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Protein kinase signaling networks in plant innate immunity

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Abstract

In plants and animals, innate immunity is triggered through pattern recognition receptors (PRRs) in response to microbe-associated molecular patterns (MAMPs) to provide the first line of inducible defense. Plant receptor protein kinases (RPKs) represent the main plasma membrane PRRs perceiving diverse MAMPs. RPKs also recognize secondary danger-inducible plant peptides and cell-wall signals. Both types of RPKs trigger rapid and convergent downstream signaling networks controlled by calcium-activated PKs and mitogen-activated PK (MAPK) cascades. These PK signaling networks serve specific and overlapping roles in controlling the activities and synthesis of a plethora of transcription factors (TFs), enzymes, hormones, peptides and antimicrobial chemicals, contributing to resistance against bacteria, oomycetes and fungi.

Introduction

In the past century, research on plant immunity was mainly focused on microbial pathogens causing devastating plant diseases and plant resistance (R) genes conferring specific protection in narrow host range [1]. The fact that most plants are resistant to most microbial invasions was unexplained, despite ample knowledge that diverse microbial and non-pathogenic signals activate rapid plant signaling including protein phosphorylation [2**]. Identification of *Arabidopsis* FLS2 RPK as the bacterial flagellin PRR provided the first evidence for MAMP perception by surface receptors in plant innate immune signaling, analogous to the toll-like receptor signaling in mammals and humans [2**,3]. Critical experiments demonstrated that PRR signaling confers plant resistance to diverse pathogens [2**,4*], and non-host bacteria proliferate when signaling via multiple MAMP receptors is blocked [5]. Recent studies have identified new RPKs recognizing MAMPs and plant-derived signals that elicit conserved, dynamic and differential signaling processes leading to broad-spectrum microbial resistance. The earliest protein phosphorylation events directly linked to RPKs and associated PK signaling partners are beginning to be elucidated.

Although genetic screens were successful in the identification of specific PRRs, functional dissection of the primary signaling events involving protein phosphorylation requires integrated experimental approaches combining cellular and biochemical analysis, functional genomic screens, phosphoproteomics, and genetics [6–17**]. This review highlights the latest progress in unraveling the dynamic and complex PK networks in plant innate immunity. Emphasis is placed on the primary and transient signaling events closely linked to RPKs that initiate a spectrum of downstream signaling cascades. Long-term and late

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responses connected to calcium-activated PKs and MAPK cascades are also integrated into the networks.

RPK signaling

RPKs for MAMPs and plant-derived signals

The best characterized plant PRRs are leucine-rich repeat (LRR) RPKs, including FLS2 recognizing a conserved 22 amino-acid peptide (flg22) in bacterial flagellin and EFR binding to 18 amino-acid epitope (elf18) of bacterial elongation factor EF-Tu [2**,4*]. The rice LRR-RPK XA21 shares similar structure and is activated by a sulphated 17-amino acid peptide (AxY^S22) conserved in strains of *Xanthomonas* [18**]. CERK1 belongs to a distinct subfamily of RLK (receptor-like kinase) with LysM motifs for glycan binding in the N-terminal ectodomain, and is required for immune signaling triggered by fungal chitin [19,20]. Interestingly, both CERK1 and another *Arabidopsis* LysM protein (LYM3) lacking the kinase domain bind bacterial peptidoglycans (PGNs) and contribute to PGN perception and bacterial immunity. An *Arabidopsis* type III chitinase may process PGNs to facilitate ligand delivery and perception (T Nürnberger *et al.*, personal communication) (Figure 1). MAMP perception and RPK signaling could be tissue-specific as revealed by an interesting study demonstrating differential flg22, PGN and chitin responses in distinct root zones and root insensitivity to elf26 [21].

Besides MAMPs, plant-derived peptides and cell wall fragments, acting as secondary danger signals to prolong or amplify immune responses, are also perceived by RPKs to enhance resistance to bacterial and fungal pathogens. Wounding, stress hormones and MAMPs significantly activate *Arabidopsis* *PROPEP* genes [2**,22**] encoding the secreted peptides (PEP1–6) that bind redundant LRR-RPKs PEPR1,2 to trigger immune signaling [23,24*]. Wall-associated RPKs (WAK1,2) bind pectin and oligogalacturonides (OGs) and modulate both immunity and development [25*,26] (Figure 1).

Although MAMPs and plant-derived danger signals trigger conserved innate immune signaling, these ligands are recognized by distinct RPKs that may also specify distinct kinetics and outputs in downstream responses. Analyses of chimeric RPKs indicate that the cytoplasmic kinase domains of FLS2 and EFR are functionally equivalent [25*,27]. However, OGs signaling displays unique features, including brief MAPK activation, no ethylene (ET) release and reduced gene activation. Chimeric RPKs with EFR and WAK1 kinase domains recapitulate RPK signaling specificity [25*]. Comprehensive studies with chimeric LysM RPKs consisting of *Arabidopsis* chitin receptor CERK1 and the *Lotus* Nod factor RPKs (NFR1) demonstrate that limited alterations in the kinase domain of LysM RPKs specify their predominant functions in signaling for nodulation or defense [19].

Unexpectedly, new findings provide evidence that the CLV3 (CLAVATA3) peptide expressed and secreted from the stem cells in the shoot apical meristem (SAM) binds and activates FLS2. The *fls2* and *clv3* mutants abolish the robust and unique immunity in the SAM. CLV3p-FLS2 signaling acts independently from the stem cell signaling pathway mediated through CLV1 and CLV2 receptors, and is uncoupled from the FLS2-mediated growth suppression. Endogenous CLV3p perception in the SAM by FLS2 breaks the previously defined self and nonself discrimination in innate immunity. The dual CLV3p perceptions illustrate co-evolution of plant peptide and RPK signaling for both development and immunity [22**].

Novel RLK signaling

By screening 200 *Arabidopsis* mutants for genes activated 48 hours after bacterial pathogen inoculation, a lethal *bir1* mutant for LRR-RLK at 22°C was isolated. The *bir1* plant displays

constitutive long-term immune responses, including elevated SA (Salicylic Acid), H₂O₂, *PR1,2* expression, and cell death, which are suppressed at 27°C and by the *sobir1* mutation in another LRR-RLK. *SOBIR1* overexpression activates cell death and long-term defense responses. BIR1 interacts with redundant LRR-RLKs, BAK1 and BKK1 [28*]. The *bak1 bkk1* double mutant is lethal under light [29], but independently of temperature, suggesting additional BAK1/BKK1 functions [30]. A semidominant mutant of SNC4, a unique RLK with putative glycerophosphoryl diester phosphodiesterase domains, activates SA, JA (Jasmonic Acid), H₂O₂ and cell death via known immune regulators, NDR1, MPK4 substrate MKS1, and JA biosynthesis gene *OPR3* [31]. It remains to be determined whether BIR1, SOBIR1 or SNC4 recognize any ligands with short ectodomains, and how they prevent or promote autoimmunity. Although most RPK and RLK mutations affect general defense signaling and broad pathogen resistance, the mutation in FERONIA (FER), a CrRLK, leads to spontaneous H₂O₂ and cell death, specific powdery mildew resistance and aberrant pollen tube reception, unraveling unforeseen similarities between these distinct processes [32] (Figure 1).

