PERKing up our understanding of the proline-rich extensin-like receptor kinases, a forgotten plant receptor kinase family

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Research review

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Summary

Proline-rich extensin-like receptor kinases (PERKs) are an important class of receptor-like kinases (RLKs) containing an extracellular proline-rich domain. While they are thought to be putative sensors of the cell wall integrity, there are very few reports on their biological functions in the plant, as compared with other RLKs. Several studies support a role for PERKs in plant growth and development, but their effect on the cell wall composition to regulate cell expansion is still lacking. Gene expression data suggest that they may intervene in response to environmental changes, in agreement with their subcellular localization. And there is growing evidence for PERKs as novel sensors of environmental stresses such as insects and viruses. However, little is known about their precise role in plant immunity and in the extracellular network of RLKs, as no PERK-interacting RLK or any coreceptor has been identified as yet. Similarly, their signaling activities and downstream signaling components are just beginning to be deciphered, including Ca^{2+} fluxes, reactive oxygen species accumulation and phosphorylation events. Here we outline emerging roles for PERKs as novel sensors of environmental stresses, and we discuss how to better understand this overlooked class of receptor kinases via several avenues of research.

Introduction

Receptor-like kinases (RLKs), the largest receptor family in plants, play a major role in the perception by the plant cells of internal and external signals and in the regulation of multiple cellular processes. Among them, proline-rich extensin-like receptor kinases (PERKs) constitute a small family of RLKs containing an extracellular proline-rich domain, and, as such, are thought to be putative sensors of the cell wall integrity. In contrast to other putative cell wall sensors, such as *Catharanthus roseus* like (Cr)-RLK1Ls, wall-associated kinases (WAKs), leucine-rich repeat (LRR)-RLKs or other proteins linked to the cell wall (Rui & Dinnemy, 2019), rare are the reports exploring the biological functions of PERKs (Fig. 1). And most of these few studies suggest that PERKs regulate mainly plant growth and development (Borassi *et al.*, 2016). However, during the last 3 yr, a combination of different approaches, including genome wide identification of PERKs and gene mining for proline-based signaling proteins in different species, plus the discovery of PERKs as key genes of the response to abiotic and biotic stresses, suggests that these RLKs exert more complex roles (Ishan *et al.*, 2017; Feng *et al.*, 2019; Qanmber *et al.*, 2019; Chen *et al.*, 2020; Dievart *et al.*, 2020; Uemura *et al.*, 2020; Xue *et al.*, 2021). Moreover, their signaling activities and downstream signaling components are just beginning to be deciphered. Finally, while many RLKs are involved in pathogen perception through highly interconnected and dynamic networks of receptor–coreceptor complexes (Brustolini *et al.*, 2017; Smakowska-Luzan *et al.*, 2018; Delplace *et al.*, 2020), the question of the place of PERKs in such an arrangement has not yet been addressed.

In this review, we will describe the recent progress made to understand the functions of PERKs beyond their established roles in plant development, and we will focus on emerging evidence for their function in perception of environmental cues. We will also address the question of their putative association with other receptors/proteins and implication in receptor networks to sense diverse environments and generate adapted and controlled responses. Finally, we propose some research avenues for a better understanding of this overlooked receptor subfamily.

