1 Introduction

Plants have evolved complex immune systems to fight against biotic threats, such as pathogen invasion, insect herbivory and weed parasitism. At the cell surface, plasma membrane (PM)-anchored pattern recognition receptors (PRRs) perceive extracellular danger signals either released from attacking pathogens (microbe-associated molecular patterns, MAMPs) or from the plant itself because of cellular damages (damage-associated molecular patterns, DAMPs). DAMPs have been further categorized by Gust et al[1] in two groups: "classic" DAMPs which are passively released upon cell injury (e.g. extracellular ATP (eATP), oligogalacturonides (OGs) and cutin monomers) as well as phytocytokines which are actively produced by the plant in response to imminent danger.

Collectively, perception of MAMPs, DAMPs and phytocytokines by cognate PRRs activate the first stage of the plant immune network, pattern-triggered immunity (PTI), launching sequential phosphorylation-dominated signal transduction events for defense outputs, and ultimately defeating the invaders.

1.1 Self- and non-self-molecular patterns

MAMPs, originally termed pathogen-associated molecular patterns (PAMPs) in 1989, are conserved molecular structures produced by foreign microorganisms[2]. The most intensively studied MAMPs are flg22 (the N-terminal 22-amino acid peptide epitopes from bacterial flagellin)[3], elf18 (the N-acetylated 18-amino acid peptide from bacterial Elongation Factor-Tu)[4], csp22 (the 22-residue N-terminal consensus sequences of bacterial cold shock proteins)[5], and chitin oligosaccharides (a major component of fungal cell walls)[6].

Phytocytokines have been further divided by Hou et al[7] into (1) secreted peptides whose precursor proteins contain a signal peptide: e.g. Hydroxyproline-rich systemin (HypSys)[8], Small Phytocytokines Regulating Defense and Water Loss (SCREW)[9], PAMP-induced secreted peptide 1/2 (PIP1/2)[10], serine-rich endogenous peptide 12 (SCOOP12)[11], phytosulfokines (PSKs)[12], inflorescence deficient in abscission (IDA)[13], Rapid Alkalization Factors (RALFs)[14,15] and Regeneration Factor 1 (REF1)[16]; and (2) non-secreted peptides, whose precursors lack a signal peptide for secretion: e.g. systemin[17], plant elicitor peptide (PEP1)[18], *Z. mays* immune signaling peptide 1 (ZIP1)[19], and soybean GmPEPs[20] (Table 1).

Some above-mentioned danger cues like flg22, chitin, PEPs, SCREWs and RALFs are widely perceived throughout the plant kingdom due to their conserved structures. The recognition of others, however, is restricted to certain plant species. For example, elf18

is only recognized by *Brassicaceae* and the perception of csp22 is restricted to *Solanaceae*. Phytocytokines like SCOOPs are encoded by the *Brassicaceae*-specific *PROSCOOP* gene family, whereas systemin peptides are only found in some members of the *Solanaceae* family, including tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum*), bell pepper (*Capsicum annuum*), and black nightshade (*Solanum nigrum*) (Table 1). Plant species-specific pattern recognition is primarily due to the cognate membrane-bound PRRs, which specifically bind the corresponding ligands to launch plant pattern-triggered immunity.

Table 1 Perception of microbe- and plant-derived molecular patterns and paired PRRs