RPK complex phosphorylation and signaling

The earliest MAMP signaling event is the robust FLS2 and BAK1 association within 1 second. Although flg22 binding is normal in *bak1*, multiple conserved MAMP responses, including MAPK activation, oxidative burst, gene expression, growth repression and pathogen immunity, are compromised [2**,16**,30]. A valuable cell system enabled *in vivo* [³³P]-phosphate pulse labeling to demonstrate the phosphorylation of FLS2-BAK1 complex within seconds. Significantly, BAK1 phosphorylation is shared in FLS2, EFR and PEPR1 signaling [16**] (Figure 1). Paradigm-shifting findings validate BAK1 as a dual-specificity PK and uncover specific T450 requirement in flg22-mediated growth repression uncoupled from BR signaling [33], whereas Y610 differentially modulates some defense gene expression and immunity [34]. The recent isolation of novel *bak1* mutant alleles, *bak1-5* and *bak1^{elg}*, provide exciting new tools for dissecting the differential roles of BAK1 in plant growth, cell death and innate immunity [35,36].

Immediately after FLS2-BAK1 activation, *Arabidopsis* BIK1 (a receptor-like cytoplasmic kinase) plays a pivotal role in MAMP signaling via multiple RPKs. BIK1 interacts with FLS2 and BAK1, and flg22 triggers FLS2- and BAK1-dependent BIK1 phosphorylation [12**,17**]. BAK1 phosphorylates BIK1 at T237, which is critical for BIK1 functions [12**]. S236 is also important for BIK1 activity [17**]. Moreover, BIK1 phosphorylates the kinase domains of FLS2 and BAK1 [12**], and BIK1-FLS2 interaction decreases after flg22 signaling [12**,17**] (Figure 1). Whether FLS2 phosphorylates BIK1, how BIK1 is involved in chitin signaling without BAK1 [17**,30], whether T242, essential for fungal defense [37], functions in MAMP signaling, and what the BIK1 targets are, remain questions that deserve further investigation.

Quantitative mass spectrometry, co-immunoprecipitation and yeast-two-hybrid methods have identified new components in RPK complexes, including *Arabidopsis* H⁺-ATPase, FER [10], SCD1 (Stomatal Cytokinesis-Defective1) [11], and rice ATPase XB24 [38] (Figure 1). Mutant and overexpression studies seem to suggest their often complex and negative roles in RPK signaling. Their further characterizations promise to unveil the molecular and biochemical mechanisms underlying negative control of RPK signaling.

Ca²⁺ and PK signaling

Ca²⁺ signals and early events

Cytosolic Ca²⁺ rise is one of the primary events in plant innate immunity. Diverse MAMPs elicit different Ca²⁺ signatures in amplitude and duration that could contribute to some

specificity in downstream responses despite a strong overlap in MAMP-induced gene regulation [39,40]. Reflecting the reality of multiple MAMP releases by microbes simultaneously, it is noteworthy that flg22/elf18 and flg22/LOS (Lipo-OligoSaccharide) combinations amplify Ca^{2+} signals to potentiate plant defenses [39]. How MAMP perception is linked to cytosolic Ca^{2+} elevation remains elusive [41]. An exciting study shows that *Arabidopsis* PEP2/3 activate PEPR1 and its guanylyl cyclase activity to induce CNGC2-dependent Ca^{2+} rise [15**]. However, FLS2 and EFR induction of anion channels requires Ca^{2+} channel activity independently of CNGC2 [9], suggesting the involvement of additional Ca^{2+} channels in MAMP signaling.

Recent research advances suggested that Ca^{2+} signaling via CDPKs (Ca^{2+} -dependent PKs), CBL/CIPKs (Calcineurin B-Like/CBL-Interacting PKs) and calmodulin (CAM) are involved in different aspects of plant immunity (Figure 2). A functional genomic screen identified closely related *Arabidopsis* CPK4, 5, 6, 11 as global regulators of early transcriptional reprogramming in MAMP signaling, acting within 5–30 minutes after elicitation [8**]. These CDPKs also regulate flg22-induced oxidative burst, potentially by directly phosphorylating the NADPH oxidase RBOH like the potato homologs, StCDPK4 and StCDPK5 [42]. Importantly, CDPKs and MAPK cascades differentially or synergistically control conserved early target genes in MAMP signaling [8**]. The identification of the targeted TFs will provide new insights into the CDPKs and MAPK cascades interplay. Moreover, Ca^{2+} blockers reduce MAPK activation by flg22 independently of CPK5, 6, 11 [8**], suggesting that other Ca^{2+} sensors also modulate MAPK cascades. The *in vitro* CAM activation of MAPK phosphatase1 (MKP1) [43] that inhibits MPK6 [44**] reveals more complex interplays between Ca^{2+} signaling and MAPKs, an interesting challenge for future research.

Complex late Ca^{2+} and PK signaling

Several studies indicated that SA elevation occurring late in immune responses is modulated by different Ca^{2+} sensors in a complex manner. *Arabidopsis* CPK1 overexpression promotes SA accumulation and *PR* genes expression [45] while the CAM-binding transcription activator CAMTA3 (also called AtSR1) inhibits these responses and represses *WKY33* and *WKY70* [46,47]. The CAM-binding protein CBP60g specifically contributes to MAMP-induced SA accumulation within 3–9 hours after bacterial inoculation, and is important for resistance to pathogenic bacteria [48]. The key SA-signaling activator NPR1 is phosphorylated and activated by PKS5/CIPK11 to induce *WKY38* and *WKY62* within 4–12 hours after bacterial inoculation [49]. CPK1 overexpression also induces camalexin production. The molecular mechanisms underlying CPK1's defense roles remain to be elucidated from its localization in peroxisomes and lipid bodies [45]. Interestingly, the tobacco homolog of CPK1, NtCDPK2, is phosphorylated by an upstream PK in response to Cf9-Avr9 interaction, adding another layer of regulation in Ca^{2+} signaling [50]. In the late induction phase of rice culture cells exposed to the fungal MAMP TvX/EIX, OsCIPK14/15 promote *PR* gene expression, phytoalexin biosynthesis and cell death, probably through OsCBL4 Ca^{2+} sensor [51]. Integrating these diverse Ca^{2+} interplays on SA elevation from different experimental systems and physiological contexts will be a great future challenge to advance our understanding of Ca^{2+} signaling in innate immunity.

Ca^{2+} in wounding and herbivore signaling

Plant responses to wounding and herbivores share conserved features as MAMP signaling [2**,24*,52]. Novel findings uncover CAM activation of MPK8 with MKK3 co-regulation after wounding to limit ROBHD-dependent H_2O_2 propagation by repressing *ROBHD*, *OXII* and *ZAT12* within 30 minutes [53**]. Based on the *Nicotiana attenuata* model, herbivore attacks activate a MAPK cascade leading to *WKY3/6* induction required for JA accumulation

[52,54]. A screen of 19 *Arabidopsis cpk* mutant leaves exposed to *Spodoptera littoralis* larvae for 24 hours identified CPK3 and CPK13 as regulators of *PDF1.2* expression, likely through heat shock TF HSFB2a without affecting JA and ET levels [55]. Since CPK3 can induce the flg22-responsive gene *NHL10*, it may also be involved in MAMP signaling [8**]. Thus, multiple CPKs from three subgroups play redundant and specific roles in plant defense. Considering the 34 *Arabidopsis* CDPKs, 25 CIPKs and 8 putative CAM-regulated PKs, future research will reveal more Ca^{2+} -regulated PKs involved in plant immunity. These PKs may be activated in different cellular locales by localized Ca^{2+} signals to regulate specific responses or converge to common targets for a fine-tuned regulation of downstream responses. Only few substrates of Ca^{2+} -regulated PKs are known in innate immunity. Their identification will greatly enhance our understanding of Ca^{2+} -mediated PK network in plant defense.

