 2004).

GSK-3/Shaggy-Like Protein Kinases

The glycogen synthase protein kinase 3 (GSK-3)/SHAGGY protein kinases are Ser/Thr kinases that carry out multiple functions in metazoans (Doble and Woodgett, 2003). GSK-3 was first characterized for its ability to phosphorylate and inactivate the enzyme glycogen synthase (Embi et al., 1980; Woodgett and Cohen, 1984). The *GSK-3/SHAGGY*-like gene family in Arabidopsis contains at least 10 members, which are divided into three classes (Dornelas et al., 1998; Dornelas et al., 1999).

In Arabidopsis, the *GSK-3/SHAGGY*-like family members are expressed in the embryo (Dornelas et al., 1999), the pollen (Tichtinsky et al., 1998), and in floral organs

(Dornelas et al., 2000). Analyses of real-time reverse transcriptase PCR expression profiling indicated that many members of this gene family are expressed across multiple tissues while others are tissue-specific and/or responsive to abiotic stresses (Charrier et al., 2002).

Functional analyses revealed that the *GSK-3/SHAGGY*-like family members play both developmental and stress-responsive roles in Arabidopsis. *AtSK11* (*ASK*α) and *AtSK12* (*ASK*γ) antisense lines show morphological phenotypes in flowers, including increased perianth organs and altered gynoecium development (Dornelas et al., 2000). *AtGSK1* can rescue a salt-sensitive yeast strain (Piao et al., 1999), and its overexpression results in a constitutive expression of salt-stress genes and an enhanced tolerance to salt stress (Piao et al., 2001).

The best-characterized member in Arabidopsis is *BIN2/UCU1/DWF12*. Two alleles of *BR-INSENSITIVE 2* (*BIN2*) were uncovered in a screen of dwarf BR-insensitive plants (Li et al., 2001). Both alleles are dominant and have a phenotype similar to *bri1*. Subsequent cloning of *BIN2* revealed it as a GSK-3/SHAGGY-like family member (Li and Nam, 2002). The two *BIN2* alleles act as gain-of-function mutations and plants with either allele have BR-deficient phenotypes. Reduced *BIN2* expression can partially rescue weak *bri1* mutants, suggesting *BIN2* is a negative regulator of BR signaling (Li and Nam, 2002).

Additional alleles of *BIN2* have been isolated. Three alleles of *ULTRACURVATA1* (*UCU1*) have been cloned and are named for the phenotype of strongly downward rolled leaves (Perez-Perez et al., 2002). In these plants, cell expansion is reduced, resulting in dwarfed plants that are also BR-insensitive. Studies in a different ecotype uncovered *dwarf12* (*dwf12-1D* and *dwf12-2D*), which has identical mutations as *ucu1-1* and *bin2-1*, respectively (Choe et al., 2002). Interestingly, six of the seven point mutations described thus far occur in a domain highly conserved with animal GSK-3s called the TREE domain, a possible phosphorylation site (Choe et al., 2002). However, in vitro phosphorylation target site studies do not suggest that the TREE domain is a direct BRI1 target (Oh et al., 2000), and BRI1 was not found to interact physically with BIN2 (Li and Nam, 2002). Future studies should identify what role, if any, this domain may play in the regulatory control of BIN2/UCU1/DWF12.

Two genes have been isolated that are candidate *BIN2/UCU1/DWF12* substrates. The *brassinazole-resistant 1-1D* (*bzr1-1D*) mutant suppresses a BR-deficient phenotype and is a positive regulator of the BR-signaling pathway (Wang et al., 2002). *BZR1* is a member of a plant-specific gene family that comprises five members in Arabidopsis. *bri1-EMS-suppressor1* (*bes1*), a mutant with a similar phenotype as *bzr1-1D* also has been identified (Yin et al., 2002). These two genes are closely related to each other. Furthermore, yeast two-hybrid and pull-down assays indicate that BES1 and BIN2 interact specifically and that BIN2 is able to phosphorylate BES1 (Yin et al., 2002). This suggests that BIN2 phosphorylation of BES1 acts as a regulatory mechanism to reduce the activity of BES1 at low BR concentrations. BIN2 also directly interacts with BZR1 in vitro and negatively regulates BZR1 accumulation in vivo, likely by phosphorylation (He et al., 2002). However, BIN2 phosphorylation of BZR1 and BES1 does not appear to be dependent on a priming phosphorylation or a scaffold protein, as has been reported in animal GSK-3s (Zhao et al., 2002b). This suggests that a novel activation mechanism may exist in the plant GSK-3/SHAGGY-like protein kinases.

PINOID

Mutations of the *PINOID* (*PID*) gene encodes a Ser/Thr kinase that displays a phenotype similar to plants treated with an auxin-transport inhibitor (Christensen et al., 2000) (Figure 4). *PID* encodes a functional protein kinase that contains all the typical subdomains of protein kinases. Constitutive overexpression of *PID* demonstrated a role in auxin signaling (Christensen et al., 2000; Benjamins et al., 2001). Further work demonstrated a role for *PID* in mediating localization of *Pin-Formed1* (*PIN1*, a transporter-like membrane protein) to create auxin gradients during patterning processes (Friml et al., 2004). *PID* also has been shown to play a role in the formation of cotyledons during embryogenesis (Furutani et al., 2004; Treml et al., 2005).