Category of origins	ligands	Major receptor	PRR type	Co-receptor	Reference
Bacterial flagellin	flg22	FLS2 (*Arabidopsis*)	LRR-RLK	BAK1	21,22
Bacterial flagellin	flgII-28	FLS3 (*Solanaceae*)	LRR-RLK	BAK1	23
Bacterial EF-Tu	elf18	EFR (*Brassicaceae*)	LRR-RLK	BAK1	24,25
Bacterial cell wall	PGN	LYM1/LYM3 (*Arabidopsis*); OsLYP4/OsLYP6 (Rice)	LysM-RLP	CERK1	26,27
Bacterial cold shock protein	csp22	CORE (*Solanaceae*)	LRR-RLK	SlSERK3A/3B (putative)	28,29
Fungal cell wall	chitin	LYK5 (*Arabidopsis*); OsCEBiP (Rice)	LysM-RLK; LysM-RLP	CERK1	30,31
Fungal xylanase	EIX	LeEix1/LeEix2 (Tomato)	LRR-RLP	BAK1	32,33
Botrytis cinerea pectinases	Polygalacturonases (PGs)	RLP42 (*Arabidopsis*)	LRR-RLP	SoBIR1/BAK1	34
Oomycetes	nlp20	RLP23 (*Arabidopsis*)	LRR-RLP	SoBIR1/BAK1	35
Plant cell wall component	OGs	not WAK (*Arabidopsis*)	Unknown	Unknown	36
Plants (*Arabidopsis;* soybean; tomato et al)	AtPEP; GmPEP914; REF1, et al	PEPR (*Arabidopsis*, et al); PORK1 (Tomato)	LRR-RLK	BAK1	16,20,37–39
Maize	ZIP1	Unknown	Unknown	Unknown	19
Tomato	systemin	SYR1	LRR-RLK	SlSERKs	17,29,40
Plants	PIP1/2	RLK7 (*Arabidopsis*)	LRR-RLK	BAK1 (putative)	10
Plants	SCREWs	NUT (*Arabidopsis*)	LRR-RLK	BAK1	9
Solanaceae	HypSys	Unknown	Unknown	Unknown	8
Plants	IDA	HAE, HSL2 (*Arabidopsis*)	LRR-RLK	SERKs	13,41
Plants	RALF1/22/23	FER (*Arabidopsis*)	CrRLK1L	LLG	14,15,42,43
Brassicaceae	SCOOP12	MIK2 (*Arabidopsis*)	LRR-RLK	BAK1	11,44
Plants	PSK	PSKR1 (*Arabidopsis*)	LRR-RLK	BAK1	12,45

1.2 Pattern-triggered immune signaling is initiated by PRR complexes

At the frontline of PTI signaling, cell-surface localized PRRs, namely receptor like kinases (RLKs) or receptor like proteins (RLPs; lacking cytoplasmic kinase domains), are well characterized for their essential roles in signaling initiation shortly after ligand binding. Plant RLKs consist of an extracellular ligand binding domain (ECD), a single-pass transmembrane domain (TM), and an intracellular kinase domain (KD)[46].

1.2.1 LRR-RLKs

LRR-RLKs, whose ECDs contain leucine-rich repeat (LRR) motifs, form the largest RLK family in plants[47]. *Arabidopsis* flagellin-sensing 2 (AtFLS2), one of the most studied LRR-RLKs, recognizes bacterial flagellin by its flg22 epitope and triggers antibacterial immunity[22,48,49]. Once flg22 binds to the extracellular LRR domain of FLS2, the co-receptor BAK1/SERK3 is instantly recruited to form a FLS2-BAK1 heterocomplex[21,50]. The detailed molecular mechanism underlying this flg22-induced FLS2-BAK1 immune complex formation has been elaborated by a crystal structure of the $FLS2^{LRR}$-flg22-$BAK1^{LRR}$ complex, which further revealed that flg22 stabilizes the FLS2-BAK1 heterodimer by serving as a "molecular glue" between their ECDs[51]. After the rapid FLS2-BAK1 heterodimerization, subsequent reciprocal transphosphorylation between their contacting cytosolic KDs kicks off the intracellular signal transduction cascade and finally confers resistance to bacterial pathogens[50].

Besides AtFLS2, orthologs of FLS2 were found to perceive flg22 in tomato (*Solanum lycopersicum*)[52], *Nicotiana benthamiana*[53], rice (*Oryza sativa*)[54], and grapevine (*Vitis vinifera*)[55] as well. In order to combat the polymorphisms of flagellin peptides, tomato has co-evolved a second flagellin receptor, called flagellin-sensing 3 (FLS3), to specifically detect a different flagellin epitope called flgII-28, a 28-amino acids peptide derived from the central region of the flagellin protein[23]. Although SlFLS2 and SlFLS3 share the co-receptor SlBAK1/SlSERK3, some immune outputs of SlFLS3 are different from those of SlFLS2, suggesting that SlFLS2/flg22 and SlFLS3/flgII-28 pathways may use specific molecular mechanisms to activate flagellin-triggered immunity in tomato[56].