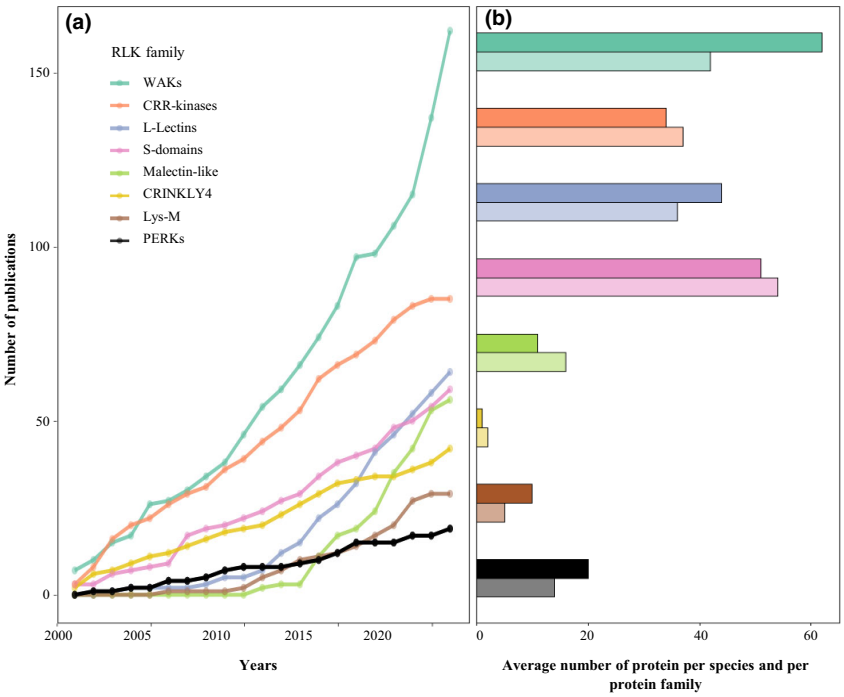

Fig. 1 Literature survey of receptor-like kinase (RLK) studies over the last 20 yr. (a) Cumulative number of articles published from 2001 to 2021 on families of RLKs harboring different domain organizations. WAK, wall-associated kinase; CRR-kinases, cysteine-rich repeat kinase; L-lectin, L type lectin; Lys-M, lysin motif; PERK, proline-rich extensin-like receptor kinase. Data were collected from PubMed on the basis of representative keywords. The LRR RLKs have not been included in this analysis as they represent the most intensively studied group (11 publications in 2001 and 907 in 2021, for example), minimizing the relative importance of the other groups. (b) Average number of proteins per species belonging to monocotyledons (darker colors) or dicotyledons (light colors) for each RLK family (data from Dievart *et al.*, 2020). Concerning the PERKs, it should be noted that our data based on the species listed in Supporting Information Table S1 indicate an average number of 17 genes for monocotyledons and 10.7 genes for dicotyledons.

Origin, diversity and expression of PERKs in plant species

To date, 207 PERK genes have been identified in 15 plant species (Qanmber *et al.*, 2019). The PERK family probably emerged from the common ancestor of the Streptophytes and then diversified within land plants (Dievart *et al.*, 2020; Supporting Information Fig. S1). Interestingly, only the aero-terrestrial Charophytes (with the exception of *Penium margaritacereum*) seem to possess PERK proteins, indicating that PERKs are present only in species living in terrestrial habitats. This species distribution echoes the role of PERKs as biotic/abiotic stress sensors in nonaquatic habitats. In land plants, PERKs are retrieved in all species, with the exception of *Anthoceros* species (Borassi *et al.*, 2016; Qanmber *et al.*, 2019; Figs 2, S1), but are characterized by a high duplication level specific to Angiosperms, Eudicots and even Brassicaceae in the case of PERK11/12/13, 5/6/7 and 1/2/3. PERKs diversity has been investigated in at least 16 Brassica species (Chen *et al.*, 2020) as well as in monocots and dicots (Qanmber *et al.*, 2019), and the overall number of genes per plant species varies from 17 to 27 in *Brassica* species (Chen *et al.*, 2020) to 55 in *Camelina sativa* probably as a result of the well-preserved hexaploid genome structure resulting from a whole-genome triplication event (Chen *et al.*, 2020) (Fig. 1).

