Plant mitogen-activated protein kinase signaling cascades

Guillaume Tena*, Tsuneaki Asai†, Wan-Ling Chiu‡ and Jen Sheen§

Mitogen-activated protein kinase (MAPK) cascades have emerged as a universal signal transduction mechanism that connects diverse receptors/sensors to cellular and nuclear responses in eukaryotes. Recent studies in plants indicate that MAPK cascades are vital to fundamental physiological functions involved in hormonal responses, cell cycle regulation, abiotic stress signaling, and defense mechanisms. New findings have revealed the complexity and redundancy of the signaling components, the antagonistic nature of distinct pathways, and the use of both positive and negative regulatory mechanisms.

Addresses
Department of Molecular Biology, Massachusetts General Hospital, Department of Genetics, Harvard Medical School, Wellman 11, 50 Blossom Street, Boston, Massachusetts 02114, USA
*e-mail: guillaume@molbio.mgh.harvard.edu
†e-mail: asai@molbio.mgh.harvard.edu
‡e-mail: wchiu@molbio.mgh.harvard.edu
§e-mail: sheen@molbio.mgh.harvard.edu

Current Opinion in Plant Biology 2001, 4:392–400

1369-5266/01/$ – see front matter

Abbreviations

At	*Arabidopsis thaliana*
CTR1	CONSTITUTIVE TRIPLE RESPONSE 1
DSP	dual specificity MAPK phosphatase
EDR1	ENHANCED DISEASE RESISTANCE 1
ERK	extracellular signal-regulated kinase
MAPK (or MPK)	mitogen-activated protein kinase
MAPKK	MAPK kinase
MAPKKK	MAPKK kinase
MEK/MKK	MAPK kinase
MEKK	MAPKK kinase
MKP	MAPK phosphatase
MMK	*Medicago* MAPK
Ms	*Medicago sativa*
NPK1	*Nicotiana* protein kinase
Nt	*Nicotiana tabacum*
PTP	phosphotyrosine phosphatase
SA	salicylic acid
SAMK	stress-activated MAPK
SAR	systemic acquired resistance
SIMK	salt-induced MAPK
SIMKK	SIMK kinase
SIPK	salicylic-acid-inducible protein kinase
WIPK	wound-inducible protein kinase

Introduction

Plants possess integrated signaling networks that mediate the perception of and responses to the hormones, nutrients, and environmental cues and stresses that govern plant growth and development. Our current knowledge of plant signal transduction pathways has come from the identification of the sensors and receptors that perceive the signal, and of the transcription factors and target genes that coordinate the response [1]. What is missing from our understanding of most plant signal transduction pathways, however, is the identity of the regulatory components that link sensors/receptors to target genes and other cellular responses.

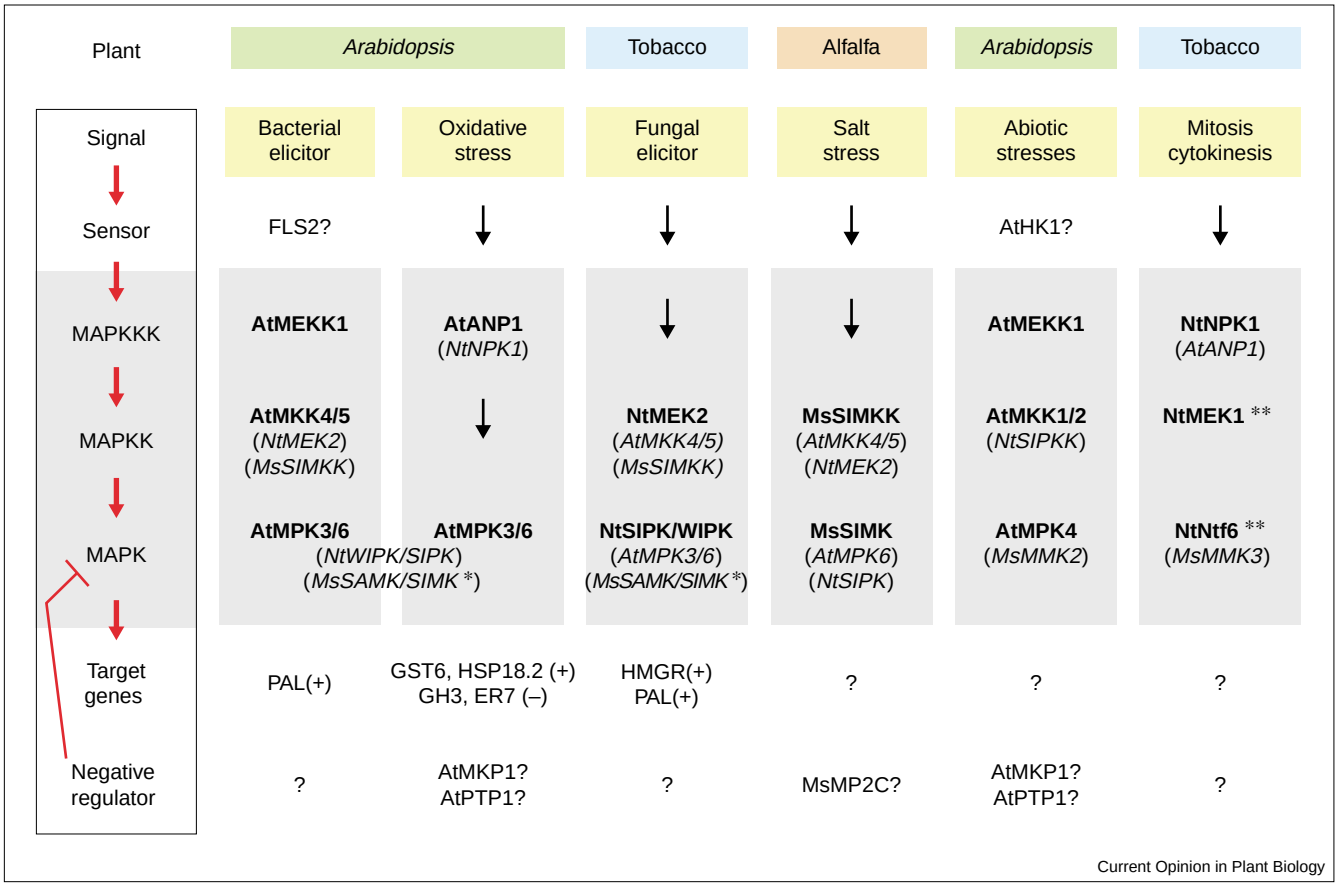
In the past few years, it has become apparent that mitogen-activated protein kinase (MAPK) cascades play some of the most essential roles in plant signal transduction pathways from cell division to cell death (Figure 1). MAPK cascades are evolutionarily conserved signaling modules with essential regulatory functions in eukaryotes, including yeasts, worms, flies, frogs, mammals and plants. The recent enthusiasm for plant MAPK cascades is backed by numerous studies showing that plant MAPKs are activated by hormones, abiotic stresses, pathogens and pathogen-derived elicitors, and are also activated at specific stages during the cell cycle [2]. Until recently, studies of MAPK cascades in plants were focused on cDNA cloning [3,4] and used a MAPK in-gel assay, MAPK and tyrosine-phosphate antibodies, and kinase inhibitors to connect signals to MAPKs [2]. The recent completion of the *Arabidopsis thaliana* (At) genome sequence presents a new opportunity to identify and isolate the large gene families encoding MAPKs and their immediate upstream regulators, MAPK kinases (MAPKKs) and MAPKK kinases (MAPKKKs) on the basis of sequence conservation (Table 1). Various combinations of this large set of genes provide the diversity and specificity necessary to transmit a broad spectrum of signals in plants. The challenge ahead is to develop additional tools to better define the components of plant MAPK cascades and to determine the specific roles of individual MAPK cascade genes in particular signal transduction pathways.