PRRs of the LRR-RLK family also sense endogenous plant peptides. The first reported phytocytokine is tomato systemin (TomSys), an 18-amino acids peptide isolated from tomato leaves in 1991[17]. However, it was not until 2018 that the LRR-RLK systemin receptor 1 (SYR1) was identified to be a genuine receptor for systemin perception, as it not only binds systemin with high affinity and specificity, but also engages in defense against mechanical wounding and herbivore attacks [40,57]. SYR2, a low-affinity systemin-binding LRR-RLK that shares 89% sequence identity with SYR1, has recently been

reported to antagonize SYR1-mediated systemin signaling by competing for interaction with the co-receptor SlSERK3A to balance plant defense and growth[57]. Another wound-inducible endogenous plant peptide is Pep1, the first *Arabidopsis* phytocytokine identified in 2006, which is released from the C-terminus of PROPEP1 after the cleavage by Ca^{2+}-dependent METACASPASE4 (MC4)[18,58]. The crystal structure of $PEPR1^{LRR}$-Pep1 suggests a recognition mechanism that is conserved with flg22-mediated FLS2 activation: Binding of Pep1 to $PEPR1^{LRR}$ leads to the PEPR1-BAK1 heterodimerization, which activates subsequent intracellular phosphorylation cascades to enhance disease resistance [38]. Not limited to Arabidopsis, the PEPR/Pep module is widely present in monocots (e.g. maize, rice) and eudicots (e.g. tomato, soybean) and contributes to balancing growth-defense trade-offs [39,59]. The tomato PEPR1/2 ortholog receptor-like kinase1 (PORK1) originally was characterized as a LRR-RLK required for systemin-mediated responses[60]. It is phylogenetically related to systemin receptors, but does not induce systemin-triggered SlSERK3A phosphorylation[29]. Recently, Yang et al discovered that PORK1 is the receptor of the tomato Pep1 ortholog REF1, which controls plant regeneration after wounding[16]. Thus, wound responses, particularly wound-induced defense and tissue repair, are under the tight control of SYR1/systemin and PORK1/REF1 signaling pathways, respectively.

1.2.2 LysM-RLKs

Plant LysM-RLKs, which have three lysin motifs in their ECD, are important for sensing microbial GlcNAc-containing molecules (linear 1,4-β-linked polymers of N-acetyl-D-glucosamine) such as fungal chitin[61]. Chitin perception in Arabidopsis is achieved by a tripartite complex of AtLysM-RLKs, comprising LysM-containing receptor kinase 4 (LYK4), LYK5, and chitin elicitor receptor kinase (CERK1)[30,62,63]. AtLYK5 is the primary chitin receptor because it binds chitin with a much higher affinity than AtCERK1[30]. AtLYK4 and AtLYK5 interact constitutively and act partly redundantly in chitin signaling[30,63,64]. When chitin binds AtLYK5, AtLYK5 and AtCERK1 dimerize and AtCERK1 is activated by auto-phosphorylation[65]. Neither AtLYK4 nor AtLYK5 are enzymatically active kinases, so AtCERK1 is recruited not for chitin binding but rather acts as a co-receptor providing kinase activity to the receptor complex. Thus, chitin-induced AtCERK1 phosphorylation is required for AtLYK5 internalization and to transduce signals to cytosol[30,62–64,66–68].

LysM receptors appear to work differently in crop plants. Rice deploys the chitin elicitor binding protein (OsCEBiP; LysM-RLP)-OsCERK1 complex to sandwich chitin oligomers[31]. Legumes utilize two sets of LysM receptors to discern mutualistic microbes

from pathogens. For example, *Lotus japonicus* uses LjNFR1 and LjNFR5 to perceive Nod factors (acetylated chito-oligosaccharides) as endosymbiosis signals, while long-chain chitin oligomers (e.g. CO6-CO8) are perceived by LjLYS6/LjCERK6 to regulate antifungal immunity[69]. Tomato LYK4 (Solyc02g089900) is highly expressed in fruits. Similar to the mechanism in Arabidopsis, SlLYK4 is a kinase-inactive protein which only binds chitin, and SlCERK1 (as known as SlLYK1; Solyc07g049180) trans-phosphorylates SlLYK4 to mediate chitin signaling[70].