Interestingly, a yeast two-hybrid screen found two proteins that bind Ca^{2+} and interact with PID in a Ca^{2+}-dependent manner (Benjamins et al., 2003). While this interaction stimulates autophosphorylation activity of PID, the Ca^{2+}-binding proteins are not a substrate. Recently, two protein kinases similar to PID (WAG1 and WAG2) were found to be root-tip expressed protein kinases that negatively regulate a root-waving response (Santner and Watson, 2006) (Fig. 4).

TOUSLED

TOUSLED (*TSL*) encodes a Ser/Thr protein kinase that is important in development of both floral and vegetative tissue (Roe et al., 1993). The N-terminal region of *TSL* contains an essential nuclear localization signal and domains important for oligomerization, which are essential for kinase activity (Roe et al., 1997). TSL kinase activity is higher during G2/M-phase and during G1-phase cells compared to S-phase cells, suggesting a role for TSL during the cell cycle (Ehsan et al., 2004).

Histidine Protein Kinases

In plants, there are eight canonical histidine protein kinases in the genome. Histidine protein kinases are members of two-component signaling relays. Two-component systems have been shown to be important in both cytokinin and ethylene signal transduction in plants. Histidine protein kinases and two-component systems are covered in

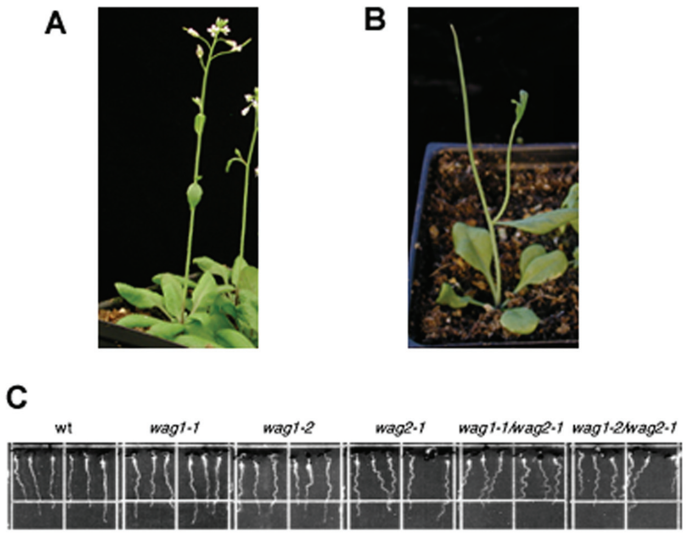

Figure 4. Phenotypic characteristics in Arabidopsis mutants of *Pinoid* and related genes. **(A)** A wt plant showing a typical flowering shoot. **(B)** *Pinoid* mutants display a pin-formed flowering shoot that is devoid (as shown) or nearly devoid of any flowers. **(C)** Mutations in the WAG1 and WAG2 protein kinases do not have an obvious shoot phenotype. However, WAG1 and WAG2 negatively regulate root waving as seen in the single knockout mutants and more clearly in the double knockout mutants. (**C**) From Santner and Watson, 2006; © 2006 Blackwell Publishing Ltd, used with permission.

another chapter of *The Arabidopsis Book* (Schaller et al., 2002).

Light-Responsive Protein Kinases

Two groups of protein kinases are important in light-signaling pathways in plants. Phytochromes are responsible for monitoring the red and far-red regions of the spectrum. *PHYA* is a potential Ser/Thr protein kinase in higher plants. Phytochromes are covered in more detail in another chapter of *The Arabidopsis Book* (Wang and Deng, 2004).

Phototropins (*phot1* and *phot2*) are involved in the responses to unidirectional blue-light sources. It is likely that both *phot1* and *phot2* are capable of autophosphorylation by Ser/Thr kinase domains in response to blue-light illumination. Phototropins are covered in more detail in another chapter of *The Arabidopsis Book* (Liscum, 2002).

PROTEIN PHOSPHATASES IN ARABIDOPSIS

Protein phosphorylation and dephosphorylation are involved in almost all cell-signaling events. Reversible protein phosphorylation depends on coordinated actions of protein kinases and protein phosphatases. While both protein kinases and protein phosphatases exist as large protein families in Arabidopsis, protein kinases greatly outnumber protein phosphatases. This numerical imbalance gives rise to the question of how a limited number of protein phosphatases are fulfilling the job of maintaining the phosphorylation status of a cell. Protein phosphatases regulate a diverse array of processes in Arabidopsis, including auxin transport, ABA signaling, and RLK signaling. Based on phospho-amino-acid-substrate specificity, protein phosphatases are divided into two major groups: protein Ser/Thr phosphatases and protein tyrosine phosphatases.

