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Protein kinase signaling networks in plant innate immunity

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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.

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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]. Crucial 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 [6,7,8••,9–11,12••,13,14,15••,16••,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 responses connected to calcium-activated PKs and MAPK cascades are also integrated into the networks.

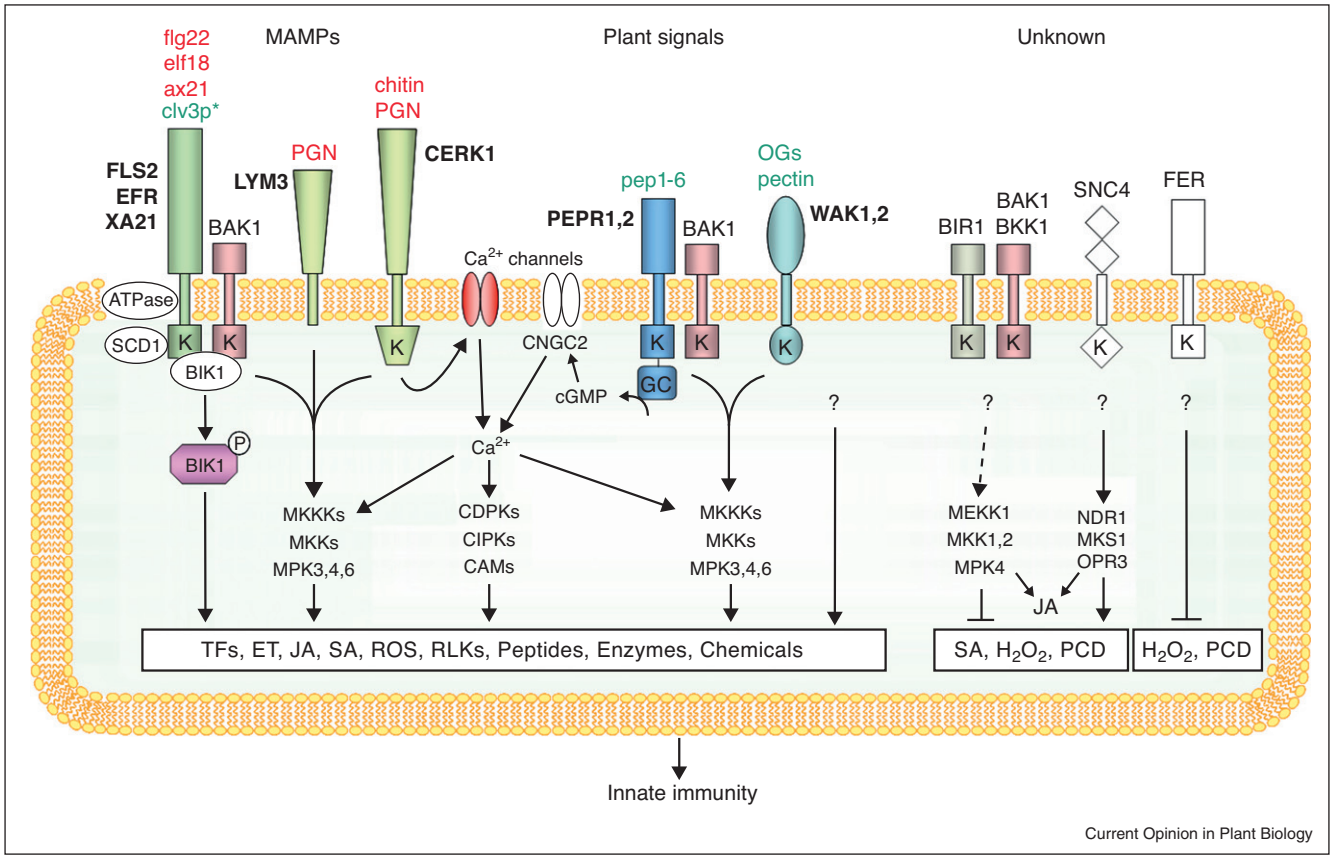
RPK signaling

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 (flg*22*) 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 sulfated 17-amino acid peptide (AxY^{S}*22*) 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

Figure 1



RPK network signaling in innate immunity. RPKs perceive MAMPs (red), secondary plant (green) and unknown signals, and activate conserved and convergent Ca^{2+} 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.

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 h 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), H_2O_2, *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), H_2O_2 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 H_2O_2 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 s. 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* [^{33}P]-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-dependent and BAK1-dependent BIK1 phosphorylation [12••,17••]. BAK1 phosphorylates BIK1 at T237, which is crucial 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.