Although the varied compositions of individual PRR complexes have not been fully investigated, a simplified signaling competent unit has been proposed to date, which consists of at least one membrane-bound RLK/RLP for ligand binding, co-RKs for complex activation by phosphorylation, and receptor-like cytoplasmic kinases (RLCKs) for intracellular signal amplification[71] (Fig.1.1).

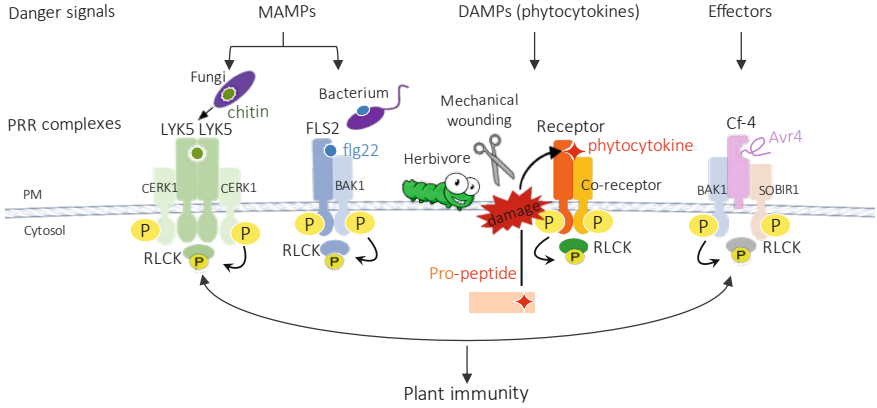

Figure 1.1: A variety of danger signals activate PRR complexes to initiate immune signaling.
Schemes from left to right: MAMPs are perceived by LysM-RLK complexes (e.g. fungal chitin) or by LRR-RLK complexes (e.g. bacteria flagellin); DAMPs including phytocytokines released from the damaged plants, are perceived by their cognate PRR complexes (e.g. SYR1/systemin, FER/RALF). The effector avirulence protein 4 (Avr4) secreted from fungal pathogen *Fulvia fulva* is perceived by Cf4 (tomato RLP)/SOBIR1 complex, which was proposed to be biomolecular equivalent of genuine RLKs[143]. Once major receptors sense ligands, the co-receptors (e.g. BAK1/SERK) are recruited to form receptor-coreceptor heterodimer which allows reciprocal trans-phosphorylation, leading to full activation of PRR complexes. The activated PRR complexes will then phosphorylate downstream RLCKs for signaling amplification and induce plant immune responses. The yellow circle with "P" inside represents phosphorylation.

1.3 RLCKs are activated by PRR complexes through phosphorylation

RLCKs, which function downstream of PRRs, resemble RLKs in structure but lack ECDs[72]. Many RLCKs are attached to the plasma membrane by N-terminal

myristoylation and/or palmitoylation, allowing for interaction with the cytosolic KDs of PRR complexes[73].

One representative and well-studied example is *Arabidopsis* Botrytis-induced kinase 1 (BIK1) from the RLCK-VII family. BIK1 is a central regulator downstream of FLS2, EFR, PEPR and LYK5[74,75]. BIK1 interacts with FLS2-BAK1 in unstimulated plants. Upon flg22 perception, BAK1 phosphorylates BIK1, which in turn phosphorylates both FLS2 and BAK1. Afterwards, the flg22-activated BIK1 dissociates from the receptor complex and regulates other signaling components by phosphorylation[74,76]. In the presence of Pep1, PEPRs directly phosphorylate BIK1 and Pep-PEPRs-BIK1 signaling mediates ethylene-induced immunity[75]. Unlike its role as a positive regulator of RLK-dependent immune signaling, BIK1 negatively regulates RLP23/nlp30-mediated immune activation[77].