A general background to MAPK signaling cascades has been provided by excellent reviews on organisms from yeast, to mammals, to plants [2,5,6]. In this review, we offer the first glance at the coding capacity for MAPK cascade genes in the *Arabidopsis* genome (Table 1), and focus on the most recent progress in the identification of plant MAPK, MAPKK, and MAPKKK genes in specific signal transduction pathways (Figure 1). We also discuss the emerging feature of antagonistic interactions between MAPK signaling cascades (Figure 2).

Plant MAPK cascade genes

Known eukaryotic MAPKs can be divided into three main subfamilies on the basis of their structural characteristics, which often correlate with their functions in distinct signal transduction pathways [7]. However, all plant MAPK genes described so far belong to a single group, the so-called extracellular signal-regulated kinase (ERK) subfamily. In mammals, members of this subfamily are mainly responsible for the transduction of mitogenic signals but, in plants, ERKs seem to have evolved in such a way as to be able to transmit a broader range of stimuli [8].

Figure 1



MAPK cascades in diverse plant signal transduction pathways. A general schematic presentation of signal transduction pathways is shown on the left. FLS2 is the putative receptor for the flagellin peptide elicitor flg22. AtHK1 is the putative histidine kinase osmosensor. The functionally defined MAPK-cascade components are shown in bold. MAPK, MAPKK and MAPKKK homologs in three plant species, tobacco (Nt), alfalfa (Ms) and *Arabidopsis* (At) are shown. *Alfalfa MsSAMK is MsMMK4, MsSIMK is MsMMK1. **Tobacco NtMEK1 is the same as NtNQK1, and tobacco NtNTF6 is NtNRK1. The negative regulators shown here are limited to known MAPK-specific phosphatases: dual-specificity MAPK phosphatase (AtMKP1), phosphotyrosine phosphatase (AtPTP1), and protein phosphatase 2C (MsMP2C). ER7, auxin-inducible enhancer; GH3 auxin-inducible promoter; GST, glutathione-*S*-transferase; HMGR, 3-hydroxy-3-methylglutaryl CoA reductase; HSP, heat shock protein; PAL, phenylalanine ammonia-lyase; (+) positive regulation; (–) negative regulation.

In terms of physiological roles, the best-characterized plant MAPK proteins are from *Medicago sativa* (alfalfa), *Nicotiana tabacum* (tobacco) and *Arabidopsis*. A whole-genome survey of the MAPK genes and the genes encoding their upstream regulators (MAPKKs and MAPKKKs) is, however, currently possible only in *Arabidopsis* (Table 1). According to our sequence search criteria with highly conserved signature motifs, there are 23 MAPK and 9 MAPKK genes in the *Arabidopsis* genome (Table 1). The sequences of putative MAPKKK genes [4] are more divergent than those of other members of the MAPK cascades. In the *Arabidopsis* genome, a comprehensive database search has identified more than 25 MAPKKK gene candidates on the basis of sequence conservation in the kinase domain (Table 1). The MAPKKK gene candidates can be divided into at least two main subfamilies, typified by their mammalian homologs: Raf-like (e.g. *AtCTR1* and *AtEDR1*) and MEKK-like (e.g. *AtANP1* and *AtMEKK1*) [9,10•,11••–13••]. The true functional proof of any MAPK cascade component requires a combination of physiological, genetic and/or biochemical data. A global survey of the MAPK cascade gene expression patterns using customized microarrays in *Arabidopsis* could help to establish their functionality. A more systematic nomenclature of MAPK cascade genes would also facilitate information exchange among research groups working on different plant species (see Figure 1 and Table 1).

MAPK cascades in hormonal responses

Extensive studies have linked MAPKs with mitogenic stimuli in mammalian cells, thus providing the generic name for this subclass of protein kinases. In plants, auxin (as a mitogen) has previously been linked to MAPK activation in tobacco BY-2 cells [14]; however, at that time, the extreme sensitivity of plant MAPKs to mechanical stress was not recognized. Other researchers were unable to show auxin activation of MAPK using the same system, but did stimulate a strong MAPK-like activation by simply re-suspending BY-2 cells in fresh medium or treating the

Table 1

MAPK cascades components in the *Arabidopsis* genome.