Protein Ser/Thr Phosphatases

Further classification of the protein Ser/Thr phosphatases is based on substrate specificity, divalent cation requirements, and inhibitor sensitivity, as summarized in Table 3 (Smith and Walker, 1996).

Based on sequence and structural analysis, the type one (PP1), type 2A (PP2A), and type 2b (PP2B) protein phosphatases are related enzymes and, hence, are defined as the PPP family. The type 2C protein phosphatases (PP2C) and other Mg^{2+}-dependent Ser/Thr phosphatases, are closely related and share no sequence homology with PPP and, thus, are defined as the PPM family (Barford, 1996; Barford et al., 1998).

Types 1 and 2 Protein Phosphatases

The PPP family enzymes are multimeric holoenzymes. The core catalytic region (around 280 aa) is very conserved

Table 3. Classification of the protein Ser/Thr phosphatases.

		Substrate specificity	**Sensitivity to inhibitor I & II**	**Divalent cations requirement**	**Okadaic acid sensitivity**	**Cantharidin sensitivity**
Type I	PP1	β-subunit of phosphorylase kinase	Sensitive to nanomolar conc.	No	0.1-1.0 nM	No
Type II	PP2A	α-subunit of phosphorylase kinase	insensitive	No	10-100 nM	Yes
	PP2B			Ca^{2+}	No	No
	PP2C			Mg^{2+}	No	No

among the PPP family members. However, the non-catalytic N- or C- terminal regions are variable. Assembling with a diverse array of associated regulatory subunits, the holoenzymes are unique in their functions. PP1s are highly conserved and ubiquitous phosphatases across all eukaryotes. In animals, the PP1C core catalytic subunit, which assembles with different regulatory subunits, is implicated in cell-cycle regulation, apoptosis, glycogen metabolism, and neuronal activities (Bollen, 2001; Gallego and Virshup, 2005).

In plants, there is only limited knowledge about PP1 so far (Smith and Walker, 1996; Lin et al., 1998; Lin et al., 1999; Luan, 2003). An initial survey identified eight PP1-type protein phosphatase catalytic subunit genes in Arabidopsis (Smith and Walker, 1993; Lin et al., 1998), which later were confirmed by whole-genome analysis (Kerk et al., 2002). Expression pattern analyses demonstrated that PP1 catalytic subunits are ubiquitously expressed in Arabidopsis (Lin et al., 1998). Most of the functional inferences of PP1 catalytic subunits are based on inhibitor approaches. As shown in Table 3, PP1s are sensitive to okadaic acid and insensitive to cantharidin, which is used to distinguish PP1 activity from other phosphatases in the cell. PP1 activity was found to be involved in the regulation of membrane channels, cell cycle control, and developmental regulation in plants (Smith and Walker, 1996; Luan, 2003). However, no loss-of-function phenotypes have been described yet for the genes encoding the PP1 catalytic subunits.

PP2As are heterotrimeric enzymes formed by a catalytic core subunit (C subunit) that associates with a scaffolding A subunit and a regulatory B subunit. The Arabidopsis genome encodes 5 C subunits, 3 A subunits, and 17 B subunits. With different combinations of A, B, and C subunits, there could be up to 255 PP2A holoenzymes (Kerk et al., 2002). While both A subunits and C subunits are highly conserved across all eukaryotes, B subunits are quite varied, which is consistent with their regulatory functions. B subunits are further classified into B, B', and B", based on molecular weight (Luan, 2003). In animals, regulatory B subunits regulate the subcellular localization, substrate specificity, and activity of heterotrimeric PP2As (Li et al., 2002; Gallego and Virshup, 2005). In Arabidopsis, there is still only limited knowledge about the regulatory functions of the B subunits.

In Arabidopsis, PP2As play essential roles in plant development and auxin signal transduction. *rcn1* (*root curl in Naphthylphthalamic acid*) was identified in a screen for potential auxin-transport mutants by a root curling assay. *rcn1* roots tightly curl in the presence of the auxin-transport inhibitor Naphthylphthalamic acid (NPA), while the wild-type roots grow in straight lines. Auxin efflux in *rcn1* mutants was shown to be more sensitive to NPA than in wild-type seedlings (Garbers et al., 1996). *RCN1* encodes an A subunit of PP2A. Initial functional analysis showed that RCN1 was able to complement the yeast *tpd3*, a mutation in a PP2A A subunit (Garbers et al., 1996). Plants treated with the PP2A inhibitors okadaic acid and cantharidin can partially phenocopy the *rcn1* mutant, suggesting that decreased PP2A activity is responsible for the *rcn1* phenotype. In vitro enzyme assays demonstrated that RCN1 is a positive regulator of PP2A activity, as PP2A activity in an *rcn1* protein extract is much lower than that in wild-type. Inhibitor assays also showed that *rcn1* seedlings are more sensitive to okadaic acid and cantharidin, which is consistent with the reduced PP2A activity in the *rcn1* mutant (Deruere et al., 1999).