In addition to BIK1, the *Arabidopsis* RLCK-VII family includes 45 members which have been assigned to nine subgroups (RLCK VII-1 to RLCK VII-9) based on sequence similarity [78]. Due to functional redundancy of RLCKs in each of these subgroups, Rao et al generated higher order mutants of each subgroup to study their either specific or common functions in plant immunity[78]. It was shown that the RLCK VII-4 subfamily including the PBS1-LIKE 19 (PBL19) and the PTI-compromised receptor-like cytoplasmic kinase 1/2 (PCRK1/2), is required specifically for early chitin-induced responses[78]. PBL27 from RLCK VII-1 was previously reported to selectively regulate chitin-induced MAPK activation[79]. However, neither Rao et al nor Bi et al were able to reproduce this result even when testing the *rlck vii-1* quintuple mutant, suggesting that RLCK VII-4, instead of RLCK VII-1 members, are specific for chitin-activated MAPK cascades[78,80]. In RLCK VII-7, PBL30 and PBL31 play positive roles in RLP-mediated PTI but have less prominent function in RLK-mediated immune responses[81]. While most characterized RLCKs serve as positive regulators, PBL13 in RLCK subfamily VII-6, which is characterized by unique C-terminal repeats, has been reported to negatively regulate PTI[74,79,82–84].

The functions of RLCKs in crops have gradually emerged in recent years. The rice ortholog of AtPBL27, OsRLCK185 functions downstream of CEBiP-CERK1 to activate a MAPK cascade in response to chitin[85], while both peptidoglycan (PGN) and chitin can trigger the dissociation of OsRLCK176 from OsCERK1[86]. The tomato BIK1 ortholog, Tomato Protein Kinase 1b (TPK1b; Solyc06g005500) is induced by pathogen infection and mechanical wounding. It not only functions in ethylene-dependent fungal resistance but also results in resistance against herbivorous insects[87]. Systemin induces

TPK1b phosphorylation by using the SYR1-SlSERK complex[29]. Given that TPK1b can also be phosphorylated by PORK1[60], it seems to integrate systemin and REF1 signaling pathways. TPK1b thus resembles AtBIK1 in that it coordinates multiple signaling pathways downstream of different PRR complexes. TPK1b-related kinase 1 (TRK1;Solyc06g005520) interacts with SlLYK1 and SlMYC2 (Solyc08g076930), which links chitin perception with JA signaling, to function in chitin-induced fungal resistance[88]. Another wound-inducible RLCK is tomato ZED1-related kinase 1 (SlZRK1; Solyc06g060680) acting as a negative player in wound signaling. Loss of *SlZRK1* resulted in increased JA accumulation and enhanced resistance to mite herbivory[89]. Some tomato RLCKs are involved in immune responses to elicitors released from *Pst* (*Pseudomonas syringae*), such as SlPti1a (Solyc12g098980) and SlPti1b (Solyc05g053230)[90,91]. In flagellin-triggered immunity, the tomato FLS2/FLS3-interacting receptor-like cytoplasmic kinase 1 (Fir1; Solyc12g099830) interacts with SlJAZ3 (Solyc03g122190), a negative regulator of JA signaling[92]. The SIRIPK (Solyc07g041940) positively regulates plant resistance to a broad-spectrum of diseases without compromising yield[93]. Taken together, the available data indicate that RLCKs regulate responses to different signals, and that functional divergence of RLCKs may contribute to the distinction of RLK/RLP-activated immune pathways.

1.4 Phosphatases act as phosphorylation erasers to modify immune signaling.

On the flip side of phospho-signaling, phosphatases counteract kinases to fine-tune phosphorylation relay. Plant phosphatases achieve de-phosphorylation of target proteins mainly by two potential mechanisms: either by (1) direct de-phosphorylation of substrates, or indirectly by (2) inactivation of kinases resulting in decreased phosphorylation of cognate kinase substrates[94]. According to substrate specificity, catalytic mechanism and inhibitor sensitivity, phosphatases have been classified into PPPs (phosphoprotein phosphatases), PPMs (metallo-dependent protein phosphatases), PTPs (protein tyrosine phosphatases), and aspartate (Asp)-dependent phosphatases. PP2Cs (protein phosphatase 2Cs) account for the majority of PPMs. The emerging role of PP2Cs as negative regulators of PTI has lately been reported, primarily in Arabidopsis and tomato.