In *Arabidopsis thaliana*, the PERK family encompasses 15 members, with an extra copy (AT1G70450) without the extensin-like extracellular domain (ECD). They harbor an extensin-like ECD, which is composed of Ser/Pro (3–5) repetitions and lacks the Tyr-Val-Tyr sequence needed for extensin crosslinking (Borassi *et al.*, 2016). This domain shows some variability, mainly in the two first exons of the *PERKs*, which leads to different lengths of ECD. Although PERKs lack a signal peptide, they are predicted to be localized in the plasma membrane, possibly thanks to a stretch of positively charged amino acid residues (Nakhamchik *et al.*, 2004) or unidentified signal peptides. However, while BnPERK, AtPERK4 or GmKHAK2 show a plasma membrane localization (Silva & Goring, 2002; Bai *et al.*, 2009; Uemura *et al.*, 2020), AtPERK13 has also been shown to be anchored in the cell wall (Hwang *et al.*, 2016). Their intracellular domain consists of a conserved serine/threonine kinase domain (Silva & Goring, 2002; Nakhamchik *et al.*, 2004) which has been shown to be active *in vitro* for BnPERK1 and AtPERK4 (Borassi *et al.*, 2016).

Extraction of gene expression data from databases and targeted literature revealed very diverse PERK expression profiles (Fig. 3). Expression of most of the PERKs was first found to be specific to floral organs such as pollen grain, pollen tube or floral buds (Nakhamchik *et al.*, 2004; Chen *et al.*, 2020). However, *AtPERK8* and *AtPERK13/RHS10* (Root Hair Specific 10) are mainly expressed in roots and root hairs, respectively (Nakhamchik *et al.*, 2004; Won *et al.*, 2009; Humphrey *et al.*, 2015). Additionally, while some PERKs, like *AtPERK1*, are ubiquitously expressed in vascular tissues of cotyledons and in leaves and roots, others, like *AtPERK4*, *5*, *6*, *7*, are rather tissue-specific, suggesting diverse roles.

Recent data showed that PERK expression is also regulated in response to environmental cues. *AtPERK13* is induced under phosphate, nitrogen and iron deprivations (Xue *et al.*, 2021). PERK expression in *Gossypium hirsutum* is sensitive to, and generally downregulated by, heat, cold, salt or drought (Qanmber *et al.*, 2019). An exposition to a moderately low temperature (18°C) leads to an expression change of a rice *PERK* gene, *Z15* (Feng *et al.*, 2019). Importantly, PERKs are also regulated in response to biotic stresses. *BnPERK1* is rapidly induced in response to wounding and in the presence of the pathogen *Sclerotinia*

Fig. 2 Number of proline-rich extensin-like receptor kinases (PERKs) identified by phylogenetic analysis in Viridiplantae. The tree represents the evolution of Viridiplantae with the number of PERK proteins identified indicated by green bars. The blue bars indicate the ploidy level for each species. Because diploid species are represented by a haploid genome, the ploidy is set to 1.

sclerotiorum (Silva & Goring, 2002). However, with the exception of *AtPERK9* (average fold-change of 2, GENEINVESTIGATOR), PERK expression does not seem to be affected by cell wall-derived signals such as oligo-galacturonides, chitin, mixed-linked glucans or even damage-associated molecular patterns such as pathogen-associated molecular patterns-induced peptide 2 (Pep2) (Rebaque *et al.*,

2021). By contrast, *G. hirsutum* PERKs are coexpressed with several genes related to defense responses and plant–pathogen interactions. Finally, coexpression of particular PERKs was found, whether they are phylogenetically close or not (Fig. 3; Nakhamchik *et al.*, 2004; Qanmber *et al.*, 2019; Chen *et al.*, 2020).

All these data suggest that PERKs beyond their roles in development may intervene in response to environmental changes, in good agreement with their subcellular localization.

PERKs in plant growth and development

Until recently, PERKs were primarily associated with plant developmental processes (Borassi *et al.*, 2016) (Table 1). The first report concerning a PERK developmental function concerned *BnPERK1* from *Brassica napus*. Antisense *BnPERK1* Arabidopsis lines showed loss of apical dominance, defects in floral organs and increase in secondary branching (Haffani *et al.*, 2006). Likewise, the Arabidopsis mutant inflorescence growth inhibitor 1 (*igi1*), affected in the *AtPERK12* gene, exhibited increased shoot branching and reduced plant growth (Hwang *et al.*, 2010).