MAPK cascade signaling

The transient activation of MAPK cascades is also one of the first conserved defense responses, starting at 1 minute, suggesting an early triggering role in some of the later metabolic changes and transcriptional reprogramming resulting in resistance. Most research in innate immunity gravitates towards 3 *Arabidopsis* MAPKs, partially redundant MPK3/6 and MPK4, representing the last steps of at least 2 activation cascades. The progress to precisely dissect the roles of these PKs has been slowed by the lethal mutant phenotypes, and their involvement in several fundamental and interconnected pathways in development, cell division, metabolism, hormone regulations and abiotic stresses, covered by excellent reviews [56–59]. Interesting recent findings relevant to innate immunity in *Arabidopsis* are highlighted in temporal relationships.

Primary responses

Pure MAMPs typically induce fast and transient activation of MPK3, MPK4 and MPK6 lasting no more than 1 hour in *Arabidopsis*. Plant signals OGs activate MPK3/6 for less than 10 minutes, which is correlated with a common core of primary defense gene regulation in 1 hour without ET production. Flg22 activates MPK3/6 up to 1 hour and more secondary defense genes for 3–12 hours. Flg22 and PGN, but not OGs and chitin, activate the late SA-mediated *PR1* by 12–24 hours [2**,6,25*,40,60,61]. MAPK activations may need to reach a threshold in duration and magnitude, and combine with Ca^{2+} signaling [8**], to activate secondary and late responses. This threshold is also controlled by phosphatases, as both PP2Cs and MKP1 down-regulate MPK3/6 activity 10–15 minutes after elicitation in protoplasts and leaves [44**,62]. Significantly, *mkp1* seedlings and adult plants are more resistant to pathogenic bacteria via MPK6-specific functions [44**].

A phosphoproteomics approach identified the novel protein PHOS32, which is phosphorylated 4 minutes after flg22 elicitation and is presumably a MPK3/6 target as supported by *in vitro* assays [7]. Agrobacteria-activated MPK3 phosphorylates the bZIP TF VIP1 at S79, and promotes its nuclear translocation within 5 minutes. Besides facilitating T-DNA transfer, VIP1 binds to VIP1 response elements and enhances *MYB44* and *TRXH8* expression within 10–20 minutes after flg22 stimulation. VIP1 may heterodimerize with other TFs to control more primary defense genes [58,63]. The TF ERF104 interacts with MPK6, and its phosphorylation and release in 5–15 minutes requires both flg22 and endogenous ET in protoplasts. *ERF104* overexpression induces a sub-group of defense and stress genes but reduces immunity. As *erf104* plants show similarly compromised immunity, long-term genetic manipulations may cause unpredictable complexity [64*].

Flg22-activated MPK3/6 phosphorylate ACC synthases (ACS2/6) to increase their stability and activity after 15 minutes for ET accumulation detectable by 2 hours. Constitutively

active MKK4/5/9 activate MPK3/6 and ET production [61,65]. Constitutively active MKK9 alone cannot promote primary ET signaling, which requires simultaneous MKK9 activation and CTR1 inhibition by ET to promote the stability and activity of TFs EIN3/EIL1 via bifurcate MAPK pathways [66]. Transient expression of constitutively active MKK4/5/9 mimics transcriptome reprogramming triggered by flg22 within 30–60 minutes. Analyses of conditional mutants support the central roles of MEKK1/MKKs-MKK4/5/9-MPK3/6 cascades in primary transcription regulation [6,8**,67] (G Tena *et al.*, unpublished). *In silico* [66,68] and protein chip [14] analyses have provided a wealth of TF candidates as MPK3/6 substrates for systematic functional validation (Figure 3).

MPK4 is activated by flg22 within 10 minutes [69], and its primary activity is in part regulated by PP2C phosphatases [62]. Nevertheless, the eventually lethal *mpk4* mutant [70] has been used to demonstrate its critical roles in multiple late immune responses, including SA and camalexin accumulation through distinct mechanisms [71*,72]. Future efforts may reveal new aspects of primary MPK4 signaling events in innate immunity by uncoupling it from the long-term and complex *mpk4* phenotypes.

Long-term responses

Extensive genetic studies show that the MEKK1-MKK1/2-MPK4 cascade plays a long-term role negatively regulating temperature-dependent acquired resistance, by keeping in check endogenous levels of SA and ROS (Reactive Oxygen Species), allowing JA and ET responses, and preventing programmed cell death (PCD) [59,69–73]. Unlike MEKK1 and other MKKK (MKK kinase) activation of MPK3/6 and MPK4 that requires kinase activity [5,6,67,74] (G Tena *et al.*, unpublished), the structural presence of MEKK1 but not its kinase activity is sufficient to prevent the long-term and PCD phenotypes [69]. Transgenic plants expressing *NahG* [59] and crosses with *sid2* [75] to block SA accumulation only slightly rescue the *mpk4* and *mkk1,2* morphological defects, indicating that at least two pathways need continuous MPK4 activity or responsiveness for normal plant development. The essential role of MPK4 in cell cycle control may partially explain *mpk4* lethality and growth defects [70]. Microarray analyses with *mekk1*, *mkk1,2* and *mpk4* mutants support their overlapping as well as distinct functions [75,76]. Dissecting the complex functions of MEKK1 and other MKKKs will require new strategies and tools.

Many MPK4 functions are mediated by suppressing its substrate MKS1 as most long-term *mpk4* phenotypes are eliminated in the *mpk4 mks1* double mutant in the absence of stimulation by MAMPs or pathogens. Consistently, constitutive *MKS1* overexpression elevates *PR* gene expression, inhibits growth, enhances resistance to bacterial and oomycete pathogens, but reduces resistance to a fungal pathogen [56,59,71*]. Interestingly, *bir1* shares similar temperature-dependent phenotypes as *mekk1*, *mkk1,2* and *mpk4*, and prevents MPK4 but not MPK3/6 activation by flg22 at 22°C. At 27°C, when BIR1 functions are not required to maintain normal growth and to prevent autoimmunity, MPK4 is accessible to flg22 activation [28*]. MAMP activation of primary MPK4 and MPK3/6 act independently from long-term MPK4 functions [67] (Figure 1, 3).

Uncoupled from SA synthesis, it is suggested that MKS1 phosphorylation by MPK4 leads to the release of MKS1-WKY33 complex to activate the promoter of *PAD3*, a gene indispensable for biosynthesis of the anti-fungal camalexin. Although *wky33* abolishes long-term camalexin accumulation and reduces flg22 induction of *PAD3* and *CYP71A13* at 2 hours, *mks1* does not affect camalexin accumulation [71*,72]. Compelling evidence supports essential roles of MKKKs-MKK4/5/9-MPK3/6 in activating *Arabidopsis* camalexin biosynthesis genes and accumulation [74,77], while OsMKK4-OsMPK6 controls the synthesis of rice phytoalexins [78]. Unlike the transient activation after treatment with pure MAMPs, infection with the fungal pathogen *Botrytis cinerea* induces a strong and

prolonged activation of MPK3/6 that lasts up to 36 hours [74]. This long-term induction is the trigger for camalexin production, which is nearly abolished in a rescued *mpk3,6* double mutant, in which MPK4 is constitutively activated [74]. Recent evidence shows that the long-term induction (6–24 hours) of *WKY33*, and also of *CYP71A13* and *PAD3*, two genes essential for camalexin biosynthesis, is reduced in the *mpk3,6* double mutant. *WKY33* is directly phosphorylated by MPK3/6 and binds to the promoters of *WKY33* and *PAD3*. Importantly, the MPK3/6 phosphorylation sites are necessary for the full *WKY33* function *in vivo* [79**]. Although flg22 activates MPK3/6 and *PAD3* induction in 1 hour, camalexin does not accumulate in leaves [8**,40], but is exuded from roots after 24 hours [21], suggesting additional tissue-specific factors in long-term MPK3/6 activation besides *WKY33* phosphorylation. Interestingly, orthologous tobacco SIPK, NTF4 and WIPK also phosphorylate NbWKY8 closely related to *WKY33*, and activate metabolic genes critical for immunity [80].