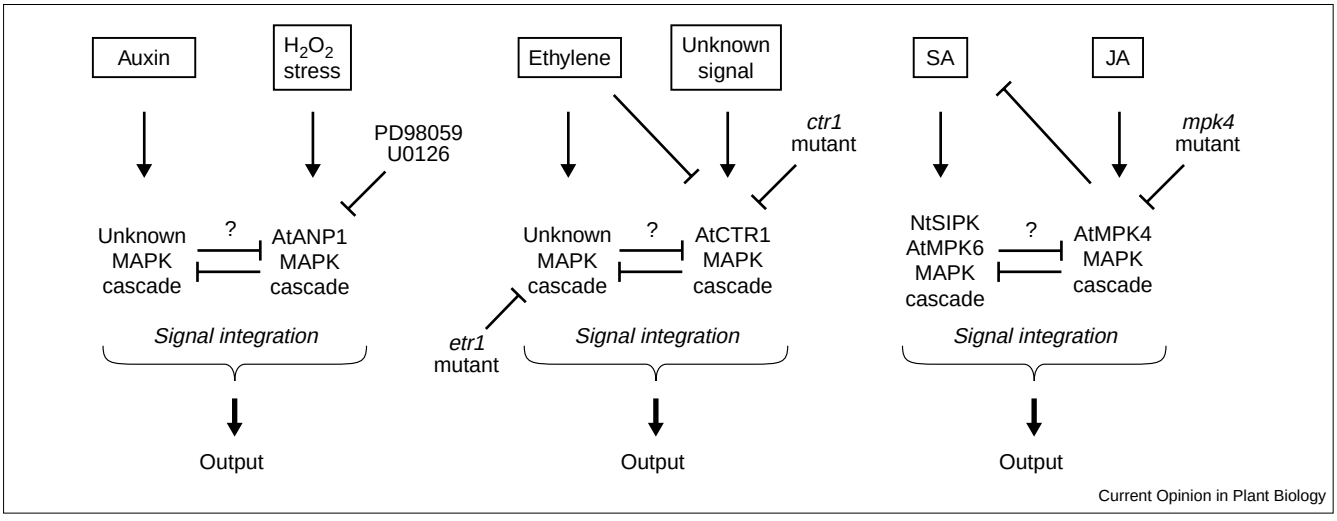
	Total number	Minimal signature motif	cDNA described	Function described	Subfamilies		
						Members	Act. Loop
MAPK	23	TxYVxxRWYRAPE	9	3: AtMPK3, 4, 6 [10•,11••,43••]	Group 1: AtMPK3, AtMPK6	3	TEY
					Group 2: AtMPK4, AtMPK5	5	TEY
					Group 3: AtMPK1, AtMPK2, AtMPK7	4	TEY
					Group 4: AtMPK8, AtMPK9	8	TDY
					Group 5: AtMHK	3	TEY
						Members	
MAPKK	9	[ST]xxGTxxYMxPER	5	4: AtMEK1, AtMKK2 AtMKK4, 5 [25] (T Asai, G Tena, unpublished data)	Group 1: AtMEK1, AtMKK2	3	
					Group 2: AtMKK4, AtMKK5	2	
					Group 3: AtMKK3	1	
					Group 4: No described cDNA	3	
						Putative members	
MAPKKK	>25	MEKK family: DIWSxGCTxxExxTxxxP Raf family: GVxxWELxTxxxPW	10	6: AtCTR1, AtEDR1 AtMEKK1, AtANP1, 2, 3 [9,11••–13••]	MEKK-like Group 1: AtANP1	3	
					MEKK-like Group 2: AtMEKK1	4	
					MEKK-like Group 3: AtMAP3Kalpha	3	
					Raf-like Group 1: AtCTR1, AtEDR1	6	
					Raf-like Group 2: No described cDNA	6	
					Raf-like Group 3: AtMAP3Ktheta1	3	
					Other Groups?	?	

An overview of the MAPK cascade genes in the *Arabidopsis* genome. The MAPK and MAPKK genes are defined by the minimal signature motifs. An estimated number and putative signature motifs are given for MAPKKK genes because of the divergence of the encoded proteins and the lack of sufficient functional data. The minimal signature motifs for classification were determined through alignment of the most conserved structural sequences using all available plant gene sequences for a given class of proteins. The minimal signature motifs shown here are sufficient to identify all members of the MAPK and MAPKK gene families in the *Arabidopsis* genome.

cells with a high concentration of 2,4-D (2,4-dichlorophenoxyacetic acid), which induced cytosolic acidification [15].

The use of an *Arabidopsis* leaf protoplast transient expression assay provided critical evidence that an oxidative stress MAPK cascade, involving AtANP1 and both AtMPK3 and AtMPK6, negatively regulates early auxin responses [11••]. The assay used well-defined auxin-responsive promoters and enhancers fused to a reporter gene. Using the same system, H_2O_2 was shown to trigger this stress MAPK signaling cascade and activate transcription, thus demonstrating that the same cascade can mediate both negative and positive regulation of transcription [11••]. The possibility that another MAPK cascade is involved in activating auxin

Figure 2



Antagonistic MAPK signaling pathways. Three hypothetical interactions of MAPK cascades are shown in auxin, ethylene, and jasmonate (JA) signaling. PD98059 and U0126 are mammalian MEK inhibitors.

responses has, however, not been excluded. More recently, using *Arabidopsis* seedling roots, a rapid transient activation of an unknown MAPK by biologically active auxin has been shown unequivocally [16•]. This auxin-induced MAPK activation — but not the one induced by salt stress — is almost abolished in the *axr4* mutant, which is impaired in its root growth response to auxin. Surprisingly, the inhibitors of the mammalian mitogenic MEKs, PD98059 and U0126, do not inhibit but greatly enhance auxin-dependent MAPK activation, although the same inhibitors repress the auxin-responsive reporter gene *BA3::GUS* in a transgenic *Arabidopsis* line [16•]. This unexpected result could be partially explained by the observation that these inhibitors can also block stress MAPK cascades in plants (see below and T Asai, J Sheen, unpublished data). It is consistent with the earlier finding that a stress MAPK cascade inhibits auxin responses [11••,17]. The link between auxin activation of MAPK and auxin-dependent gene expression requires further investigation. It appears that antagonistic MAPK cascades exist in the same cells and the final physiological output is more dependent on the balance of the qualitative and quantitative activities of multiple MAPK pathways rather than a simple linear on/off switch (Figure 2).

The role of MAPK cascades in ethylene responses is still unresolved. CTR1 (CONSTITUTIVE TRIPLE RESPONSE 1), a homolog of the mammalian MAPKKK Raf, has been shown genetically to act as a negative regulator in ethylene signaling: the recessive *ctr1* mutant displays striking phenotypes that mimic constitutive ethylene responses [9]. Although genetic data have provided impressively detailed information on the ethylene signaling pathway from the perception of ethylene to the activation of nuclear gene expression, to date, no components of a MAPK cascade have been identified downstream of CTR1 [18]. Curiously, ethylene treatment induces a MAPK-like activity in *Arabidopsis* leaves and this MAPK-like activity is greatly enhanced in the *ctr1* mutant ([19]; Y Kovtun, J Sheen, unpublished data). This situation is similar to the enhancement of auxin-dependent MAPK activation by MEK inhibitors in *Arabidopsis* roots [16•]. Apparently, the inhibition of one MAPK cascade can enhance the activation of another antagonistic and distinct MAPK cascade. The genetically defined negative regulators could be positive regulators of an antagonistic signaling pathway, as demonstrated in the case of auxin and H_2O_2 signaling [11••]. The final physiological responses to ethylene may depend on the combination of multiple outputs from different kinase cascades (Figure 2).