Interestingly, most of the PERKs are involved in the polar growth of either roots or pollen tubes, potentially through a modulation of the cell wall composition. At*PERK13* was found to act as a negative regulator of root hair growth, and the ECD, including a few proline residues, is essential for its function (Cho, 2016; Hwang *et al.*, 2016). Other PERKs act as negative regulators of root growth and might act as regulators of cell expansion by modulating the transport of cell wall material (Bai *et al.*, 2009; Humphrey *et al.*, 2015). Some other PERKs are necessary for pollen tube growth, such as PERK5 and PERK12 (Borassi *et al.*, 2021). The double mutant *perk5 perk12* showed reduced pollen tube growth, in conjunction with nonmethylated pectin accumulation. Four *Brassica rapa* genes (orthologs of *AtPERK6* and *12*) might be linked to male sterility as they were downregulated during anther development and in male sterile mutants (Chen *et al.*, 2020).

These data established the role of many PERKs in plant development clearly and point to a putative functional link between these developmental roles and the cell wall composition. However, evidence demonstrating that PERKs act on the cell wall composition to allow or repress cell expansion in certain organs is still lacking. Even if we do not yet know at which level they act, either sensors or regulators, they are crucial for proper cell development.

PERK downstream signaling

While the surveillance and maintenance of cell wall integrity have been extensively studied at the cellular level, its functional roles in the context of plant development and in response to biotic and abiotic stresses require further investigation (Rui & Dinneny, 2019). Owing to their molecular organization, PERKs represent good sensor and integrator candidates in this context. However, they have not been considered as such, or identified as major regulators of such processes until very recently. Importantly, the