Arabidopsis PP2Cs with 76 members have been divided into 10 clades (A-J)[95]. For example, PP2C38 belonging to clade D acts as a negative player in BIK1-mediated immunity. In the resting state, PP2C38 dephosphorylates FLS2-BIK1 or EFR-BIK1 to keep them inactive. Once cognate elicitors are perceived, BIK1 phosphorylates and inactivates PP2C38, leading to its dissociation from PRR complexes[96]. Similarly,

Poltergeist-like 4 and 5 (PLL4 & 5) from PP2C clade C repress PTI by dephosphorylating EFR in the absence of ligand. Ligand-induced phosphorylation of PLL4 by BIK drives its dissociation from the receptor complex, thereby alleviating its inhibitory activity[97]. While PP2C phosphatases typically act on phospho-serine or phospho-threonine, *Arabidopsis* CERK1-interacting protein phosphatase 1 (CIPP1; clade F) dephosphorylates CERK1 at tyrosine 428 upon chitin perception, thereby interrupting chitin signaling[98.]

In tomato, a list of 97 SlPP2C members falls into 13 clades (A to M)[99]. Through the analysis of publicly available RNA-seq data, a subset of PP2C immunity-associated candidate (Pic; to date Pic1-Pic14) proteins, whose transcript abundance was differentially changed by MAMPs, has been identified[99]. SlPic1 (Solyc07g066260; clade E) negatively regulates flg22- and csp22-induced immunity through dephosphorylating RLCK SlPti1b[100]; Closely related and partially redundant SlPic3 (Solyc01g105280; clade F) and SlPic12 (Solyc10g047290; clade F) negatively modulate tomato resistance to *Pst*[101]. SlPic6 (Solyc05g052520; clade B) and SlPic14 (Solyc06g082080; clade B) interact with MAPKs via their KIM domains and suppress MAPK activation, suggesting their functional redundancy in suppressing plant immunity[102,103]. SlPic14 has further been reported to dephosphorylate MKK2 to inhibit NLR-triggered immunity (NTI)[103].

Although the currently available data suggest that phosphatases primarily act as negative regulators of immunity, in fact, plant phosphatases also play positive roles in stress signaling, possibly in PTI. It is reported that the overexpression of ZmPP2C55 improved drought stress tolerance[104]. Ectopic expression of Rice PP2Cs, OsBIPP2C1 (*Oryza sativa* L. BTH-induced protein phosphatase 2C 1) or OsBIPP2C2, elevated the disease resistance in tobacco plants[105,106]. The AtPP2C clade D member, Senescence-Suppressed Protein Phosphatase (SSPP), which positively enhanced salt stress tolerance by increasing ROS scavenging capacity, displayed a significantly upregulated reporter (*pFRK1::luciferase*) expression after flg22 treatment, indicating that it may also positively regulate PTI responses[107].

In conclusion, plant phosphatases, particularly PP2C family members, can negatively regulate plant immunity on the one hand; While on the other hand, more functional studies are required to verify their potential positive contribution in plant immunity.

1.5 Early immune responses depend on phosphorylation of signaling players

Upon danger perception, the auto- or trans-phosphorylation of PRR complexes leads to their activation and releases RLCKs to phosphorylate downstream substrates. Thereafter, a series of cellular responses regulated by reversible phosphorylation occur at the PM, where the signal gets amplified and transmitted to cytosol, and later propagates to the nucleus for transcriptional reprogramming[108,109].