MAPK cascades in cell cycle regulation

To elucidate the function of MAPK cascades in cell cycle regulation, synchronized alfalfa and tobacco BY-2 suspension culture cells have been used to analyze the expression, activity and localization of an alfalfa MAPK (*Medicago* MAPK [MMK3]) and a tobacco MAPKKK (*Nicotiana* protein kinase [NPK1]) [13••,20]. Both MMK3 and NPK1 are transiently activated after the removal of propyzamide, which arrests cells in metaphase. Systematic immunostaining experiments show that the subcellular localization of NPK1 in BY-2 cells is dynamic. In mitosis, the protein moves from the nucleus during prophase to a thin equatorial zone of the phragmoplast. MMK3 shows similar localization in fava bean and alfalfa root-tip cells at various stages of mitosis. Both studies suggest that, during cytokinesis, a MAPK cascade functions in cell-plate formation. In an elegant experiment using transgenic cell lines expressing a dominant-negative NPK1 under the control of an inducible promoter, multinucleated cells were observed, revealing a clear defect in cytokinesis [13••]. The use of dominant-negative NPK1 fusions with green fluorescent protein (GFP) provided a visual correlation of the cytokinesis defect and revealed a partial inhibition of lateral expansion of the phragmoplast [13••]. In transgenic tobacco plants, cotyledon guard cells show the most striking aberrant phenotypes in cytokinesis.

Using the yeast two-hybrid system, a putative upstream activator of NPK1, NACK1 (with sequence similarity to kinesin-like proteins), and two possible downstream components of this cascade, NtNQK1 and NtNRK1, have been isolated [13••]. As NPK1 activity is modulated by phosphorylation, its regulation by cyclin-dependent protein kinases is also an attractive possibility. Using yeast two-hybrid, pull-down and kinase assays, it has been shown that NtNTF6 (which is identical to NtNRK1) and NtMEK1 (identical to NtNQK1) are components of a MAPK cascade, possibly acting downstream of NPK1. Interestingly, transcription of *NtMEK1* and activation of NtNTF6 are both found in cells whose division has been induced by auxin and cytokinin treatment [21]. The identification of phosphorylation substrates will uncover the molecular action of NPK1 in cytokinesis. Whether MAPK cascades control other steps of the cell cycle in plants, as they do in yeasts, frogs and mammals, and whether a MAPK cascade is required in cytokinesis await further studies.

MAPK activation in abiotic stress signaling

Plants possess sophisticated protection mechanisms to cope with various environmental stresses such as cold, freezing, heat, drought, ozone, UV light, salinity, osmotic shock, and mechanical wounding [22–24]. Accumulating evidence indicates that plants rapidly activate MAPKs when exposed to multiple abiotic stress stimuli [2,8]. The identity, roles, and specificity of MAPK cascades in the regulation of diverse abiotic stress responses, and their link to defense mechanisms (see below) and growth regulation, are the focal points of intensive research. The current lack of any MAPK cascade mutants in abiotic stress signaling has promoted the use of alternative research strategies, including molecular cloning on the basis of sequence homology, biochemical purification, specific antibody recognition, the use of inhibitors, *in vitro* kinase measurements, yeast mutant complementation and two-hybrid assays, protoplast transient expression and transgenic plant analyses.

Extensive two-hybrid analysis and mutant complementation in yeast has allowed the first *Arabidopsis* MAPK cascade, consisting of AtMEKK1, AtMEK1/AtMKK2, and AtMPK4, to be proposed [25]. This hypothetical cascade is supported by the finding that AtMPK4 is activated by AtMEK1 *in vitro* [26]. Using specific MAPK antibodies, recent analyses have identified AtMPK4 and AtMPK6 as the MAPKs activated by low temperature, low humidity, hyper-osmolarity, touch, and wounding in *Arabidopsis* plants [10•]. In analogy to the yeast SLN1 osmosensing and HOG1 MAPK signaling pathways [6], it is tempting to speculate that the AtMEKK1, AtMEK1/AtMKK2, and AtMPK4 signaling cascade is involved in abiotic stress responses with a putative upstream hybrid histidine kinase osmosensor, AtHK1 in *Arabidopsis* (Figure 1; [3]). As AtMPK4 and AtMPK6 show distinct levels and kinetics of activation, they may serve different functions [10•].

High NaCl or hyperosmotic conditions activate SIMK (salt-induced MAPK) in alfalfa cells and SIPK (salicylic-acid-inducible protein kinase) in tobacco cells [27•,28]. The specific upstream regulator of SIMK is SIMK kinase (SIMKK; an ortholog of AtMKK4,5), which was identified by a yeast two-hybrid screen [27•]. This result raises the possibility that different MAPKs may interact with specific MAPKKs and multiple MAPK cascades may be induced by the same signal. Whether the abiotic-stress-activated AtMPKs (AtMPK4 and AtMPK6) are regulated by the same or different MAPK signaling cascades and whether they serve similar or distinct functions in abiotic stress signaling requires functional analysis in plant cells. In transfected parsley protoplasts, SIMK is hardly activated by NaCl unless SIMKK is co-transfected [27•]. Thus, SIMKK seems to be a limiting factor in this putative MAPK cascade. This well-established plant transient expression system will aid in the functional analysis of SIMK and SIMKK *in vivo*.

Although each stress stimulus may involve a distinct perception process and trigger specific responses, there appear to be some common underlying mechanisms for abiotic stress signaling. The convergent points include the production of second messengers, such as calcium and H_2O_2, and/or the reliance on common signaling cascades and transcription factors [11••,22,29]. A connection between the activation of a plant MAPK cascade and one common second messenger, H_2O_2, which is generated by diverse stress stimuli, has been demonstrated using the *Arabidopsis* protoplast transient expression assay. The study shows that functional redundancy is found in MAPK cascades in plants, as also found in yeast and mammals. For instance, H_2O_2 activates AtMPK3 and AtMPK6, which act downstream of the redundant MAPKKKs, ANP1, ANP2 and ANP3. The versatile system also allows the link from the H_2O_2 signal to the MAPK cascade and to the target genes. It has been shown that H_2O_2 activates the *GST6* and *HSP18.2* promoters but represses the auxin-responsive *GH3* promoter. The same effect can be mimicked by the constitutively active versions of ANP1, ANP2 and ANP3. Interestingly, the abscisic-acid-/drought-/cold-inducible *RD29A* promoter is not activated by this oxidative-stress-induced MAPK cascade. It is further demonstrated that transgenic tobacco plants expressing the constitutively active version of NPK1, a tobacco ortholog of ANP1, exhibit tolerance to multiple stresses such as cold, heat, drought and high salinity. As described above, activation of this MAPK cascade results in the repression of the auxin-responsive *GH3* promoter, providing a molecular link between stress and hormone signaling (Figure 2; [11••]).