All three A subunits are functional, with RCN1 playing a major function and PP2AA2 and PP2AA3 playing relatively minor functions (Zhou et al., 2004b). The *pp2aa2/pp2aa3* double mutants display only minor defects, such as slightly reduced root elongation. However, the *rcn1/ppaa2* and *rcn1/pp2aa3* double mutants have an extremely severe developmental and growth defect phenotype. At the seedling stage, the mutants have greatly reduced hypocotyls and root elongation. The hypocotyls and roots show radial expansion, resulting from irregular cell expansion in the epidermis and cortical cells. In the adult mutant plants, small rosette leaves are clustered together, and the inflorescence blots are much shorter than in the wild-type. Moreover, the *rcn1/pp2aa2* double mutants are infertile (Zhou et al., 2004b). These systemic analyses suggest that all three PP2A A subunits are functionally overlapping but not equivalent.

The irregular cell expansion in both the *rcn1/pp2aa2* and *rcn1/pp2aa3* double mutants is reminiscent of the ton2/fass mutant phenotype. The *ton2/fass* is the only PP2A B regulatory subunit that has been characterized in Arabidopsis so far (Camilleri et al., 2002). In the *ton2* mutants, defects in cortical microtubule organization cause irregular cell-size and cell-shape phenotypes. A yeast two-hybrid assay demonstrated that TON2 interacts with the A subunits of PP2A, indicating that protein phosphatase 2A may be involved in the control of cortical cytoskeleton organization (Camilleri et al., 2002).

Type 2C Protein Phosphatases

The PP2Cs belong to the PPM family of Ser/Thr phosphatases. Their catalytic activity requires the divalent cations Mn^{2+} or Mg^{2+}. PP2Cs are monomeric enzymes that exist in all eukaryotes. PPM phosphatases do not share any sequence homology with PPP phosphatases. However, the protein structures of these two families of phosphatases are fairly similar, suggesting similar catalytic mechanisms and a convergent evolution (Das et al., 1996).

In the Arabidopsis genome, there are about 69 PP2Cs, which is much more complex and abundant than in other organisms (Kerk et al., 2002). Except for six genes, the 69 PP2Cs can be subdivided into 10 groups (Schweighofer et al., 2004). Catalytic domains of individual PP2Cs have various N- or C-terminal extensions (e.g., putative MAPK docking site, membrane localization signaling anchor, phospho-protein interacting FHA domain) that may contribute to their regulation, substrate specificity, and subcellular localization (Schweighofer et al., 2004).

Although relatively few have been functionally characterized, PP2Cs are implicated in diverse developmental and stress responsive signaling pathways in Arabidopsis.

Abscisic Acid Insensitive 1 and 2. There are three Ser/Thr protein phosphatases known to be involved in regulation of the ABA-signaling pathway: *Abscisic Acid Insensitive 1* (*ABI1*) and *ABI2*, *Homology to ABI1/ABI2* (*HAB1*), and *AtPP2CA*. The *abi1-1* and *abi2-1* mutants were identified in an EMS mutant screen for ABA insensitive seed germination. Both mutants have reduced seed dormancy, and seed germination is less sensitive to inhibitory concentrations of ABA. At the vegetative stage, both mutants display seedling growth that is insensitive to ABA, reduced drought tolerance, and abnormal stomata regulation (Leung et al., 1994; Leung et al., 1997). ABI1 and ABI2 are highly homologous at the protein sequence level. A biochemical analysis showed that both *ABI1* and *ABI2* encode functional PP2Cs. *abi1-1* and *abi2-1* both carry the same metal-binding site mutation (G>D) that abolishes most of the PP2C protein phosphatase activity of the enzyme (Bertauche et al., 1996; Sheen, 1998). Based on the dominant nature of the original *abi1-1* and *abi2-1* mutations, the pleiotropic phenotype is believed to be due to dominant negative effects (Bertauche et al., 1996; Leung et al., 1997; Rodriguez, 1998).

Isolation and analysis of intragenic recessive revertant alleles of *abi1-1* and *abi2-1* showed that ABI1 and ABI2 are negative regulators of ABA signaling (Gosti et al., 1999; Merlot et al., 2001). All the revertant alleles carry missense mutations in the conserved catalytic domain. In vitro phosphatase assays demonstrated that these revertants either have greatly reduced or no PP2C activity, indicating that these revertants are loss-of-function alleles. In contrast to *abi1-1*, all the revertants of *abi1-1* (*abi1-1R*) are hypersensitive to ABA, as evidenced by increased inhibition of seed germination, enhanced seed dormancy, and drought tolerance with exogenous ABA application. However, why the *abi1-1* dominant negative allele shows an opposite ABA sensitivity than the recessive *abi1-1R* remains unclear. One possibility is that a dominant negative mutation may trap target protein kinases and, thus, inhibit their function in the ABA signaling. The recessive revertants, on the other hand, may lose their ability to trap and dephosphorylate the target protein kinases, thereby allowing the hyperphosphorylated protein kinases to function in the ABA signaling pathway. T-DNA insertional null mutants of ABI1 and ABI2 have been reported (Kuhn et al., 2005; Yoshida et al., 2006). Future, detailed analyses of such mutants are needed to help solve this puzzle.