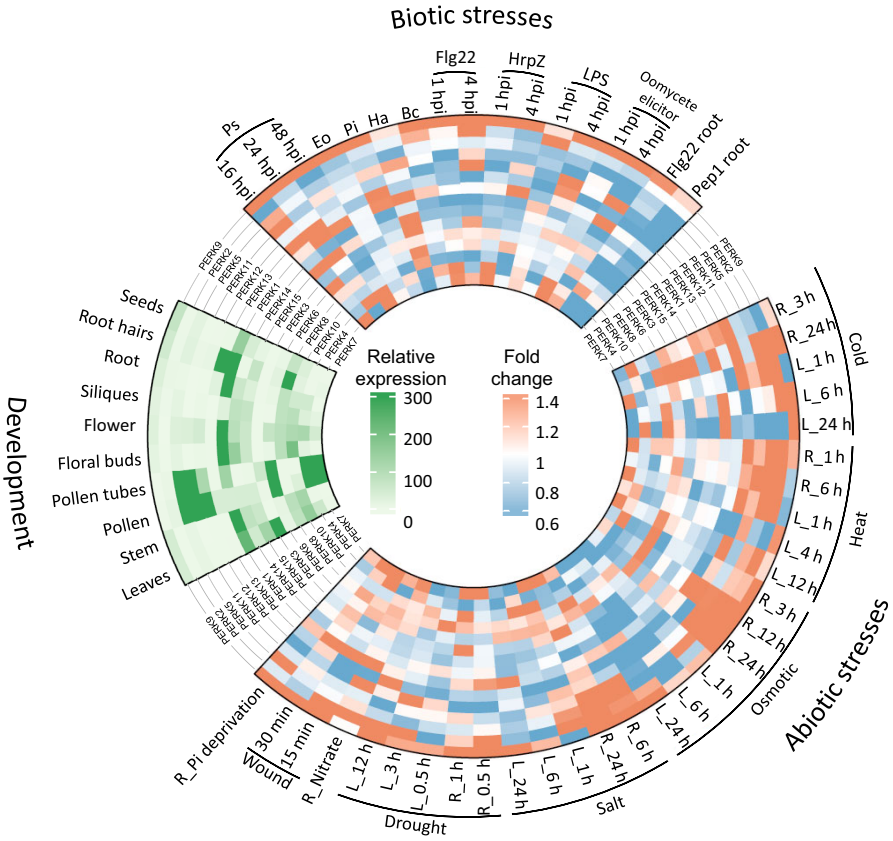

Fig. 3 Relative expression of *Arabidopsis thaliana* proline-rich extensin-like receptor kinases (PERKs) during plant development and in response to biotic and abiotic stresses. Expression in response to stress is expressed in fold changes in comparison to control. Data were collected from the ePlant database (https://bar.utoronto.ca/eplant/) and enriched with published data. Ps, *Pseudomonas syringae*; Eo, *Erysiphe orontii*; Pi, *Phytophtora infestans*; Ha, *Hyaloperonospora arabidopsidis*; Bc, *Botrytis cinerea*; LPS, lipopolysaccharides; Osmo, osmotic; Ni, nitrogen; Pi, phosphate; L, leaves; r, root; hpi, h post-infection.

Table 1 Proline-rich extensin-like receptor kinases (PERKs) for which a putative function has been reported.

Biological process	Gene name	Organism	Gene expression	Function	Ligand/co-receptor	Dowstream signaling	References
Stem length, apical dominance and flower development	*AtPERK1*, *AtPERK3*, *AtPERK6*, *AtPERK7 and AtPERK13*, *BnPERK1*	*Arabidopsis thaliana*	*PERK1* expresssion induced by wounding and lowered in the *ice1* mutant	Stem length, apical dominance and flower development? (observed using antisense *BnPERK1* in Arabidopsis)	ND	ND	Haffani *et al*. (2006); Silva & Goring (2002)
Abscisic acid (ABA)-induced root cell elongation	*AtPERK4*	*Arabidopsis thaliana*	Induced specifically by ABA and expressed in roots and flowers	Regulator in the ABA-mediated inhibition of root growth	ND	ABA and Ca signaling	Bai *et al*. (2009)
Plant height and branching	*AtPERK12 (IGI1)*	*Arabidopsis thaliana*	Strongly expressed in the anther and inhibited by auxin	Regulator of axillary bud development	ND	Strigolactone biosynthesis pathway, auxin pathway	Hwang *et al*. (2010)
Root cell expansion	*AtPERK8*, *AtPERK9*, *AtPERK10*	*Arabidopsis thaliana*	Expressed in the whole seedlings, roots, some floral tissues and seeds	Negative regulation of root growth	ND	KIPK1 and -2 and KCBP interact with these PERKs and are possible signaling components	Humphrey *et al*. (2015)
Pollen tube growth	*AtPERK5*, *AtPERK12*	*Arabidopsis thaliana*	Highly expressed in mature pollen	Regulators of pollen tube growth and cell wall polysaccharide assembly	ND	ROS	Borassi *et al*. (2021)
Root hair growth	*AtPERK13*	*Arabidopsis thaliana*	Expressed mainly in the root	Negative regulation of root hair growth	*ND*	ROS and RHD2, RNA catabolism (RNS2)	Hwang *et al*. (2016); Cho (2016)
Anther development and male sterility	*Bra001723.1*, *Bra037558.1*, *homolgs AtPERK6*	*Brassica rapa*	Several *BrPERK* genes expressed in different tissues, while some *BrPERKs* expressed only in buds.	Putative role in anther development and male sterility	ND	ND	Chen *et al*. (2020)
Low temperature	*Z15*	*Camelina sativa*	Expression induced by moderate low temperature (18°C)	Tolerance to low temperature (18°C)	ND	*OsWRKY71*, *OsMYB4*	Feng *et al*. (2019)
Pi and nitrogen deprivation	*AtPERK13*	*Arabidopsis thaliana*	Expression induced by Pi, nitrogen and iron deficiencies	Control of root hair growth	ND	ROS	Xue *et al*. (2021)
Wounding and *S. sclerotiorum* infection	*BnPERK1*	*Camelina sativa*	Induced by wounding and *Sclerotinia sclerotiorum* infection	Wounding response?	ND	ND	Silva & Goring (2002)
Infection by geminiviruses	*AtPERK1*	*Arabidopsis thaliana*	Expressed in all tissues, predominantly in flowers, not affected by virus or nuclear shuttle protein (NSP) treatment	Binding with the NSP from geminivirus	ND	ND	Florentino *et al*. (2006)
Resistance to Spodoptera sp. Herbivory	GmHAK2	*Glycine max*	Expression reduced in response to ALSV inoculation	Response to herbovory danger signals	Spodoptora elicitor/GmHAK1	Ethylene pathway	Uemura *et al*. (2020)