To understand the complete picture of oxidative stress signaling, ranging from signal perception to nuclear transcription events, the upstream and downstream components of this cascade — such as the H_2O_2 sensor and the transcription factors activated by AtMPK3/6 — will need to be identified. As H_2O_2 acts as a second messenger in tomato wounding responses, multiple levels of MAPK activation could be involved in wounding signaling cascades, both before and after jasmonic acid synthesis [24,30]. Using the MEK inhibitor, PD98059, a link between ozone, calcium and the oxidative-stress-activated MAPK cascade involving SIPK (an AtMAPK6 ortholog) has been shown in tobacco [31]. UV-C and genotoxic stress also activate unidentified MAPKs in *Arabidopsis* [32•]. A major question is how the same AtMPK3 and AtMPK6 respond to diverse upstream signals — abiotic stresses, H_2O_2 and pathogen elicitors (see below) — and trigger distinct responses in *Arabidopsis* cells. It is possible that different signaling complexes with distinct MAPKKs and MAPKKKs, as well as scaffold proteins, could be involved (Figure 1).

MAPK cascades in pathogen defense

MAPK activation by pathogens, pathogen-derived elicitors and defense-related second messengers is as complicated as MAPK activation by abiotic stress [2,8]. Two tobacco MAPKs, SIPK and WIPK (wound-inducible protein kinase), are activated by various pathogen-related signals through both race-specific and non-race-specific elicitation mechanisms [33–35]. As both of these MAPKs are also activated by diverse abiotic stresses, pathogen defense signaling is a part of the integrated stress-signaling network in plants. SIPK and WIPK may provide convergence points for many distinct signaling cascades in plant defense and stress responses [28,31,34–37]. Orthologs of SIPK and WIPK in *Arabidopsis* (AtMPK6 and AtMPK3, respectively) and alfalfa (SIMK and SAMK [stress-activated MAPK], respectively) are also activated by both biotic and abiotic stresses, further supporting this idea [3,8,10•,11••,38,39]. The question then is how can these MAPKs mediate the induction of stimulus-specific defense responses. Recent studies suggest that different stimuli activate these MAPKs to different levels and with different kinetics. Thus, these MAPKs may participate in distinct signaling complexes [35,38,39].

The recent identification of NtMEK2, which interacts with SIPK and WIPK in yeast cells, has been particularly informative [40••]. NtMEK2 is activated concurrently with

SIPK and WIPK in tobacco cells treated with cryptogein — a fungal elicitor that induces hypersensitive cell death and the expression of defense genes. Using an elegant conditional gain-of-function approach, the authors demonstrate that a constitutively active NtMEK2 activates endogenous SIPK and WIPK in transiently transformed tobacco leaf cells. Importantly, the constitutively active NtMEK2 induces hypersensitive cell death and the expression of defense genes in the absence of cryptogein. Other constitutively active tobacco MAPKKs neither activate SIPK or WIPK nor induce the defense responses [40••]. These results strongly suggest that the MAPK cascade containing NtMEK2, SIPK and WIPK is specifically involved in the expression of pathogen defense responses in tobacco. It remains to be shown whether a tobacco MAPKKK acts upstream of NtMEK2 and whether the activation of this MAPK cascade will enhance pathogen resistance in plants.

In *Arabidopsis*, AtMPK6 is activated with different kinetics by a flagellin peptide (flg22), hexameric chitin fragments, xylanase or pectic fragments [39]. A recent study using a *GST1*::*LUC* transgenic *Arabidopsis* line and the MEK inhibitor, PD98059, offers interesting insight into redox signaling and MAPK-dependent transcription after infection by an avirulent strain of *Pseudomonas syringae* [41]. The authors of this work speculate that a specific MAPK cascade may contribute both to the establishment of plant disease resistance and to the development of cellular protection mechanisms. The use of the *Arabidopsis* protoplast transient expression system has enabled the identification of a complete MAPK cascade (Figure 1; T Asai, G Tena, unpublished data) that is responsible for the action of flg22 elicitor and its receptor FLS2, a leucine-rich-repeat receptor-like kinase ([42••]; T Romeis, pp 407–414). The expression of the components of this MAPK cascade confers pathogen resistance in the leaves of intact *Arabidopsis* plants (T Asai, J Sheen, unpublished data). Future research will reveal whether the flg22 MAPK cascade also responds to other pathogens and pathogen-derived elicitors in *Arabidopsis*.

An even more complex scenario is evident in alfalfa, which shows activation of four MAPKs (SIMK, MMK2, MMK3 and SAMK) by multiple pathogen-derived elicitors [38]. It remains to be determined whether single or multiple MAPK cascades activate these MAPKs and whether any of the activated MAPK serve a physiologically significant role in pathogen defense in alfalfa.

Interestingly, the recent isolation of two MAPK-cascade mutants in *Arabidopsis* reveals negative regulatory roles of putative MAPK cascades in plant defense mechanisms ([12••,43••,44•]; T Romeis, pp 407–414). As discussed above for auxin and ethylene signaling, antagonistic MAPK cascades are also important in plant defense (Figure 2). The *edr1* mutant contains a mutation in a putative MAPKKK gene that belongs to the *CTR1* subfamily and exhibits enhanced pathogen resistance [12••]. As the mutant plants do not display constitutive expression of the pathogenesis-related gene *PR1*, the resistance is not caused by constitutive activation of a plant immune response, known as systemic acquired resistance (SAR). Instead, defense responses are induced by pathogen signals more rapidly in *edr1* plants than in wild-type plants. The recessive nature of the *edr1* mutation implies that EDR1 acts as a negative regulator in the expression of defense responses. It is likely that upon pathogen attack, two signaling events must occur in plant cells to induce defense responses. One is to inhibit negative regulators such as EDR1 and the other is to activate positive regulators [12••,44•]. The *Arabidopsis* MAPK cascade activated by flg22 could be one of the positive regulators. Whether EDR1 truly activates a MAPK cascade and whether it responds to specific stimuli remain open questions.

The isolation of the *Arabidopsis mpk4* mutant reveals another unexpected role of MAPKs in plants [43••]. Unlike the *edr1* mutant, the *mpk4* mutant exhibits constitutive SAR, leading to increased pathogen resistance. The constitutive SAR is caused by elevated levels of salicylic acid (SA), an endogenous signaling molecule necessary for the induction of SAR. The kinase activity of AtMPK4 is required for complementation of the *mpk4* mutant under normal growth conditions. These results suggest that the kinase activity of AtMPK4 suppresses cellular SA levels in wild-type plants and negatively regulates SAR. It is still unclear, however, whether the kinase activity of AtMPK4 is really required to suppress SAR in wild-type plants, although the authors demonstrate that a kinase-inactive mutant of AtMPK4 is unable to complement the constitutive SAR phenotype of the *mpk4* mutant. To confirm the role of AtMPK4 as a repressor of SAR, it is critical to determine whether wild-type plants inactivate AtMPK4 after pathogen attack thereby increasing cellular SA concentrations. AtMPK4 may also act as a positive regulator of the expression of jasmonate-responsive genes such as *PDF1.2* and *THI2.1*. In the *mpk4* mutant, induction of these genes by jasmonate is suppressed ([43••]; Figure 2). This result is unlikely to be due to the antagonizing effect of SA because the suppression is also observed in the *mpk4* mutant expressing an SA hydroxylase, NahG, unless a low concentration or transient elevation of SA is sufficient to block jasmonate signaling. Whether AtMPK4 kinase activity is required for the induction of these genes is unknown. It will be interesting to determine which aspect of the wounding response is impaired in the *mpk4* mutant as a result of jasmonate insensitivity, and which MAPKKK and MAPKK act upstream of AtMPK4. The mutant also offers a tool to directly test the hypothetical MAPK cascade — AtMEKK1, AtMEK1/AtMKK2 and AtMPK4 — proposed to be important for multiple abiotic stress signaling [10•].