Homology to ABI1/ABI2. Based on sequence homology, HAB1 is closely related to ABI1 and ABI2. Although sequence homology does not always indicate the same function, HAB1 does share a similar function as ABI1 and ABI2 (Rodriguez et al., 1998). Expression of HAB1 is ABA inducible. Transgenic plants constitutively expressing HAB1 have reduced ABA sensitivity, while *hab1* T-DNA insertional mutants show ABA hypersensitivity. These results suggest that HAB1 also is a negative regulator of ABA signaling. However, how HAB1 differentially regulates ABA signaling remains unclear (Rodriguez et al., 1998; Saez et al., 2004).

AtPP2CA. *AtPP2CA* can rescue the cAMP phosphodiesterase defective sterile mutant *pde1* in *Schizosaccharomyces pombe* (Kuromori and Yamamoto, 1994). Like ABI1/2 and HAB, AtPP2CA belongs to the A-type PP2C phosphatases (Schweighofer et al., 2004). A maize protoplast transient transfection experiment demonstrated that, like ABI1, AtPP2CA can block ABA-inducible gene expression (Sheen, 1998). AtPP2CA can be induced, in an ABA-dependent manner, by different abiotic stress conditions, such as drought, high salt, and low temperature.

To elucidate the function of AtPP2CA in abiotic-stress responses, antisense AtPP2CA transgenic plants were generated (Tahtiharju and Palva, 2001). Cold acclimatization in the antisense lines was much faster than in the control plants, but no differences were observed in drought tolerance (Tahtiharju and Palva, 2001). Cold-induced genes were highly up-regulated in the antisense lines, which further confirms the role of AtPP2CA in cold tolerance. In addition, the antisense lines were hypersensitive to ABA, which indicates that AtPP2CA plays a negative regulatory role in ABA-mediated cold acclimatization (Tahtiharju and Palva, 2001).

Recently, *ABA-hypersensitive germination 3* (*ahg3*) was cloned and found to be due to point mutation in *AtPP2CA*. Expression analysis demonstrated that *ahg3* is highly expressed in seeds (Yoshida et al., 2005). Independently, AtPP2CA was identified by screening a library of 35S:cDNA transgenic lines for changes of ABA sensitivity. While *35::AtPP2CA* plants were insensitive to applied ABA during seed germination, AtPP2CA T-DNA insertion alleles were hypersensitive to ABA application during seed germination (Kuhn et al., 2005). These results support the proposal that AtPP2CA is a strong negative regulator of ABA signaling.

How AtPP2CA function is regulated in Arabidopsis is still largely unknown. A yeast two-hybrid screen showed that AtPP2CA is an interaction partner of AKT2, a potassium channel inward rectifier (Vranova et al., 2000); this physical interaction was confirmed by in vitro pull-down assays. AtPP2CA modulates the AKT2 potassium-channel activity when coexpressed in COS cells and ocytes of Xenopus, which provides the evidence for a functional interaction between these two proteins (Cherel et al., 2002). However, more data are needed to generalize the regulatory mechanism of AtPP2CA's function in Arabidopsis.

KINASE ASSOCIATED PROTEIN PHOSPHATASE. KAPP (kinase associated protein phosphatase) was the first downstream regulator of an RLK to be characterized. It was identified by screening an Arabidopsis cDNA expression library for interactions with an RLK protein kinase domain (Stone et al., 1994).

KAPP is a unique multi-domain protein. It has a type I membrane anchor at the N-terminus, a kinase interaction domain (KID)-forkhead-associated (FHA) domain in the central part, and a PP2C domain at the C-terminus (Stone et al., 1994). Transient expression experiments showed that the N-terminal signal anchor is functional in targeting KAPP to the cowpea mesophyll protoplast membrane (Shah et al., 2002). However, this localization pattern needs to be confirmed with a stable transgenic line. The functional relevance of KAPP's membrane association in Arabidopsis remains unclear.

The interactions between KAPP and RLKs are mediated by KID. The KAPP KID domain, which contains 239 residues (aa 98-336), interacts with the RLK catalytic domain in a phosphorylation-dependent manner (Stone et al., 1994; Braun et al., 1997). Subsequent serial deletion and in vitro binding assays demonstrated that the minimal functional phosphoprotein binding unit of KAPP consists of 119 residues that span amino acids 180 to 298 (Li et al., 1999). In the KID domain, there is a 52-residue region (aa 208-259) that shares homology with the FHA domain (Hofmann and Bucher, 1995). Site-directed mutagenesis of four highly conserved residues (G211, S226, H229, N250) within the KAPP FHA-homology region and in vitro binding assays demonstrated that the 52 amino acid core region (208-259) is essential, but not sufficient for, its interaction with phophorylated RLKs (Li et al., 1999).