Novel findings show that mutations in two MPK phosphatases (MKP1 and PTP1), targeting MPK3/6, are rescued by a null mutation in *SNC1*, which places MAPK regulation upstream of this NB-LRR R-gene [81]. Fungal elicitors induce prolonged activation of tobacco MAPKs orthologous to MPK3/6, which regulate nitric oxide (NO) and ROS via *NOA1* (*NO ASSOCIATED1*) and *RBOHB*, respectively, for differential resistance to pathogens [82]. NO biosynthesis is also modulated by MPK6 phosphorylation of nitrate reductase (*NIA2*) [83] (Figure 3). A previously unexplored role for MPK3 has been established in priming that induces a latent reinforcement of defense and stress early signaling networks after a first exposure with a danger signal. Pre-treatment with an SA analog or infection with avirulent bacteria induces a slow increase in MPK3/6 protein levels that peak after 2–3 days. This elevation of signaling component allows a stronger response when challenged with a later biotic or abiotic stress, compared with naive unprimed plants [84].

Conclusions and perspectives

Recent studies have identified new PRRs, associated PKs and negative regulators. Continued progress will reveal the ligand binding specificity, structural requirements and modifications that link phosphorylation and signaling. It is possible that more RPKs and RLKs are dual-specificity kinases like BAK1 [34]. The RPK complexes and primary signaling events appear to require more components than previously anticipated. To circumvent functional redundancy, complexity and lethality limitations in the analysis of PK functions downstream of RPKs, new experimental strategies are needed to precisely and fully elucidate the dynamic and intertwined PK signaling networks in plant innate immunity. The regulation of different classes of TFs by diverse PKs and their precise target genes, cis-elements and expression kinetics remain to be investigated. Besides the massive transcriptome reprogramming starting in 30 minutes, flg22 induces *PROPEP2/3*, ROS, ET and JA, each with specific time-course, and all of them can in turn induce later MAPKs, stress and hormonal signaling. It is essential to understand the molecular processes connecting transient and short-term MAPK cascade activation within 1–60 minutes to long-term responses observed in mutants or after 3–24 hours, or even a few days of treatment, involving indirect, divergent and peripheral pathways leading to immunity against broad-spectrum microbes, herbivores and pathogens. Network modeling has great potential power to predict and manipulate plant protections against diverse pathogens in a variety of environments [85]. To reach such practical goals in agricultural improvement and environmental protection, comprehensive and accurate data sets are a prerequisite.

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References and recommended reading

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

* of special interest

** of outstanding interest

1. Dodds PN, Rathjen JP. Plant immunity: towards an integrated view of plant-pathogen interactions. *Nat Rev Genet.* 2010; 11:539–548. [PubMed: 20585331]
2. Boller T, Felix G. A Renaissance of Elicitors: Perception of Microbe-Associated Molecular Patterns and Danger Signals by Pattern-Recognition Receptors. *Annu Rev Plant Biol.* 2009; 60:379–406. [PubMed: 19400727] This is a comprehensive review on MAMPs and PRRs with a special emphasis on the time course of events following PRR activation. The model on defense syndrome nicely summarizes plant perceptions of distinct microbial and plant damage-associated signals in evolutionary context and innate immunity.
3. Ronald PC, Beutler B. Plant and Animal Sensors of Conserved Microbial Signatures. *Science.* 2010; 330:1061–1064. [PubMed: 21097929]
4. Lacombe S, Rougon-Cardoso A, Sherwood E, Peeters N, Dahlbeck D, van Esse HP, Smoker M, Rallapalli G, Thomma BPHJ, Staskawicz B, et al. Interfamily transfer of a plant pattern-recognition receptor confers broad-spectrum bacterial resistance. *Nat Biotech.* 2010; 28:365–369. This work provides the most compelling evidence that the activity of a PRR can be transferred among plant families and confers broad-spectrum bacterial resistance in important crops.
5. He P, Shan L, Lin N-C, Martin GB, Kemmerling B, Nürnberger T, Sheen J. Specific Bacterial Suppressors of MAMP Signaling Upstream of MAPKKK in Arabidopsis Innate Immunity. *Cell.* 2006; 125:563–575. [PubMed: 16678099]
6. Asai T, Tena G, Plotnikova J, Willmann MR, Chiu W-L, Gomez-Gomez L, Boller T, Ausubel FM, Sheen J. MAP kinase signalling cascade in Arabidopsis innate immunity. *Nature.* 2002; 415:977–983. [PubMed: 11875555]
7. Merkouropoulos G, Andreasson E, Hess D, Boller T, Peck SC. An Arabidopsis Protein Phosphorylated in Response to Microbial Elicitation, AtPHOS32, Is a Substrate of MAP Kinases 3 and 6. *J Biol Chem.* 2008; 283:10493–10499. [PubMed: 18285339]
8. Boudsocq M, Willmann MR, McCormack M, Lee H, Shan L, He P, Bush J, Cheng S-H, Sheen J. Differential innate immune signalling via Ca²⁺ sensor protein kinases. *Nature.* 2010; 464:418–422. [PubMed: 20164835] The authors identified the first Ca²⁺ sensor protein kinases CDPKs as positive regulators of Ca²⁺-mediated MAMP signaling and revealed differential cross-talks between CDPKs and MAPK cascades controlling early gene reprogramming in plant immunity.
9. Jeworutzki E, Roelfsema MRG, Anshütz U, Krol E, Elzenga JTM, Felix G, Boller T, Hedrich R, Becker D. Early signaling through the Arabidopsis pattern recognition receptors FLS2 and EFR involves Ca²⁺-associated opening of plasma membrane anion channels. *Plant J.* 2010; 62:367–378. [PubMed: 20113440]
10. Keinath NF, Kierszniowska S, Lorek J, Bourdais G, Kessler SA, Shimosato-Asano H, Grossniklaus U, Schulze WX, Robatzek S, Panstruga R. PAMP (Pathogen-associated Molecular Pattern)-induced Changes in Plasma Membrane Compartmentalization Reveal Novel Components of Plant Immunity. *J Biol Chem.* 2010; 285:39140–39149. [PubMed: 20843791]