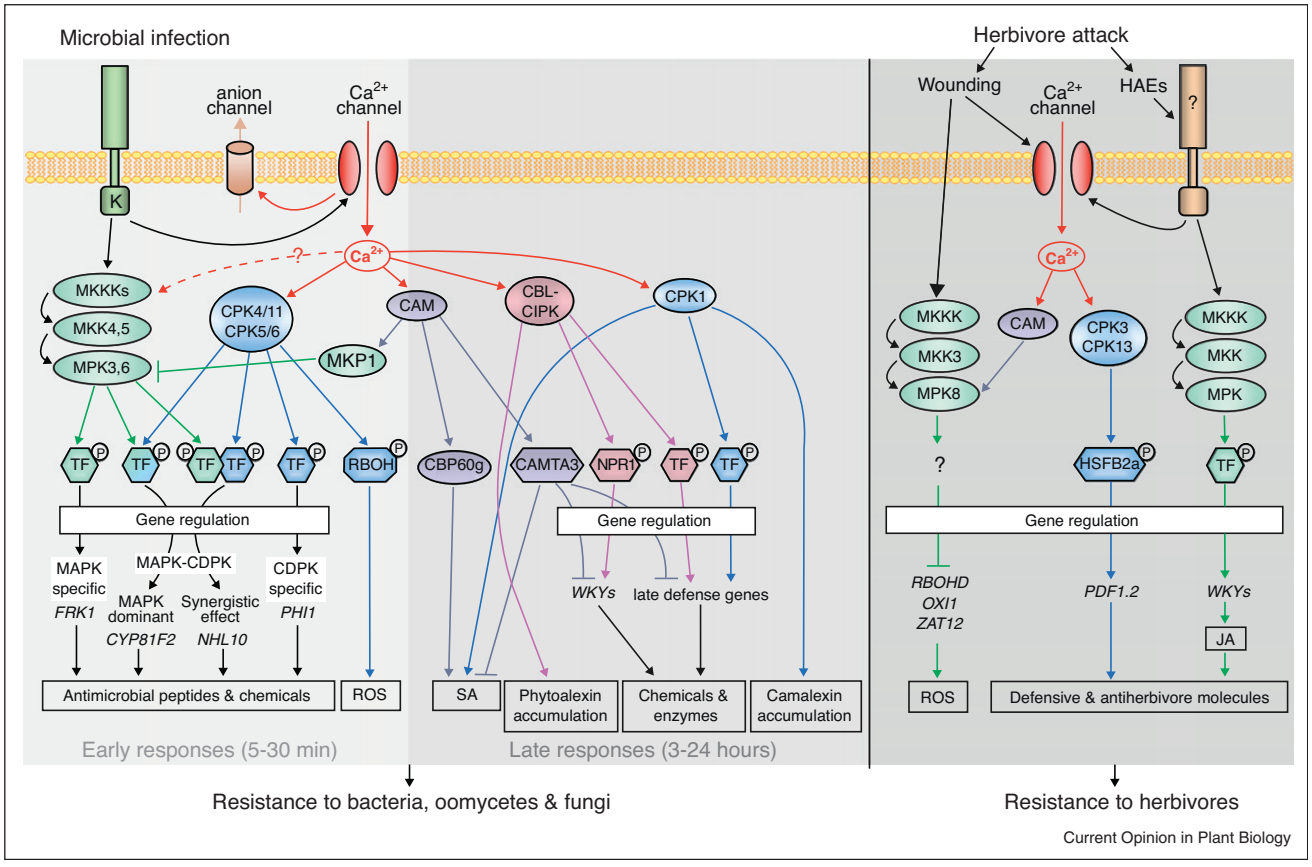
Ca^{2+} and PK signaling

Ca^{2+} signals and early events

Cytosolic Ca^{2+} rise is one of the primary events in plant innate immunity. Diverse MAMPs elicit different Ca^{2+} 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 min 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

Figure 2



Ca^{2+} signaling network through multiple PKs in plant immunity. Microbial perception quickly activates Ca^{2+} 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. Ca^{2+} 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 that is phosphorylated by *Arabidopsis* CIPK11. Herbivores can be sensed through wounding and herbivore-associated elicitors (HAEs) through unknown receptors to activate MAPK cascades and Ca^{2+} influx. Wounding-activated MPK8 through CAM and MKK3 represses genes to limit H_2O_2 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 Ca^{2+} signaling network contributes to plant resistance to bacteria, oomycetes, fungi and herbivores.

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 CBP60 g specifically contributes to MAMP-induced SA accumulation within 3–9 h 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 h 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*, *OXI1* and *ZAT12* within 30 min [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 h identified CPK3 and CPK13 as regulators of *PDF1.2* expression, probably 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 min, 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