Originally, the FHA domain was identified in a group of forkhead transcription factors. Subsequent research, however, showed that this domain exists in a wide variety of proteins from prokaryotes to eukaryotes (Hofmann and Bucher, 1995; Li et al., 2000). Supporting evidence that the FHA domain is a phosphoprotein-binding module comes from studies of the Rad53p in yeast (Sun et al., 1998). Rad53p is a protein kinase that is involved in DNA-damage responses and cell-cycle arrest in *Saccharomyces cerevisiae*. Rad53p has two FHA domains (a N-terminal FHA1 and a C-terminal FHA2 domain) that flank a central Ser/Thr kinase domain. Upon sensing DNA damage or inhibition of DNA replication, a kinase cascade is activated, and Rad9p is phosphorylated by Mec1p. The cell cycle is arrested upon recognition of phosphorylated Rad9p by Rad53p through the FHA2 domain. A mutation of the FHA2 domain can bypass the DNA damage that is induced by G2/M cell-cycle arrest, suggesting the biological function significance of this domain.

The C-terminus of KAPP is a PP2C domain. In vitro protein phosphatase assays showed that the activity of KAPP is consistent with its classification as a PP2C; Mg^{2+}/Mn^{2+} is required for its activity and it is insensitive to high concentrations of okadaic acid, which is a specific inhibitor of PP1 and PP2A phosphatases (Stone et al., 1994).

Protein phosphorylation and dephosphorylation play essential roles in regulating signal perception and transduction. In RLK-mediated signaling pathways, the regulatory mechanism of phosphorylation and dephosphorylation are not completely understood. KAPP was the first and the only phosphatase to be shown to physically interact with multiple RLKs (Stone et al., 1994; Braun et al., 1997; Stone et al., 1998; Trotochaud et al., 1999; Gomez-Gomez et al., 2001; Shah et al., 2002; Rienties et al., 2005). It has been proposed that KAPP serves as a positive regulator of RLK signaling pathways, just like the *Drosophila* SRC homology 2 (SH2) domain protein tyrosine phosphatase (PTPase), *corkscrew*. Corkscrew has been shown to interact with multiple membrane-associated receptor tyrosine kinases (RTKs) and to transduce positively RTKs' signals to the intracellular components (Perkins et al., 1996; Roberts, 1996). Alternatively, KAPP may serve as a negative regulator of RLKs' signaling pathway, to dephosphroylate and desensitize the ligand-activated RLKs (Roberts, 1996).

However, recent genetic and biochemical studies suggest that KAPP is a functional protein phosphatase and serves as a negative regulator in RLK signaling pathways. Two lines of evidence supporting this proposal come from the studies of CLV1 and FLS2. CLV1 is a LRR-RLK (Clark et al., 1997). Its primary function is to promote the shoot apical meristem to differentiate and to inhibit its proliferation by feedback down-regulation of *WUS* expression (Schoof et al., 2000; Clark, 2001). The *clv1* mutants have abnormally enlarged meristems because of an excessive proliferation of meristem tissue. To address the possible regulatory role of KAPP in a CLV1-signaling pathway, KAPP was shown to interact with CLV1 and to dephosphorylate CLV1 in vitro. Transgenic analysis showed that KAPP overexpression lines have a similar phenotype to *clv1*, and *KAPP* co-suppression lines can suppress the *clv1* weak-allele phenotype (Williams et al., 1997; Stone et al., 1998). Gel filtration experiments suggested that KAPP and Rho GTPase coexist in an active 450 kDa CLV1 complex; in the kinase inactive mutant (*clv1-10*), this complex can not be assembled (Trotochaud et al., 1999). Additional evidence that supports the proposal that KAPP is a negative regulator of RLK signaling comes from studies of *FLS2*. As noted earlier, FLS2 is a LRR-RLK involved in the binding and recognition of the bacterial elicitor flagellin (*flg22*) (Gomez-Gomez and Boller, 2000). Yeast two-hybrid experiments showed that KAPP physically interacts with FLS2. In Arabidopsis, when KAPP is overexpressed, the plants show reduced binding to *flg22* and become insensitive to flagellin treatment (Gomez-Gomez et al., 2001).

KAPP is a single-copy gene in Arabidopsis (Stone et al., 1994). No homologs of KAPP have been identified in the Arabidopsis genome (Kerk et al., 2002). However, KAPP orthologs have been identified in maize and rice; they also show binding to several RLKs (Braun et al., 1997; van der Knaap et al., 1999). Future research on the in vivo function of KAPP will be required to understand the role of this protein phosphatase.