ALSV, apple latent spherical virus; ND, not determined; Pi, phosphate; ROS, reactive oxygen species.

downstream signaling events occurring after initial signal perception are still poorly characterized (Table 1). Yet a few reports revealed some downstream responses, including changes in Ca^{2+} fluxes, reactive oxygen species (ROS) accumulation or phosphorylation events.

First, the essential role of Ca^{2+} has been described for PERK4 control of abscisic acid (ABA)-induced root growth inhibition in Arabidopsis (Bai *et al.*, 2009). The *perk4* mutant showed decreased sensitivity to ABA during cell elongation and root growth, and ABA activation of calcium channels was altered in the mutant. These data indicate that PERK4 acts at an early stage of the ABA signaling pathway to modulate root cell elongation, and that its effects are mediated by Ca^{2+} fluxes. Then, some potential downstream signaling proteins of a subclade of *PERK* genes, *PERK8*, *PERK9* and *PERK10*, shown to negatively regulate root growth, were identified by yeast two-hybrid interaction screening using their cytosolic kinase domains (Humphrey *et al.*, 2015). Two members of the AGC VIII kinase gene family, AGC1-9 and a kinesin-like calmodulin-binding protein (KCBP)-interacting protein kinase (KIPK), were found and proposed to act downstream to mediate signaling for control of root growth (Hwang *et al.*, 2016). A similar approach for PERK13 revealed a role in the negative control of ROS accumulation and led to the identification of an RNAse (RNS2) as an interacting target, leading the authors to propose a model for balanced control of root hair elongation through positive and negative regulators (Cho, 2016). Interestingly, under phosphate deficiency, *perk13* displayed increased ROS production (Xue *et al.*, 2021). ROS homeostasis was also found to be regulated in the *perk5* and *perk12* mutants, affected in pollen tube growth (Borassi *et al.*, 2021). Transcriptomic analysis of the rice *z15* mutant led to the identification of two potential downstream target genes, *OsWRKY71* and *OsMYB4*, both involved in the regulation of the cold response (Feng *et al.*, 2019). Finally, genome-wide analysis of coexpression gene modules indicated that *PERK* genes can be part of cell wall, plant–pathogen interactions, abiotic stress and phytohormone response modules/subnetworks (Ruprecht *et al.*, 2016; Qanmber *et al.*, 2019; Chen *et al.*, 2020).

In conclusion, the data accumulated until now on the signaling functions of PERKs remain fragmentary (Fig. 4) and a better understanding of the downstream signaling components is needed to get a better insight into PERK functions.

PERKs as major sensors of environmental changes?

Beyond their role in plant development, there is growing evidence for a function of the PERKs as novel sensors of environmental stresses, either abiotic or biotic (Table 1). Various studies show their possible role in the response to different abiotic stresses. A mutation in *Z15* leads to an alteration of chloroplast structure and to dramatic changes in expression of genes involved in rice cold tolerance, such as *OsMYB4* and *OsWRKY71* (Feng *et al.*, 2019), suggesting a role for *Z15* in low-temperature signaling. More recently, the effect of phosphate starvation on root hair growth was found to be mediated, at least in part, by *AtPERK13* (Xue *et al.*, 2021). Through transcriptomic analysis of *perk13* mutant and *PERK13* overexpressing lines, a dual role was observed for *AtPERK13* in the phosphate deficiency response, as it was regulating root hair growth either positively or negatively, by acting on different pathways.