11. Korasick DA, McMichael C, Walker KA, Anderson JC, Bednarek SY, Heese A. Novel Functions of Stomatal Cytokinesis-Defective 1 (SCD1) in Innate Immune Responses against Bacteria. *J Biol Chem.* 2010; 285:23342–23350. [PubMed: 20472560]
12. Lu D, Wu S, Gao X, Zhang Y, Shan L, He P. A receptor-like cytoplasmic kinase, BIK1, associates with a flagellin receptor complex to initiate plant innate immunity. *Proc Natl Acad Sci U S A.* 2010; 107:496–501. [PubMed: 20018686] This study uncovers a previously unknown function of BIK1, a RLCK, in mediating primary RPK signaling. Surprising bi-directional transphosphorylation activities between BAK1 and BIK1 are demonstrated. Phosphorylated BIK1 dissociates from FLS2 and may participate in multiple downstream signaling.
13. Pitzschke A, Hirt H. Bioinformatic and Systems Biology Tools to Generate Testable Models of Signaling Pathways and Their Targets. *Plant Physiol.* 2010; 152:460–469. [PubMed: 19915012]
14. Popescu SC, Popescu GV, Bachan S, Zhang Z, Gerstein M, Snyder M, Dinesh-Kumar SP. MAPK target networks in *Arabidopsis thaliana* revealed using functional protein microarrays. *Genes Dev.* 2009; 23:80–92. [PubMed: 19095804]
15. Qi Z, Verma R, Gehring C, Yamaguchi Y, Zhao Y, Ryan CA, Berkowitz GA. Ca²⁺ signaling by plant *Arabidopsis thaliana* Pep peptides depends on AtPepR1, a receptor with guanylyl cyclase activity, and cGMP-activated Ca²⁺ channels. *Proc Natl Acad Sci U S A.* 2010; 107:21193–21198. [PubMed: 21088220] The authors established the first link between the guanylyl cyclase activity of PEPR1 responsible for cGMP release and downstream cytosolic calcium rise. PEPR1 activation by PEP3 peptide induces a cytosolic calcium elevation depending on the cGMP-gated Ca²⁺ channel CNGC2 and activates downstream defense genes.
16. Schulze B, Mentzel T, Jehle AK, Mueller K, Beeler S, Boller T, Felix G, Chinchilla D. Rapid Heteromerization and Phosphorylation of Ligand-activated Plant Transmembrane Receptors and Their Associated Kinase BAK1. *J Biol Chem.* 2010; 285:9444–9451. [PubMed: 20103591] Pulse labeling experiments reveal the instantaneous phosphorylation of three LRR-RPKs (FLS2, EFR and PEPR1) and the shared BAK1 in response to distinct peptide signals. This valuable cell system will facilitate detailed biochemical analysis of the regulatory mechanisms and kinetics of RPK activation, and downstream primary phosphorylation events.
17. Zhang J, Li W, Xiang T, Liu Z, Laluk K, Ding X, Zou Y, Gao M, Zhang X, Chen S, et al. Receptor-like Cytoplasmic Kinases Integrate Signaling from Multiple Plant Immune Receptors and Are Targeted by a *Pseudomonas syringae* Effector. *Cell Host Microbe.* 2010; 7:290–301. [PubMed: 20413097] These authors reported the unexpected finding that BIK1 and PBS1-like kinases are critical for ROS generation and callose deposition stimulated by flg22, elf18 and chitin. BIK1 dominant-negative and phosphorylation mutants diminish ROS and *FRK1-LUC* activation by multiple MAMPs. However, the *bik1* plants also exhibit growth retardation and autoimmunity mediated by elevated SA unrelated to MAMP signaling.
18. Lee S-W, Han S-W, Sriyanum M, Park C-J, Seo Y-S, Ronald PC. A Type I-Secreted, Sulfated Peptide Triggers XA21-Mediated Innate Immunity. *Science.* 2009; 326:850–853. [PubMed: 19892983] This work reports the discovery of a 194-amino acid protein designated Ax21 (activator of XA21-mediated immunity) from *Xanthomonas* species as a new MAMP for the rice PRR XA21, a LRR-RPK. A sulfated 17-amino acid synthetic peptide derived from the N-terminal region of Ax21 is sufficient for activity.
19. Nakagawa T, Kaku H, Shimoda Y, Sugiyama A, Shimamura M, Takanashi K, Yazaki K, Aoki T, Shibuya N, Kouchi H. From defense to symbiosis: limited alterations in the kinase domain of LysM receptor-like kinases are crucial for evolution of legume-Rhizobium symbiosis. *Plant J.* 2011; 65:169–180. [PubMed: 21223383]
20. Wan J, Zhang X-C, Neece D, Ramonell KM, Clough S, Kim S-y, Stacey MG, Stacey G. A LysM Receptor-Like Kinase Plays a Critical Role in Chitin Signaling and Fungal Resistance in *Arabidopsis*. *Plant Cell.* 2008; 20:471–481. [PubMed: 18263776]
21. Millet YA, Danna CH, Clay NK, Songnuan W, Simon MD, Werck-Reichhart D, Ausubel FM. Innate immune responses activated in *Arabidopsis* roots by microbe-associated molecular patterns. *Plant Cell.* 2010; 22:973–990. [PubMed: 20348432]
22. Lee H, Chah O-K, Sheen J. Stem-cell-triggered immunity through CLV3p–FLS2 signalling. *Nature.* 2011; 473:376–379. [PubMed: 21499263] The authors made a surprising discovery that CLV3p, expressed and secreted from the stem cells and functioning as a key regulator of stem cell

homeostasis in the *Arabidopsis* SAM, can trigger similar immune signaling as flg22 via FLS2 without growth repression. CLV3p–FLS2 signaling seems to have evolved to provide constitutive immune protection in the SAM but avoid the penalty from potent growth suppression associated with MAMP signaling.

23. Krol E, Mentzel T, Chinchilla D, Boller T, Felix G, Kemmerling B, Postel S, Arens M, Jeworutzki E, Al-Rasheid KAS, et al. Perception of the Arabidopsis Danger Signal Peptide 1 Involves the Pattern Recognition Receptor AtPEPR1 and Its Close Homologue AtPEPR2. *J Biol Chem.* 2010; 285:13471–13479. [PubMed: 20200150]
24. Yamaguchi Y, Huffaker A, Bryan AC, Tax FE, Ryan CA. PEPR2 Is a Second Receptor for the Pep1 and Pep2 Peptides and Contributes to Defense Responses in Arabidopsis. *Plant Cell.* 2010; 22:508–522. [PubMed: 20179141] A thorough study uncovered the induction of *PEPR1/2* transcript levels by wounding, JA, PEPs and MAMPs. Photoaffinity labeling and binding assays clearly demonstrated differential binding affinities of PEPR1 and PEPR2 receptors with a family of plant peptide ligands. Through perception of PEPs, PEPR1 and PEPR2 contribute to defense responses in *Arabidopsis*.
25. Brutus A, Sicilia F, Macone A, Cervone F, De Lorenzo G. A domain swap approach reveals a role of the plant wall-associated kinase 1 (WAK1) as a receptor of oligogalacturonides. *Proc Natl Acad Sci U S A.* 2010; 107:9452–9457. [PubMed: 20439716] By defining the appropriate chimera design for the *Arabidopsis* PRRs, the authors circumvented the functional redundancy limitation of WAKs in genetic studies and showed that WAK1 ectodomain can activate the EFR kinase domain in response to OGs, while the EFR ectodomain activates the WAK1 kinase after elf18 induction. The kinase domains are responsible for distinct specificity in downstream signaling.
26. Kohorn BD, Johansen S, Shishido A, Todorova T, Martinez R, Defeo E, Obregon P. Pectin activation of MAP kinase and gene expression is WAK2 dependent. *Plant J.* 2009; 60:974–982. [PubMed: 19737363]
27. Albert M, Jehle AK, Mueller K, Eisele C, Lipschis M, Felix G. Arabidopsis thaliana Pattern Recognition Receptors for Bacterial Elongation Factor Tu and Flagellin Can Be Combined to Form Functional Chimeric Receptors. *J Biol Chem.* 2010; 285:19035–19042. [PubMed: 20410299]
28. Gao M, Wang X, Wang D, Xu F, Ding X, Zhang Z, Bi D, Cheng YT, Chen S, Li X, et al. Regulation of Cell Death and Innate Immunity by Two Receptor-like Kinases in Arabidopsis. *Cell Host Microbe.* 2009; 6:34–44. [PubMed: 19616764] This study identified novel LRR-RLKs, as both negative (BIR1) and positive (SOBIR1) regulators of cell death and defense responses.
29. He K, Gou X, Powell RA, Yang H, Yuan T, Guo Z, Li J. Receptor-like protein kinases, BAK1 and BKK1, regulate a light-dependent cell-death control pathway. *Plant Signal Behav.* 2008; 3:813–815. [PubMed: 19704566]
30. Shan L, He P, Li J, Heese A, Peck SC, Nürnberger T, Martin GB, Sheen J. Bacterial Effectors Target the Common Signaling Partner BAK1 to Disrupt Multiple MAMP Receptor-Signaling Complexes and Impede Plant Immunity. *Cell Host Microbe.* 2008; 4:17–27. [PubMed: 18621007]
31. Bi D, Cheng YT, Li X, Zhang Y. Activation of Plant Immune Responses by a Gain-of-Function Mutation in an Atypical Receptor-Like Kinase. *Plant Physiol.* 2010; 153:1771–1779. [PubMed: 20508139]
32. Kessler SA, Shimosato-Asano H, Keinath NF, Wuest SE, Ingram G, Panstruga R, Grossniklaus U. Conserved Molecular Components for Pollen Tube Reception and Fungal Invasion. *Science.* 2010; 330:968–971. [PubMed: 21071669]
33. Wang X, Kota U, He K, Blackburn K, Li J, Goshe MB, Huber SC, Clouse SD. Sequential Transphosphorylation of the BRI1/BAK1 Receptor Kinase Complex Impacts Early Events in Brassinosteroid Signaling. *Dev Cell.* 2008; 15:220–235. [PubMed: 18694562]
34. Oh M-H, Wang X, Wu X, Zhao Y, Clouse SD, Huber SC. Autophosphorylation of Tyr-610 in the receptor kinase BAK1 plays a role in brassinosteroid signaling and basal defense gene expression. *Proc Natl Acad Sci U S A.* 2010; 107:17827–17832. [PubMed: 20876109]
35. Jaillais Y, Belkhadir Y, Balsemao-Pires E, Dangel JL, Chory J. Extracellular leucine-rich repeats as a platform for receptor/coreceptor complex formation. *Proc Natl Acad Sci U S A.* 2011; 108:8503–8507. [PubMed: 21464298]