POLTERGEIST. *poltergeist* (*pol*) was characterized as a suppressor of *clv1* because it can suppress the massive accumulation of meristematic stem cells in *CLV* mutants. *POL* and its homologs (*PLLs, POL-like*) encode a novel subtype of PP2C, which is distinguished by the insertion of 200 unique amino acids between subdomain III and IV in the PP2C catalytic domain (Yu et al., 2003). In vitro biochemical analysis demonstrated that POL is a functional PP2C protein phosphatase and that its N-terminus may function as a negative regulatory domain for the C-terminus catalytic domain (Yu et al., 2003). Its predicted nuclear localization signal, indicates that POL may function downstream of the CLV1 signaling cascade.

pol mutants have no obvious phenotype. However, *pol* mutations are able to suppress weak *clv1* and *clv3* mutant phenotypes completely and partially suppress strong alleles of *clv1* and *clv3* (Yu et al., 2000; Yu et al., 2003). Genetic analysis of *pol clv wus* and of *clv wus* suggests that POL functions both in the CLV-WUS pathway and in a WUS-independent pathway (Yu et al., 2000; Yu et al., 2003). This is consistent with the broad expression pattern of POL and PLLs (Song and Clark, 2005). Double mutants of *pol* and *pll1* are seedling lethal (Song and Clark, 2005), which suggests that these two genes have broad roles in plant growth and development. Interestingly, *pol* and *pll1* show haploinsufficiency in rescuing the mutant phenotype of *clv*; this dosage effect indicates that POL and PLL1 function together to regulate a rate-limiting step in meristematic stem cell proliferation (Song and Clark, 2005). Further insights into understanding the role of POL will depend on the identification and characterization of POL substrates, which also will help to better elucidate the CLV signaling cascade.

Protein Tyrosine Phosphatases

Protein tyrosine phosphatases (PTPs) super-family can be classified into tyrosine-specific PTPs that act on phosphotyrosine and dual-specificity protein tyrosine phosphatase (DsPTP), which can dephosphorylate both phosphotyrosine and phosphoserine/phosphothreonine. The lack of sequence homology between PTPs and protein Ser/Thr phosphatases and the unique 3-D structure of PTPs' catalytic domains indicate that PTPs evolved independently (Fauman and Saper, 1996). However, the highly conserved structure of the catalytic domain within the PTP super-family suggests a common phosphate hydrolysis mechanism (Fauman and Saper, 1996). All the members of the PTP super-family carry the signature motif of CX_5R in their active site and the cysteine is required for PTP catalytic activity (Fauman and Saper, 1996).

There are 18 PTPs in the Arabidopsis genome (Kerk et al., 2002). The function of PTPs in plants was not known until *AtPTP1* was characterized in 1998 (Xu et al., 1998). *AtPTP1* expression is strongly induced under high-salt stress conditions. The catalytic domain of AtPTP1 shares high homology with mammalian tyrosine phosphatases. In vitro enzyme assays showed that AtPTP1 has the CX_5R signature motif essential for the tyrosine-specific phosphatases activity (Xu et al., 1998). The first DsPTP (*AtDsPTP1*) was identified the same year (Gupta et al., 1998). In animals, substrates of tyrosine phosphatases come from phosphorylation product of tyrosine kinase and MAPKK. In Arabidopsis, there are no tyrosine protein kinases that can be identified based on the genome sequence, which suggests that the major function of plant PTPs is to counteract MAPK activity (Luan, 2003). Consistent with this, AtDsPTP1 was demonstrated to be able to dephosphorylate and inactivate AtMPK4 (Gupta et al., 1998).

The first genetic inference of the function of PTP in Arabidopsis came from the genetic characterization of *AtMKP1*. *mkp1* is hypersensitive to genotoxic stress, UV-C and methyl methanesulphonate, (Ulm et al., 2001). Further research demonstrated that *mkp1* is salt tolerant. A yeast two-hybrid screen found that MKP1 interacts with MPK3, MPK4, and MPK6, which are key signaling components in a diverse set of stress and environmental signaling responses. These data indicate MKP1 may regulate plant response to salinity and genotoxic stresses through the regulation of MAPK activities (Ulm et al., 2002).

Recently, another MKP-like gene *Propyzamide-hypersensitive1* (*PHS1*) was cloned (Naoi and Hashimoto, 2004). The original *phs1-1* mutant, which carries a point mutation of Arg to Cys in the non-catalytic N-terminal, is semidominant. The *phs1-1* mutant has cortical microtubule organization defects, whereas the *phs1-2* null allele is recessive embryo lethal. In animals, the corresponding Arg is critical for the interaction between MKP and MAPK substrates, and mutation of the highly conserved Arg disrupts this interaction (Tanoue et al., 2002). By analogy, the Arg-Cys mutation in *phs1-1* may inhibit the kinase-phosphatase association. A definitive answer will have to wait for substrates for PHS1 to be identified.

PTEN, an animal tumor suppressor, is an unusual PTP. It can dephosphorylate both phosphotyrosine and phosphatidylinositol 3,4,5-triphosphate (PIP3). It has a conserved PTP phosphatase domain and a cytoskeleton-interacting tensin-like domain. PTEN functions in many cellular processes in animals, including apoptosis, cell adhesion, and cell migration (Yamada and Araki, 2001). A PTEN homolog in Arabidopsis is expressed solely in pollen grains during the tri-nuclear stage. RNAi silencing of *AtPTEN* causes pollen death after mitosis (Gupta et al., 2002). Future studies should address whether PIP3 is a physiological substrate for AtPTEN in pollen, which will help to dissect the signaling networks that regulate pollen maturation.