In response to bioagressors, it was observed in several cases that PERKs were transcriptionally regulated by infection (see earlier), suggesting their role in such responses. The first evidence for their role in response to pathogens is the physical interaction of a PERK (NsAK, NSP Associated Kinase) with the nuclear shuttle protein (NSP) of cabbage leaf curl virus (CaLCuV) and tomato geminiviruses. This protein facilitates the transport of viral DNA to the plant nucleus (Florentino *et al.*, 2006). Moreover, the *nsak* mutant was revealed to be more resistant to the virus, confirming the role of this PERK in viral infectivity. By contrast, overexpression of some AtPERKs alters host susceptibility to cyst and root-knot nematodes, probably by ECD binding to polygalacturonic acid and regulation of innate immunity (L. Torres, pers. comm.). Finally, a recent study involving mining of RLKs associated with perception of herbivore danger signals (HDS) in soybean led to the identification of *GmHAK2*, a member of the *PERK* family. Using virus-induced gene silencing, *GmHAK2* was shown to be implicated in *Spodoptera litura* resistance (Uemura *et al.*, 2020).

Altogether, these findings are in favor of PERKs having various functions in plant defense and adaptation to the biotic environment (virus, herbivores). However, only a few research lines are emerging in this area and little is known about their precise role in plant immunity in all the diversity of potential interactions.

What ligands and partners for PERKs?

As extensins constitute an essential component of the plant cell wall, the PERK ECD was hypothesized to associate with some of its compounds to bridge the inner and outer parts of the cell. Even though their ECD lacks Tyr-Val-Tyr domain that is important for crosslinking with pectins, cell wall localization of AtPERK13 confirmed this hypothesis (Borassi *et al.*, 2016; Hwang *et al.*, 2016). Moreover, AtPERK4 was shown to bind pectins, while the protein seems to be located in the plasma membrane (Bai *et al.*, 2009). These results suggest that some PERKs might perceive cell wall modifications through direct sensing of biomechanical stress. In line with this, the first PERK functionally studied was induced in response to mechanical stimuli and in response to *Sclerotinia sclerotiorum*, shown to exert plant cell wall tension (Silva & Goring, 2002; Léger *et al.*, 2021). Furthermore, it is noteworthy that many PERKs are involved in polar growth of root hairs and pollen tubes, a process requiring a fine-tuned expansion of the cell wall (Bai *et al.*, 2009; Humphrey *et al.*, 2015; Hwang *et al.*, 2016; Borassi *et al.*, 2021). Interestingly, the few receptors known to be tightly linked to the cell wall through the binding of pectins have similar functions in development. For instance, the reduced expression of several WAKs inhibits cell expansion (Lally *et al.*, 2001; Wagner & Kohorn, 2001). Likewise, the receptor FERONIA (FER) that belongs to the CrRLK1L subfamily plays a role in root cell elongation (Haruta *et al.*, 2014) and root hair development (Duan *et al.*, 2010; Zhu *et al.*, 2020). And as for PERK4, WAK1 and FER were shown to bind pectins cross-linked with the cell wall (He *et al.*, 1996; Kohorn, 2016; Feng *et al.*, 2018; Lin *et al.*, 2018). As pectins