36. Schwessinger B, Roux M, Kadota Y, Ntoukakis V, Sklenar J, Jones A, Zipfel C. Phosphorylation-Dependent Differential Regulation of Plant Growth, Cell Death, and Innate Immunity by the Regulatory Receptor-Like Kinase BAK1. *PLoS Genet.* 2011; 7:e1002046. [PubMed: 21593986]
37. AbuQamar S, Chai M-F, Luo H, Song F, Mengiste T. Tomato Protein Kinase 1b Mediates Signaling of Plant Responses to Necrotrophic Fungi and Insect Herbivory. *Plant Cell.* 2008; 20:1964–1983. [PubMed: 18599583]
38. Chen X, Chern M, Canlas PE, Ruan D, Jiang C, Ronald PC. An ATPase promotes autophosphorylation of the pattern recognition receptor XA21 and inhibits XA21-mediated immunity. *Proc Natl Acad Sci U S A.* 2010; 107:8029–8034. [PubMed: 20385831]
39. Aslam SN, Erbs G, Morrissey KL, Newman M-A, Chinchilla D, Boller T, Molinaro A, Jackson RW, Cooper RM. Microbe-associated molecular pattern (MAMP) signatures, synergy, size and charge: influences on perception or mobility and host defence responses. *Mol Plant Pathol.* 2009; 10:375–387. [PubMed: 19400840]
40. Gust AA, Biswas R, Lenz HD, Rauhut T, Ranf S, Kemmerling B, Götz F, Glawischnig E, Lee J, Felix G, et al. Bacteria-derived Peptidoglycans Constitute Pathogen-associated Molecular Patterns Triggering Innate Immunity in Arabidopsis. *J Biol Chem.* 2007; 282:32338–32348. [PubMed: 17761682]
41. Ma W, Smigel A, Walker RK, Moeder W, Yoshioka K, Berkowitz GA. Leaf Senescence Signaling: The Ca²⁺-Conducting Arabidopsis Cyclic Nucleotide Gated Channel2 Acts through Nitric Oxide to Repress Senescence Programming. *Plant Physiol.* 2010; 154:733–743. [PubMed: 20699402]
42. Kobayashi M, Ohura I, Kawakita K, Yokota N, Fujiwara M, Shimamoto K, Doke N, Yoshioka H. Calcium-Dependent Protein Kinases Regulate the Production of Reactive Oxygen Species by Potato NADPH Oxidase. *Plant Cell.* 2007; 19:1065–1080. [PubMed: 17400895]
43. Lee K, Song EH, Kim HS, Yoo JH, Han HJ, Jung MS, Lee SM, Kim KE, Kim MC, Cho MJ, et al. Regulation of MAPK Phosphatase 1 (AtMKP1) by Calmodulin in Arabidopsis. *J Biol Chem.* 2008; 283:23581–23588. [PubMed: 18579522]
44. Anderson JC, Bartels S, González Besteiro MA, Shahollari B, Ulm R, Peck SC. Arabidopsis MAP Kinase Phosphatase 1, AtMKP1, negatively regulates MPK6-mediated PAMP responses and resistance against bacteria. *Plant J.* 2011 In Press. The authors identified MAPK phosphatase MKP1 as a key negative regulator of MAMP signaling affecting both MPK3 and MPK6 phosphorylation and activation. Surprisingly, only MPK6 is specifically responsible for diverse MAMP responses and bacterial resistance in *mkp1*.
45. Coca M, San Segundo B. AtCPK1 calcium-dependent protein kinase mediates pathogen resistance in Arabidopsis. *Plant J.* 2010; 63:526–540.
46. Du L, Ali GS, Simons KA, Hou J, Yang T, Reddy ASN, Poovaiah BW. Ca²⁺/calmodulin regulates salicylic-acid-mediated plant immunity. *Nature.* 2009; 457:1154–1158. [PubMed: 19122675]
47. Galon Y, Nave R, Boyce JM, Nachmias D, Knight MR, Fromm H. Calmodulin-binding transcription activator (CAMTA) 3 mediates biotic defense responses in Arabidopsis. *FEBS Lett.* 2008; 582:943–948. [PubMed: 18298954]
48. Wang L, Tsuda K, Sato M, Cohen JD, Katagiri F, Glazebrook J. Arabidopsis CaM Binding Protein CBP60g Contributes to MAMP-Induced SA Accumulation and Is Involved in Disease Resistance against *Pseudomonas syringae*. *PLoS Pathog.* 2009; 5:e1000301. [PubMed: 19214217]
49. Xie C, Zhou X, Deng X, Guo Y. PKS5, a SNF1-related kinase, interacts with and phosphorylates NPR1, and modulates expression of WRKY38 and WRKY62. *J Genet Genomics.* 2010; 37:359–369. [PubMed: 20621018]
50. Witte C-P, Keinath N, Dubiella U, Demoulière R, Seal A, Romeis T. Tobacco Calcium-dependent Protein Kinases Are Differentially Phosphorylated in Vivo as Part of a Kinase Cascade That Regulates Stress Response. *J Biol Chem.* 2010; 285:9740–9748. [PubMed: 20118232]
51. Kurusu T, Hamada J, Nokajima H, Kitagawa Y, Kiyoduka M, Takahashi A, Hanamata S, Ohno R, Hayashi T, Okada K, et al. Regulation of Microbe-Associated Molecular Pattern-Induced Hypersensitive Cell Death, Phytoalexin Production, and Defense Gene Expression by Calcineurin B-Like Protein-Interacting Protein Kinases, OsCIPK14/15, in Rice Cultured Cells. *Plant Physiol.* 2010; 153:678–692. [PubMed: 20357140]