Negative regulation of MAPK cascades

Given the importance of MAPK signaling cascades in responding to cellular and environmental cues, one would expect inactivation of MAPKs or other components of the

cascades to be just as tightly regulated as activation of the pathways. Enzymatic dephosphorylation of either the threonine or tyrosine residue within the activation loop motif TXY (using the single letter code for amino acids, where X indicates any amino acid) can by itself result in inactivation of MAPKs.

In yeast and mammalian cells, enzymes capable of inactivating MAPKs include serine/threonine-specific protein phosphatases, such as PP2A and PP2C, and some members of the protein phosphotyrosine phosphatase (PTP) superfamily, especially dual specificity MAPK phosphatases (DSPs) [45]. Often, activation of a MAPK pathway leads to the transcriptional activation of protein phosphatases that can inactivate the same MAPK pathway, resulting in a negative-feedback control loop. In plants, several protein phosphatases have been characterized that are able to inactivate MAPKs, at least *in vitro*. These enzymes include members of the PP2C, PTP, and DSP families [26,46,47]. DSPs are members of a subfamily of PTPs that contain a short conserved signature motif around their catalytic sites. Members of the DSP subfamily are well known to inactivate MAPKs in mammalian cells. These enzymes display differences in specificity for MAPK family members, tissue-specific expression, and subcellular localization.

The conservation of the DSP signature motif throughout eukaryotes facilitated the isolation of a DSP member in *Arabidopsis*, AtDsPTP1 [48]. Although AtDsPTP1 is able to dephosphorylate and inactivate AtMPK4 *in vitro*, its biological function remains unknown. According to the presence of the DSP signature motif, there are an estimated 11 DSPs in the *Arabidopsis* genome. Thus far, the only known biological function of plant DSPs is revealed from the analysis of the T-DNA tagged MAPK phosphatase (MKP) mutant *mkp*1 [32•]. The *mkp1* null mutant does not exhibit a visible phenotype under normal growth conditions but displays hypersensitivity after UV-C and methyl methanesulfonate treatments. Exposure of wild-type *Arabidopsis* seedlings to UV-C induced a dose-dependent activation of a 49-kDa MAPK within five minutes. The level of activity of this 49-kDa MAPK is higher in the *mkp1* mutant in wild-type plants under the same treatment. On the other hand, a transgenic line that overproduces AtMKP1 is more resistant to activation of the 49-kDa MAPK by UV-C and methyl methanesulfonate. These results indicate that AtMKP1 is a crucial regulator of the MAPK pathway responding to DNA-damaging agents. Interestingly, the level of AtMKP1 mRNA remains constant during and after genotoxic stress treatments. This result suggests that post-transcriptional regulation can also be an important step in modulating AtMKP activities. It remains to be determined which MAPK cascade is regulated by AtMKP1 and how the balance between MAPK and MAPK phosphatases is achieved in the genotoxic stress response to ensure plant survival.

Conclusions and perspectives

Judging from the large number of MAPK cascade genes found in the smallest plant genome, our current understanding of MAPK-cascade functions in plants represents only a small beginning. MAPK signaling in plants involves the redundancy of signaling components, antagonism among distinct pathways and both positive and negative regulatory mechanisms. Consequently, a thorough understanding of each plant MAPK signaling cascade will require futher systematic analysis of the genes involved using genetic, genomic, and proteomic [49] approaches, as well as *in vivo* functional assays. Characterization of loss-of-function mutants of MAPK signaling components would undoubtedly advance our understanding of their functions in whole plants; however, it appears to be difficult to obtain such mutants. It is likely that some MAPK signaling components are essential for cell growth and development. It is also possible that many single-knockout mutants lack readily detectable phenotypes as a result of functional redundancy [11••]. Because of the transient nature of MAPK activation in many responses, the indirect and long-lasting phenotypes of MAPK signaling mutants could be misleading or confusing. Mutant phenotypes may not always represent the primary targets of the mutated signaling pathway [44•]. Curiously, all of the MAPK signaling mutants isolated so far — *ctr1*, *edr1*, *mpk4* and *mkp1* — indicate only a negative regulatory role of MAPK cascades in *Arabidopsis*. Therefore, it is essential to combine various assay techniques to identify the true functions of MAPK signaling cascades in plants. Besides the core MAPK cascade components and scaffold/anchoring proteins, the role of negative regulators such as various protein phosphatases and the identification of upstream signals, receptors/sensors, adaptor proteins, transcription factors, MAPK substrates and target genes will help us piece together the biological functions of a large number of plant gene products that are involved in the essential signaling network of protein phosphorylation.

Acknowledgements

We apologize to plant MAPK researchers for not being able to include all relevant papers. We would like to thank Cheri Chen for her assistance in bioinformatics and Brandon Moore for critical reading of the manuscript. The MAPK signaling cascade project in the Sheen laboratory is supported by the National Science Foundation Plant Genome Research Program DBI-0077692 and the US Department of Agriculture (USDA) Responses to Environmental Stress Program NRICGP-USDA 00-35100-9345.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. McCarty DR, Chory J: Conservation and innovation in plant signaling pathways. *Cell* 2000, 103:201-209.

2. Hirt H (Ed): *Results and Problems in Cell Differentiation: MAP Kinases in Plant Signal Transduction.* Heidelberg: Springer; 2000.

3. Mizoguchi T, Ichimura K, Yoshida R, Shinozaki K: MAP kinase cascades in ***Arabidopsis***: their roles in stress and hormone responses. In *Results and Problems in Cell Differentiation: MAP Kinases in Plant Signal Transduction.* Edited by Hirt H. Heidelberg: Springer; 2000:29-38.

4. Jouannic S, Hamal A, Leprince AS, Tregear JW, Kreis M, Henry Y: Plant MAP kinase kinase kinases structure, classification and evolution. *Gene* 1999, 233:1-11.

5. Chang L, Karin M: Mammalian MAP kinase signaling cascades. *Nature* 2001, 410:37-40.

6. Madhani HD, Fink GR: The riddle of MAP kinase signaling specificity. *Trends Genet* 1998, 14:151-155.

7. Kultz D: Phylogenetic and functional classification of mitogen- and stress-activated protein kinases. *J Mol Evol* 1998, 46:571-588.