Recently, PTPs were implicated in auxin and ABA signaling responses in Arabidopsis. *ibr5* was isolated as an indole-3-butyric acid insensitive mutant; it is also less sensitive to IAA, 2,4-D, and ABA. *IBR5* encodes a DsPTP. *IBR5* is a unique gene; no close homolog exists in the Arabidopsis genome (Monroe-Augustus et al., 2003). However, it has highly homologous analogs across monocots and dicots, indicating it has conserved functions across plant species (Monroe-Augustus et al., 2003). Identification of the IBR5 substrates will help to define the function of DsPTPs in plant hormone signaling responses. Their identification also may help to clarify the roles of MAPK in auxin and ABA signaling, since MAPKs---being putative substrates for IBR5---are implicated in auxin and ABA signaling responses, as well (Kovtun et al., 1998; Kovtun et al., 2000; Mockaitis and Howell, 2000; Lu et al., 2002).

In human and in yeast, the DsPTP CDC25 is a positive regulator of CDC2 (cyclin-dependent kinase) (Kristjansdottir and Rudolph, 2004). However, only recently have CDC25 orthologs been identified in Arabidopsis. The Arabidopsis CDC25 (AtCDC25) is composed solely of a catalytic domain. Structural analysis indicates that AtCDC25 belongs to the classical CDC25 super-family, with a central 5-strand beta sheet surrounded by helices (Landrieu et al., 2004). Unlike human CDC25, AtCDC25 does not have a long, non-conserved N-terminal regulatory domain; however, it does have one zinc-binding loop in the C-terminal that may play an equivalent regulatory role (Landrieu et al., 2004). Genetic evidence is needed to confirm the function of CDC25 in plants.

With a PKa value around 5-6 in the PTP catalytic core, the highly conserved catalytic cysteine of PTPs usually exists in a thiolate anion state, which is essential for the catalytic mechanism of PTPs (Meng et al., 2002). However, it also is very susceptible to oxidative stress; oxidation of cysteine will inhibit the catalytic activity reversibly (Meng et al., 2002; Xu et al., 2002). This is a well-designed mechanism to regulate PTP activity, as cytokines and growth factors can stimulate transient and rapid production of ROS, which is employed to regulate the activity of PTPs reversibly (Meng et al., 2002; Xu et al., 2002; Tonks, 2005). A similar regulation mechanism has been observed in Arabidopsis. AtPTP1 is reversibly inactivated by H_2O_2, which is correlated with the activation of MAPK6 by H_2O_2, suggesting that AtPTP could be a major target for oxidative stress in plants (Gupta and Luan, 2003).

As a second messenger, Ca^{2+} functions in a diverse array of signaling transduction pathways in plants. As a Ca^{2+} sensor, calmodulin may regulate a wide range of downstream proteins that help to translate differential Ca^{2+} inputs into specific physiological responses (Ng and McAinsh, 2003). It has been demonstrated that AtPTP1 interacts with CaM; depending on the substrates of AtPTP1, CaM binding can either increase or decrease the dephosphorylation activity of AtPTP1 (Yoo et al., 2004). NtMKP1 (the AtMKP1 analog in tobacco) also is regulated by CaM binding, indicating that CaM regulation of PTP activity could be a common regulatory mechanism of plant protein phosphatase activity (Yamakawa et al., 2004).

CONCLUSIONS

The Arabidopsis genome is rich with genes that encode in protein kinases and protein phosphatases. There has been an impressive advance in our understanding of the functional roles of these enzymes over the past few years. These studies reinforce the idea that protein kinases and phosphatases are major regulators of cellular and developmental processes. However, much of the work has focused on trying to understand the role of a single gene. We are just beginning to elucidate the complex networks involving protein kinase and phosphatses in cellular function. Many more questions remain to be addressed. While convergence and divergence of functions on the different levels of protein kinase cascades has been proposed, how exactly is signaling specificity maintained in each pathway? What are the spatiotemporal dynamics of the active components of in a protein phosphorylation cascade? What are the substrates and interacting regulators?

A combination of approaches will required to address these questions. Future research should go beyond the loss-of-function approaches such as T-DNA insertion mutant screening (Alonso et al., 2003), EMS mutagenized TILLING mutant screening (McCallum et al., 2000a, 2000b), and RNAi gene silencing (Wesley et al., 2001). The approaches of systems biology (e.g., whole-genome expression profiling by microarray, yeast two-hybrid screening, protein microarray proteomics, and phosphoproteomic techniques), combined with the genetics, should aid in the identification of interacting factors and substrates. For example, about 48 potential substrates for MPK3 and MPK6 have been recenlty identified by a protein microarray-based proteomics approach (Feilner et al., 2005). Phosphoproteomic approaches have also yielded tremendous information about cellular phosphorylation events (Nuhse et al., 2003, 2004; Peck, 2006). Newly developed approaches that allow comparison of multiple parallel samples will contribute to our ability to quantitatively and comparatively track cellular phosphorylation events (Shadforth et al., 2005). Future work will need to focus how these proteins are integrated into regulatory networks and how the dynamics of these networks influence plant development and responses to the environment.

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