1.5.1 Ion fluxes lead to membrane depolarization and extracellular alkalization

One of the earliest events is the drastic rise of cytosol [Ca^{2+}] upon perception of different DAMPs/MAMPs[108,110,111]. Influx of Ca^{2+} from the apoplast is often accompanied by the efflux of Cl^-, NO^{3-} and K^+, as well as the influx of H^+, leading to reduction of PM potential and extracellular alkalization[110]. Rapid membrane depolarization and alkalization of the culture medium are hallmarks of early PTI activation observed in cell cultures of parsley, Arabidopsis and tomato after application of a wide selection of elicitors, such as crude extracts of *P. sojae*, MAMPs (e.g. flg22 and chitin) or phytocytokines (e.g. systemin)[110,112–115]. Since the alkalization response can be mimicked by application of H^+-ATPase inhibitors (e.g. vanadate), and prevented by activators (e.g. fusicoccin), membrane potential changes as well as extracellular alkalization were thought to be the consequence of H^+-ATPases inhibition[116–119]. However, Jeworutzki et al showed that fusicoccin-mediated activation of H^+-ATPases did not prevent the flg22-induced depolarization in *Arabidopsis* mesophyll cells, indicating these membrane-delimited responses cannot be attributed only to the inhibition of H^+-ATPases. Instead, flg22-induced depolarization was found to involve Ca^{2+}-activated anion channels, as indicated by the observation that flg22 failed to alter membrane potential in mesophyll cells when provided with high concentration of extracellular anions [110]. The *Arabidopsis* slow anion channel 1 (SLAC1) and its homolog SLAH3, which control Cl^-/NO^{3-} transport, are known to be activated by cytosolic Ca^{2+} to regulate stomatal closure in guard cells[120,121]. However, *slac1* and *slah3* mutants exhibited Col-0 like membrane depolarization in response to flg22, showing that these anion channels are dispensable for membrane depolarization[110]. Consistently, Jeworutzki et al observed that flg22-induced extracellular alkalization was linked not only to anion efflux but also to proton influx, indicating the potential contribution of H^+-ATPases together with anion channels[110].

Till now, the exact mechanism of the elicitor-induced apoplastic alkalization remains unclear, but H^+-ATPases are likely involved. In fact, several phospho-proteomics studies identified P-type H^+-ATPases with elicitor-responsive phosphorylation changes

in plant immune responses[122–125]. One of the multiple phospho-sites of H^+-ATPases is the penultimate threonine close to its C-terminus (e.g. *Arabidopsis* AHA1^{Thr948} and AHA2^{Thr947}). Phosphorylation of this regulatory threonine promotes binding of 14-3-3 proteins resulting in enzyme activation[122,123,126,127]. This phospho-threonine showed a significant reduction in phosphorylation abundance after elicitor treatments, indicating the de-phosphorylation and inactivation of H^+-ATPases occur during PTI signaling, which correlates with the alkalization responses in plant cell cultures[122–124].

The activity of proton pumps is largely modified by phosphorylation, suggesting regulations by kinases and phosphatases. However, only a limited number of kinases and phosphatases have been reported to directly target PM H^+-ATPases. Even though Thr947 of AHA2 was the first phospho-site identified already in 1998, it took a long time to figure out what are the exact kinases or phosphatases responsible for regulating its state of phosphorylation. The classic acid growth model involves the auxin-mediated activation of AHA2 by phosphorylation at Thr947[128]. This increase in phosphorylation results from SAUR (small auxin-up RNA)-mediated inhibition of PP2C.D phosphatases, which dephosphorylate the penultimate Thr of AHA2[129]. The SAUR-PP2C.D-AHA pathway for auxin-mediated proton pump activation, however, still did not explain what kinase initiates AHA2 phosphorylation /activation in auxin signaling. It was not until 2021, that transmembrane kinases (TMKs) were reported to directly phosphorylate this penultimate residue for auxin-induced AHA2 activation, cell wall acidification and cell expansion[130]. The penultimate threonine is not the only regulatory site, as PSY1R-dependent phosphorylation of Thr881 of AHA2 also results in proton pump activation[131]. Phosphorylation can also cause inhibition of the proton pump. Perception of RALF by FERONIA induces the phosphorylation of Ser899 which inactivates the PM H^+-ATPases[42]; The *Arabidopsis* kinase PKS5 (AtCIPK11) phosphorylates Ser931, which prevents 14-3-3 from binding to Thr947 for AHA2 activation[132]. Overall, phosphorylation at different sites of PM H^+-ATPases can lead to either enzyme activation or inhibition, and under different circumstances the degree of phosphorylation can be very different.

The complex modulation of PM H^+-ATPase activity likely results in distinct downstream outputs. It was suggested that extracellular alkalization resulting from H^+-ATPase inhibition positively correlates with the induction of wound-responsive genes by systemin. On the other hand, fusicoccin-induced extracellular acidification by H^+-ATPase activation leads to the accumulation of salicylic acid (SA) and induction of pathogenesis-related (PR) genes. Thereby, the systemin-mediated wound signaling may