8. Ligterink W, Hirt H: Mitogen-activated protein (MAP) kinase pathways in plants: versatile signaling tools. *Int Rev Cytol* 2001, 201:209-275.

9. Kieber JJ, Rothenberg M, Roman G, Feldmann KA, Ecker JR: CTR1, a negative regulator of the ethylene response pathway in ***Arabidopsis***, encodes a member of the raf family of protein kinases. *Cell* 1993, 72:427-441.

10. • Ichimura K, Mizoguchi T, Yoshida R, Yuasa T, Shinozaki K: Various abiotic stresses rapidly activate ***Arabidopsis*** MAP kinases AtMPK4 and AtMPK6. *Plant J* 2000, 24:655-665.

The authors provide a thorough and systematic characterization of abiotic-stress-induced plant MAPK activation using kinase-specific antibodies in *Arabidopsis*. The yeast two-hybrid interaction analysis reported in [25] and the activation kinetics shown here suggest that AtMPK4 and AtMPK6 are activated through different signaling pathways.

11. •• Kovtun Y, Chiu WL, Tena G, Sheen J: Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. *Proc Natl Acad Sci USA* 2000, 97:2940-2945.

This paper describes the first *in vivo* functional analysis of a plant MAPK pathway that connects the H_2O_2 stress signal to both positively and negatively regulated target genes. The use of epitope-tagged kinases facilitates the analysis of functionally redundant MAPKKK genes. Analysis of transgenic plants reveals a role for H_2O_2-activated MAPK signaling in multiple stress tolerance.

12. •• Frye CA, Tang D, Innes RW: Negative regulation of defense responses in plants by a conserved MAPKK kinase. *Proc Natl Acad Sci USA* 2001, 98:373-378.

This study describes the isolation of a MAPK-signaling mutant in pathogen defense by a classic mutant screening technique. Because the mutant appears normal in the absence of pathogens but displays enhanced SA/NPR1-dependent resistance, understanding the molecular basis of this resistance may lead to significant commercial applications.

13. •• Nishihama R, Ishikawa M, Araki S, Soyano T, Asada T, Machida Y: The NPK1 mitogen-activated protein kinase kinase kinase is a regulator of cell-plate formation in plant cytokinesis. *Genes Dev* 2001, 15:352-363.

It has previously been shown that MAPK activation and localization is correlated with cytokinesis in tobacco and alfalfa cells [20,21]. This study presents strong functional evidence to show that the activity of tobacco NPK1 is essential for cell-plate lateral growth in cytokinesis by using a dominant-negative version of NPK1 under an inducible promoter. Convincing evidence is shown in both cultured cells and transgenic plants.

14. Mizoguchi T, Gotoh Y, Nishida E, Yamaguchi-Shinozaki K, Hayashida N, Iwasaki T, Kamada H, Shinozaki K: Characterization of two cDNAs that encode MAP kinase homologues in ***Arabidopsis thaliana*** and analysis of the possible role of auxin in activating such kinase activities in cultured cells. *Plant J* 1994, 5:111-122.

15. Tena G, Renaudin JP: Cytosolic acidification but not auxin at physiological concentration is an activator of MAP kinases in tobacco cells. *Plant J* 1998, 16:173-182.

16. • Mockaitis K, Howell SH: Auxin induces mitogenic activated protein kinase (MAPK) activation in roots of ***Arabidopsis*** seedlings. *Plant J* 2000, 24:785-796.

This is an interesting report that shows the rapid and transient increase of a MAPK-like activity after auxin treatment using *Arabidopsis* seedling roots. Pharmacological data suggest the involvement of both positive and negative MAPK pathways in the auxin responses.

17. Kovtun Y, Chiu WL, Zeng W, Sheen J: Suppression of auxin signal transduction by a MAPK cascade in higher plants. *Nature* 1998, 395:716-720.

18. Bleecker AB, Kende H: Ethylene: a gaseous signal molecule in plants. *Annu Rev Cell Dev Biol* 2000, 16:1-18.

19. Novikova GV, Moshkov IE, Smith AR, Hall MA: The effect of ethylene on MAP kinase-like activity in ***Arabidopsis thaliana***. *FEBS Lett* 2000, 474:29-32.

20. Bogre L, Calderini O, Binarova P, Mattauch M, Till S, Kiegerl S, Jonak C, Pollaschek C, Barker P, Huskisson NS *et al.*: A MAP kinase is activated late in plant mitosis and becomes localized to the plane of cell division. *Plant Cell* 1999, 11:101-113.

21. Calderini O, Glab N, Bergounioux C, Heberle-Bors E, Wilson C: A novel tobacco MAP kinase kinase, NtMEK1, activates the cell cycle-regulated p43Ntf6 MAP kinase. *J Biol Chem* 2001, 276:18139-18145.

22. Shinozaki K, Yamaguchi-Shinozaki K: Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signaling pathways. *Curr Opin Plant Biol* 2000, 3:217-223.

23. Zhu JK: Plant salt tolerance. *Trends Plant Sci* 2001, 6:66-71.

24. Orozco-Cardenas ML, Narvaez-Vasquez J, Ryan CA: Hydrogen peroxide acts as a second messenger for the induction of defense genes in tobacco plants in response to wounding, systemin, and methyl jasmonate. *Plant Cell* 2001, 13:179-191.

25. Ichimura K, Mizoguchi T, Irie K, Morris P, Giraudat J, Matsumoto K, Shinozaki K: Isolation of AtMEKK1 (a MAP kinase kinase kinase)-interacting proteins and analysis of a MAP kinase cascade in ***Arabidopsis***. *Biochem Biophys Res Commun* 1998, 253:532-543.

26. Huang Y, Li H, Gupta R, Morris P, Luan S, Kieber JJ: AtMPK4, an ***Arabidopsis*** homolog of mitogen-activated protein kinase, is activated ***in vitro*** by AtMEK1 through threonine phosphorylation. *Plant Physiol* 2000, 122:1301-1310.

27. • Kiegerl S, Cardinale F, Siligan C, Gross A, Baudouin E, Liwosz A, Eklof S, Till S, Bogre L, Hirt H *et al.*: SIMKK, a mitogen-activated protein kinase (MAPK) kinase, is a specific activator of the salt stress-induced MAPK, SIMK. *Plant Cell* 2000, 12:2247-2258.

This report describes the successful reconstitution in plant cells of MAPKK–MAPK interaction identified through yeast two-hybrid screening. This work, and another successful example [40••], encourages the use of the screening to organize putative plant MAPK cascades.

28. Mikolajczyk M, Awotunde O, Muszynska G, Klessig D, Dobrowolska G: Osmotic stress induces rapid activation of a salicylic acid-induced protein kinase and a homolog of protein kinase ASK1 in tobacco cells. *Plant Cell* 2000, 12:165-178.