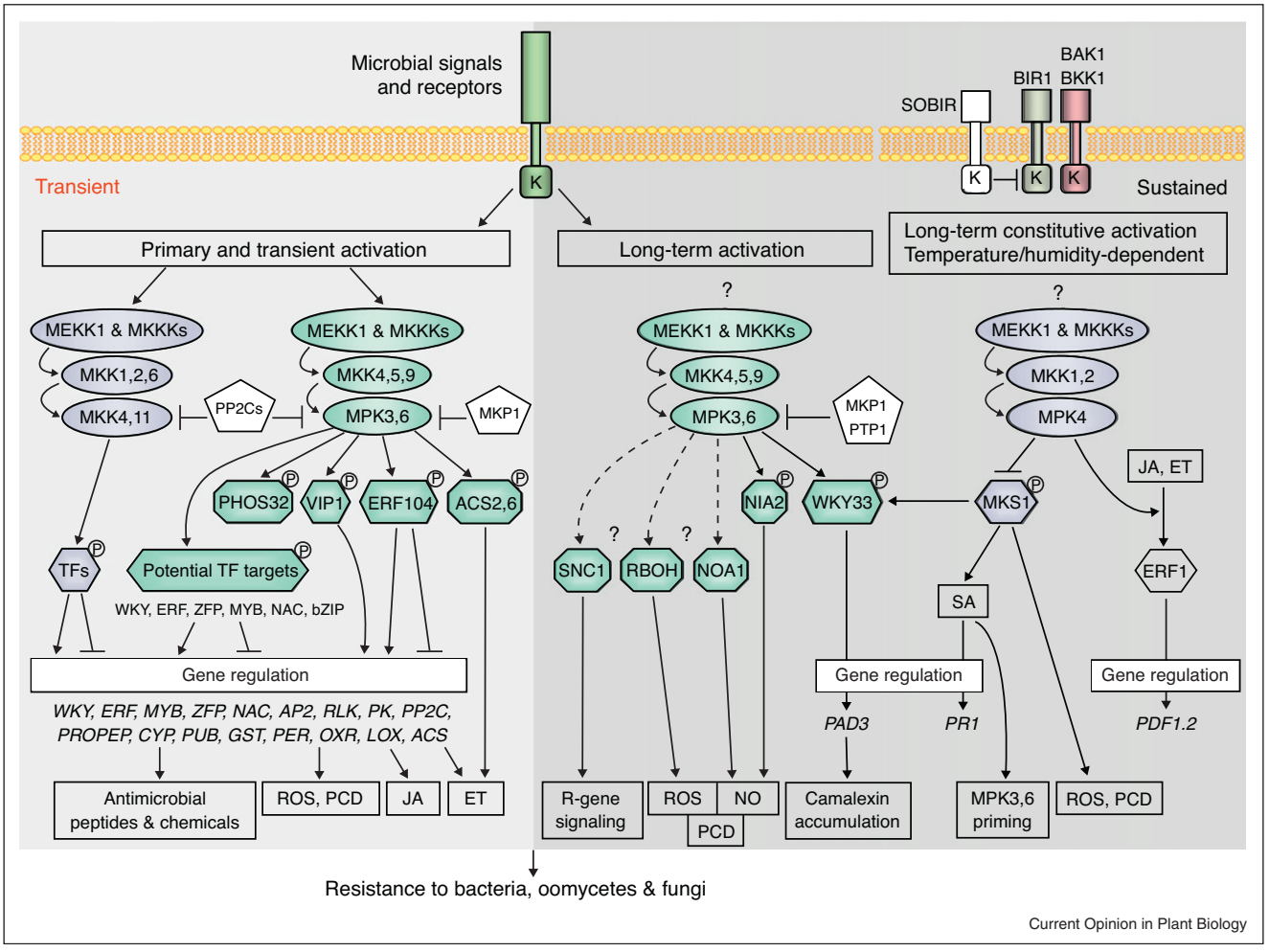
Pure MAMPs typically induce fast and transient activation of MPK3, MPK4 and MPK6 lasting no more than 1 h in *Arabidopsis*. Plant signals OGs activate MPK3/6 for less than 10 min, which is correlated with a common core of primary defense gene regulation in 1 h without ET production. Flg22 activates MPK3/6 up to 1 h and more secondary defense genes for 3–12 h. Flg22 and PGN, but not OGs and chitin, activate the late SA-mediated *PR1* by 12–24 h [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 downregulate MPK3/6 activity 10–15 min 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 min 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 min. Besides facilitating T-DNA transfer, VIP1 binds to VIP1 response elements and enhances *MYB44* and *TRXH8* expression within 10–20 min 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 min requires both flg22 and endogenous ET in protoplasts. *ERF104* overexpression induces a subgroup 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 min for ET accumulation detectable by 2 h. 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 min. Analyses of conditional mutants support the central roles of MEKK1/MKKKs-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 min [69], and its primary activity is partly regulated by PP2C phosphatases [62]. Nevertheless, the eventually lethal *mpk4* mutant [70] has been used to demonstrate its crucial roles in multiple late immune responses, including SA and camalexin

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.

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] (Figures 1 and 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 h, *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 h [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 h) 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 h, camalexin does not accumulate in leaves [8••,40], but is exuded from roots after 24 h [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 crucial 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 min, 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 min to long-term responses observed in mutants or after 3–24 h, 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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