52. Bonaventure G, VanDoorn A, Baldwin IT. Herbivore-associated elicitors: FAC signaling and metabolism. *Trends Plant Sci.* 2011 In Press.
53. Takahashi F, Mizoguchi T, Yoshida R, Ichimura K, Shinozaki K. Calmodulin-Dependent Activation of MAP Kinase for ROS Homeostasis in Arabidopsis. *Mol Cell.* 2011; 41:649–660. [PubMed: 21419340] In addition to the classical MAPK activation by a MAPKK, the authors identified a novel mechanism by direct binding of CAMs to MPK8, linking MAPK cascades and Ca²⁺ signaling. This is the first demonstration that MPK8 activation by both CAMs and MKK3 in response to mechanical wounding negatively regulates ROS propagation by repressing *RBOHD*.
54. Skibbe M, Qu N, Galis I, Baldwin IT. Induced Plant Defenses in the Natural Environment: *Nicotiana attenuata* WRKY3 and WRKY6 Coordinate Responses to Herbivory. *Plant Cell.* 2008; 20:1984–2000. [PubMed: 18641266]
55. Kanchiswamy C, Takahashi H, Quadro S, Maffei M, Bossi S, Bertea C, Zebelo S, Muroi A, Ishihama N, Yoshioka H, et al. Regulation of Arabidopsis defense responses against *Spodoptera littoralis* by CPK-mediated calcium signaling. *BMC Plant Biology.* 2010; 10:97. [PubMed: 20504319]
56. Andreasson E, Ellis B. Convergence and specificity in the Arabidopsis MAPK nexus. *Trends Plant Sci.* 2010; 15:106–113. [PubMed: 20047850]
57. Fiil BK, Petersen K, Petersen M, Mundy J. Gene regulation by MAP kinase cascades. *Curr Opin Plant Biol.* 2009; 12:615–621. [PubMed: 19716758]
58. Pitzschke A, Schikora A, Hirt H. MAPK cascade signalling networks in plant defence. *Curr Opin Plant Biol.* 2009; 12:421–426. [PubMed: 19608449]
59. Suarez-Rodriguez M-C, Petersen M, Mundy J. Mitogen-Activated Protein Kinase Signaling in Plants. *Annu Rev Plant Biol.* 2010; 61:621–649. [PubMed: 20441529]
60. Denoux C, Galletti R, Mammarella N, Gopalan S, Werck D, De Lorenzo G, Ferrari S, Ausubel FM, Dewdney J. Activation of Defense Response Pathways by OGs and Flg22 Elicitors in Arabidopsis Seedlings. *Mol Plant.* 2008; 1:423–445. [PubMed: 19825551]
61. Liu Y, Zhang S. Phosphorylation of 1-Aminocyclopropane-1-Carboxylic Acid Synthase by MPK6, a Stress-Responsive Mitogen-Activated Protein Kinase, Induces Ethylene Biosynthesis in Arabidopsis. *Plant Cell.* 2004; 16:3386–3399. [PubMed: 15539472]
62. Umbrasaite J, Schweighofer A, Kazanaviciute V, Magyar Z, Ayatollahi Z, Unterwurzacher V, Choopayak C, Boniecka J, Murray JA, Bogre L, et al. MAPK phosphatase AP2C3 induces ectopic proliferation of epidermal cells leading to stomata development in Arabidopsis. *PLoS One.* 2010; 5:e15357. [PubMed: 21203456]
63. Pitzschke A, Djamei A, Teige M, Hirt H. VIP1 response elements mediate mitogen-activated protein kinase 3-induced stress gene expression. *Proc Natl Acad Sci U S A.* 2009; 106:18414–18419. [PubMed: 19820165]
64. Bethke G, Unthan T, Uhrig JF, Pöschl Y, Gust AA, Scheel D, Lee J. Flg22 regulates the release of an ethylene response factor substrate from MAP kinase 6 in Arabidopsis thaliana via ethylene signaling. *Proc Natl Acad Sci U S A.* 2009; 106:8067–8072. [PubMed: 19416906] This study suggested that flg22 activation of MPK6 and ET are both required for ERF104 release from MPK6 and its access to target genes. Surprisingly, constitutive overexpression of ERF104 activates hundreds of genes with diverse functions in transgenic plants, but reduces immunity to bacteria and fungi. ERF104 may be involved in complex signaling with tight regulations, as altering ERF104 expression in either direction changes the responses.
65. Han L, Li G-J, Yang K-Y, Mao G, Wang R, Liu Y, Zhang S. Mitogen-activated protein kinase 3 and 6 regulate Botrytis cinerea-induced ethylene production in Arabidopsis. *Plant J.* 2010; 64:114–127. [PubMed: 20659280]
66. Yoo S-D, Cho Y-H, Tena G, Xiong Y, Sheen J. Dual control of nuclear EIN3 by bifurcate MAPK cascades in C2H4 signalling. *Nature.* 2008; 451:789–795. [PubMed: 18273012]
67. Sheen J, He P, Shan L, Xiong Y, Tena G, Yoo S-D, Cho Y-H, Boudsocq M, Lee H. Signaling specificity and complexity of MAPK cascades in plant innate immunity. In: Lorito, M.; Woo, S-L.; Sorrento, Scala F., editors. 13th International Congress on Molecular Plant-Microbe Interactions. Vol. vol 6. Italy: 2008.