29. Bowler C, Fluhr R: The role of calcium and activated oxygens as signals for controlling cross-tolerance. *Trends Plant Sci* 2000, 5:241-246.

30. Seo S, Sano H, Ohashi Y: Jasmonate-based wound signal transduction requires activation of WIPK, a tobacco mitogen-activated protein kinase. *Plant Cell* 1999, 11:289-298.

31. Samuel MA, Miles GP, Ellis BE: Ozone treatment rapidly activates MAP kinase signaling in plants. *Plant J* 2000, 22:367-376.

32. • Ulm R, Revenkova E, di Sansebastiano GP, Bechtold N, Paszkowski J: Mitogen-activated protein kinase phosphatase is required for genotoxic stress relief in ***Arabidopsis***. *Genes Dev* 2001, 15:699-709.

This important paper reports the unique *Arabidopsis* mutant lacking a dual specificity MAPK phosphatase, AtMKP1. In the absence of AtMKP1, plants are hypersensitive to DNA-damaging agents. AtMKP1 seems to act specifically on the MAPK cascade activated by genotoxic stress as *mkp1* responds to other stress signals in the same way as wild-type plants.

33. Zhang S, Klessig DF: Resistance gene N-mediated ***de novo*** synthesis and activation of a tobacco mitogen-activated protein kinase by tobacco mosaic virus infection. *Proc Natl Acad Sci USA* 1998, 95:7433-7438.

34. Romeis T, Piedras P, Zhang S, Klessig DF, Hirt H, Jones JDG: Rapid Avr9- and Cf-9-dependent activation of MAP kinases in tobacco cell cultures and leaves: convergence of resistance gene, elicitor, wound, and salicylate responses. *Plant Cell* 1999, 11:273-287.

35. Zhang S, Klessig DF: Pathogen-induced MAP kinases in tobacco. In *Results and Problems in Cell Differentiation: MAP Kinases in Plant Signal Transduction*. Edited by Hirt H. Heidelberg: Springer; 2000:65-84.

36. Seo S, Ohashi Y: Mitogen-activated protein kinases and wound stress. In *Results and Problems in Cell Differentiation: MAP Kinases in Plant Signal Transduction*. Edited by Hirt H. Heidelberg: Springer; 2000:53-63.

37. Droillard MJ, Thibivilliers S, Cazale AC, Barbier-Brygoo H, Lauriere C: Protein kinases induced by osmotic stresses and elicitor molecules in tobacco cell suspensions: two crossroad MAP kinases and one osmoregulation-specific protein kinase. *FEBS Lett* 2000, 474:217-222.

38. Cardinale F, Jonak C, Ligterink W, Niehaus K, Boller T, Hirt H: Differential activation of four specific MAPK pathways by distinct elicitors. *J Biol Chem* 2000, 275:36734-36740.

39. Nuhse TS, Peck SC, Hirt H, Boller T: Microbial elicitors induce activation and dual phosphorylation of the ***Arabidopsis thaliana*** MAPK 6. *J Biol Chem* 2000, 275:7521-7526.

40. •• Yang KY, Liu Y, Zhang S: Activation of a mitogen-activated protein kinase pathway is involved in disease resistance in tobacco. *Proc Natl Acad Sci USA* 2001, 98:741-746.
Because SIPK and WIPK are widely studied, the newly identified MAPKK that activates these MAPKs will be a useful tool for many researchers in investigating the roles of the MAPKs in both biotic and abiotic stress signaling. This study represents a major step towards understanding the molecular mechanism and significance of hypersensitive cell death in pathogen resistance.

41. Grant JJ, Yun B, Loake GJ: Oxidative burst and cognate redox signaling reported by luciferase imaging: identification of a signal network that functions independently of ethylene, SA and Me-JA but is dependent on MAPKK activity. *Plant J* 2000, 24:569-582.

42. •• Gomez-Gomez L, Boller T: FLS2: An LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in ***Arabidopsis***. *Mol Cell* 2000, 5:1003-1011.
This study provides a major breakthrough in our understanding of the perception mechanism of a general elicitor flg22, an oligopeptide derived from the most conserved region of bacterial flagellin proteins. Because flg22 induces the expression of defense genes and the activation of AtMPK6 in *Arabidopsis* cells (see [39]), the finding may lead to the identification of a link between plant receptor-like kinases and MAPK signaling.

43. •• Petersen M, Brodersen P, Naested H, Andreasson E, Lindhart U, Johansen B, Nielsen H, Lacy M, Austin MJ, Parker JE *et al.*: ***Arabidopsis*** MAP kinase 4 negatively regulates systemic acquired resistance. *Cell* 2000, 103:1111-1120.
The first transposon-tagged recessive MAPK mutant is isolated and characterized in *Arabidopsis*. The *mpk4* mutant exhibits SA-dependent, but NPR1-independent, constitutive SAR without spontaneous necrotic lesions. The mutant also shows dwarfism caused by decreased cell size and has flowers with reduced pollen production and fertility. Microarray analysis shows that only defense-related transcripts are affected by the mutation, suggesting that the constitutive SAR phenotype is not caused by a pleiotropic effect.

44. • Bent AF: Plant mitogen-activated protein kinase cascades: negative regulatory roles turn out positive. *Proc Natl Acad Sci USA* 2001, 98:784-786.
An excellent commentary that points out the interrelated nature of plant MAPK cascades and the difficulty in interpreting the results obtained with MAPK-related mutants.

45. Tonks NK, Neel BG: Combinatorial control of the specificity of protein tyrosine phosphatases. *Curr Opin Cell Biol* 2001, 13:182-195.

46. Meskiene I, Bogre L, Glaser W, Balog J, Brandstotter M, Zwerger K, Ammerer G, Hirt H: MP2C, a plant protein phosphatase 2C, functions as a negative regulator of mitogen-activated protein kinase pathways in yeast and plants. *Proc Natl Acad Sci USA* 1998, 95:1938-1943.

47. Xu Q, Fu HH, Gupta R, Luan S: Molecular characterization of a tyrosine-specific protein phosphatase encoded by a stress-responsive gene in ***Arabidopsis***. *Plant Cell* 1998, 10:849-857.

48. Gupta R, Huang Y, Kieber J, Luan S: Identification of a dual-specificity protein phosphatase that inactivates a MAP kinase from ***Arabidopsis***. *Plant J* 1998, 16:581-589.

49. Lewis TS, Hunt JB, Aveline LD, Jonscher KR, Louie DF, Yeh JM, Nahreini TS, Resing KA, Ahn NG: Identification of novel MAP kinase pathway signaling targets by functional proteomics and mass spectrometry. *Mol Cell* 2000, 6:1343-1354.