68. Heazlewood JL, Durek P, Hummel J, Selbig J, Weckwerth W, Walther D, Schulze WX. PhosphAt: a database of phosphorylation sites in *Arabidopsis thaliana* and a plant-specific phosphorylation site predictor. *Nucleic Acids Res.* 2008; 36:D1015–D1021. [PubMed: 17984086]
69. Suarez-Rodriguez MC, Adams-Phillips L, Liu Y, Wang H, Su S-H, Jester PJ, Zhang S, Bent AF, Krysan PJ. MEKK1 Is Required for flg22-Induced MPK4 Activation in *Arabidopsis* Plants. *Plant Physiol.* 2007; 143:661–669. [PubMed: 17142480]
70. Kosetsu K, Matsunaga S, Nakagami H, Colcombet J, Sasabe M, Soyano T, Takahashi Y, Hirt H, Machida Y. The MAP Kinase MPK4 Is Required for Cytokinesis in *Arabidopsis thaliana*. *Plant Cell.* 2010; 22:3778–3790. [PubMed: 21098735]
71. Petersen K, Qiu J-L, Lütje J, Fiil BK, Hansen S, Mundy J, Petersen M. *Arabidopsis* MKS1 Is Involved in Basal Immunity and Requires an Intact N-terminal Domain for Proper Function. *PLoS One.* 2010; 5:e14364. [PubMed: 21203436] The authors demonstrated that the majority of the *mpk4* phenotypes are mediated through its substrate MKS1 as the *mks1 mpk4* double mutant eliminates many long-term immune responses observed in *mpk4*. The N-terminal domains of MKS1 are specifically required for nuclear localization and multiple functions in immunity.
72. Qiu J-L, Fiil BK, Petersen K, Nielsen HB, Botanga CJ, Thorgripsen S, Palma K, Suarez-Rodriguez MC, Sandbech-Clausen S, Lichota J, et al. *Arabidopsis* MAP kinase 4 regulates gene expression through transcription factor release in the nucleus. *EMBO J.* 2008; 27:2214–2221. [PubMed: 18650934]
73. Gao M, Liu J, Bi D, Zhang Z, Cheng F, Chen S, Zhang Y. MEKK1, MKK1/MKK2 and MPK4 function together in a mitogen-activated protein kinase cascade to regulate innate immunity in plants. *Cell Res.* 2008; 18:1190–1198. [PubMed: 18982020]
74. Ren D, Liu Y, Yang K-Y, Han L, Mao G, Glazebrook J, Zhang S. A fungal-responsive MAPK cascade regulates phytoalexin biosynthesis in *Arabidopsis*. *Proc Natl Acad Sci U S A.* 2008; 105:5638–5643. [PubMed: 18378893]
75. Qiu J-L, Zhou L, Yun B-W, Nielsen HB, Fiil BK, Petersen K, MacKinlay J, Loake GJ, Mundy J, Morris PC. *Arabidopsis* Mitogen-Activated Protein Kinase Kinases MKK1 and MKK2 Have Overlapping Functions in Defense Signaling Mediated by MEKK1, MPK4, and MKS1. *Plant Physiol.* 2008; 148:212–222. [PubMed: 18599650]
76. Pitzschke A, Djamei A, Bitton F, Hirt H. A major role of the MEKK1-MKK1/2-MPK4 pathway in ROS signalling. *Mol Plant.* 2009; 2:120–137. [PubMed: 19529823]
77. Su T, Xu J, Li Y, Lei L, Zhao L, Yang H, Feng J, Liu G, Ren D. Glutathione-Indole-3-Acetonitrile Is Required for Camalexin Biosynthesis in *Arabidopsis thaliana*. *Plant Cell.* 2011; 23:364–380. [PubMed: 21239642]
78. Kishi-Kaboshi M, Okada K, Kurimoto L, Murakami S, Umezawa T, Shibuya N, Yamane H, Miyao A, Takatsuji H, Takahashi A, et al. A rice fungal MAMP-responsive MAPK cascade regulates metabolic flow to antimicrobial metabolite synthesis. *Plant J.* 2010; 63:599–612. [PubMed: 20525005]
79. Mao G, Meng X, Liu Y, Zheng Z, Chen Z, Zhang S. Phosphorylation of a WRKY Transcription Factor by Two Pathogen-Responsive MAPKs Drives Phytoalexin Biosynthesis in *Arabidopsis*. *Plant Cell.* 2011 In Press. This work provides compelling *in vitro* and *in vivo* evidence that MPK3 and MPK6 directly phosphorylate the transcription factor WKY33 during *Botrytis cinerea* infection, which supports the long-term biosynthesis of anti-microbial compound camalexin. Moreover, WKY33 directly binds to its own promoter for autoregulation and to the promoter of *PAD3*, one of the camalexin biosynthesis genes.
80. Ishihama N, Yamada R, Yoshioka M, Katou S, Yoshioka H. Phosphorylation of the *Nicotiana benthamiana* WRKY8 Transcription Factor by MAPK Functions in the Defense Response. *Plant Cell.* 2011; 23:1153–1170. [PubMed: 21386030]
81. Bartels S, Anderson JC, González Besteiro MA, Carreri A, Hirt H, Buchala A, Métraux J-P, Peck SC, Ulm R. MAP KINASE PHOSPHATASE1 and PROTEIN TYROSINE PHOSPHATASE1 Are Repressors of Salicylic Acid Synthesis and SNC1-Mediated Responses in *Arabidopsis*. *Plant Cell.* 2009; 21:2884–2897. [PubMed: 19789277]
82. Asai S, Ohta K, Yoshioka H. MAPK Signaling Regulates Nitric Oxide and NADPH Oxidase-Dependent Oxidative Bursts in *Nicotiana benthamiana*. *Plant Cell.* 2008; 20:1390–1406. [PubMed: 18515503]

83. Wang P, Du Y, Li Y, Ren D, Song C-P. Hydrogen Peroxide-Mediated Activation of MAP Kinase 6 Modulates Nitric Oxide Biosynthesis and Signal Transduction in Arabidopsis. *Plant Cell*. 2010; 22:2981–2998. [PubMed: 20870959]
84. Beckers GJM, Jaskiewicz M, Liu Y, Underwood WR, He SY, Zhang S, Conrath U. Mitogen-Activated Protein Kinases 3 and 6 Are Required for Full Priming of Stress Responses in Arabidopsis thaliana. *Plant Cell*. 2009; 21:944–953. [PubMed: 19318610]
85. Sato M, Tsuda K, Wang L, Collier J, Watanabe Y, Glazebrook J, Katagiri F. Network Modeling Reveals Prevalent Negative Regulatory Relationships between Signaling Sectors in Arabidopsis Immune Signaling. *PLoS Pathog*. 2010; 6:e1001011. [PubMed: 20661428]

Figure 1.

RPK network signaling in innate immunity. RPKs perceive MAMPs (red), secondary plant (green) and unknown signals, and activate conserved and convergent Ca²⁺ signaling and MAPK cascades to control the activities and synthesis of a plethora of transcription factors (TFs), enzymes, hormones, ROS, PCD, peptides and antimicrobial chemicals, contributing to plant immunity.

Figure 2.

Ca²⁺ signaling network through multiple PKs in plant immunity. Microbial perception quickly activates Ca²⁺ influx that regulates early signaling events occurring within minutes, including anion efflux, ROS production and gene expression involved in the biosynthesis of antimicrobial chemicals and peptides. These responses mainly mediated through CDPKs are co-regulated by MAPK cascades, that can be further modulated by CAM. Ca²⁺ rise also regulates late responses within hours and days, including the production of SA, phytoalexin, camalexin and other defense compounds through gene regulation. These responses are modulated positively or negatively by CAM, CBL-CIPKs and CDPKs. Most PK substrates are unknown, except the key SA-signaling activator NPR1 which is phosphorylated by *Arabidopsis* CIPK11. Herbivores can be sensed through wounding and herbivore-associated elicitors (HAEs) through unknown receptors to activate MAPK cascades and Ca²⁺ influx. Wounding-activated MPK8 through CAM and MKK3 represses genes to limit H₂O₂ propagation. Other MAPKs, CPK3 and CPK13 induce gene expression to produce antiherbivore molecules in JA-dependent and JA-independent pathways. This complex and fine-tuned Ca²⁺ signaling network contributes to plant resistance to bacteria, oomycetes, fungi and herbivores.

Figure 3.

MAPK networks in MAMP perception downstream of receptors. Fast and transient activation of at least two MAPK cascades induces primary responses (left). Direct targets, phosphorylated in minutes, have been identified for MPK3,6. Modulation of transcription factor (TF) activity by MAPKs induces a massive gene expression reprogramming, ultimately leading to increased resistance to pathogens through various biological responses such as synthesis of antimicrobial peptides and chemicals, programmed cell death (PCD), and production of reactive oxygen species (ROS), nitric oxide (NO) and stress hormones. A long-term activation of MAPKs (center) by microbes also induces biological responses, most notably the accumulation of camalexin through release and direct phosphorylation of WKY33 and modulation of *PAD3* gene in leaves. A continuously active MAPK cascade, consisting of MEKK1 and other MKKKs, MKK1/2 and MPK4 (right), has a sustained requirement to control salicylic acid (SA), PCD, ROS and *PR1* gene levels through the direct phosphorylation of MKS1, and to allow JA and ET responses, independently of MAMP perception. Abbreviations: PP2C, protein phosphatase 2C; *CYP*, cytochrome P450; *PUB*, plant U-box E3-ligase; *GST*, glutathione-S-transferase; *PER*, peroxidase; *OXR*, FAD-binding oxidoreductase; *LOX*, lipoxygenase.