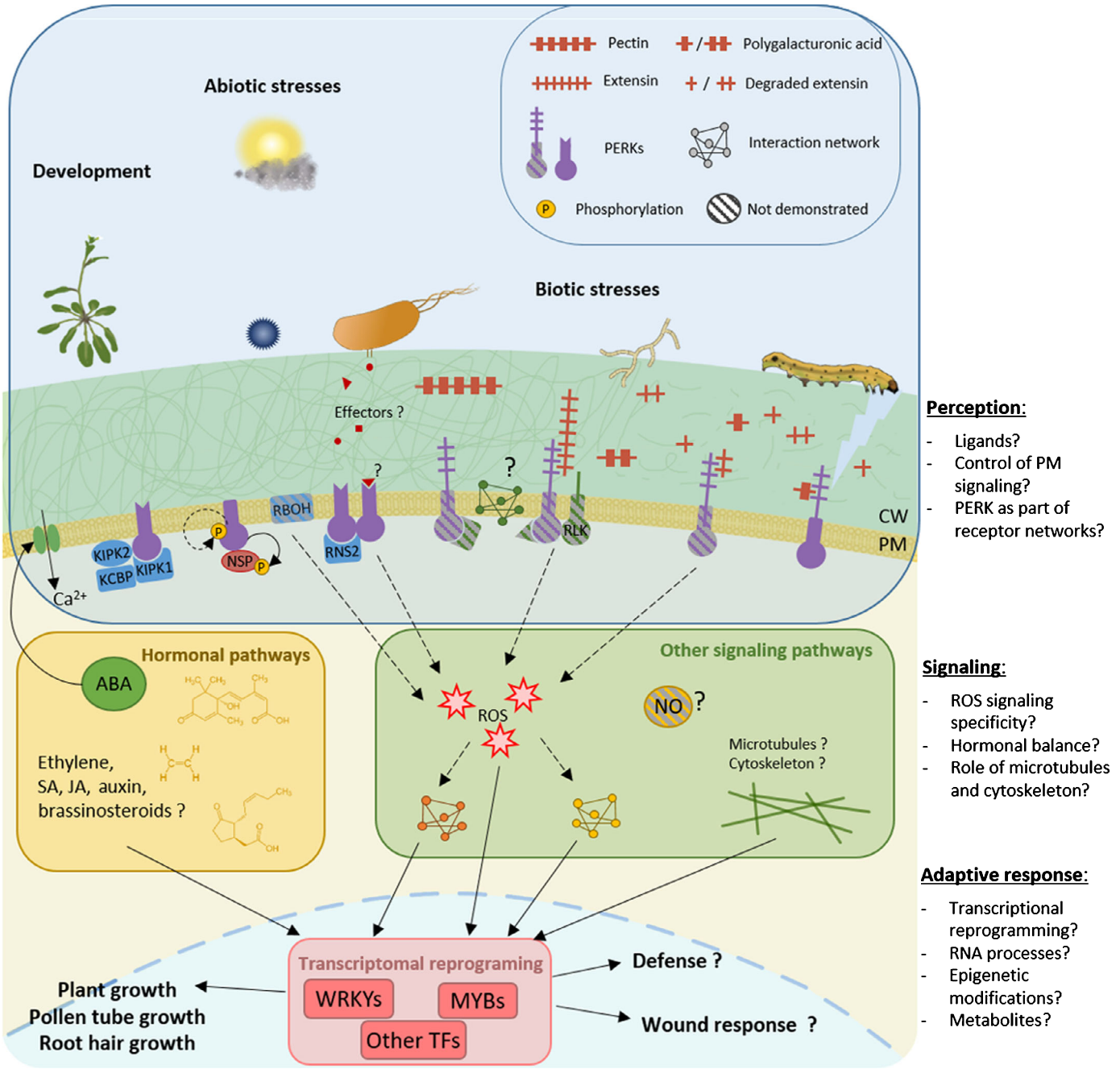

Fig. 4 Putative proline-rich extensin-like receptor kinase (PERK)-dependent pathways during plant development and in response to environmental cues. While PERKs are thought to exert different biological functions in plants and to be putative sensors of the cell wall integrity, their ligands remain mostly unknown. PERKs can bind pectins, extensins or other cell wall components thanks to their extensin-like domain. PERKs might also be involved in perception of pathogen effectors, as for other receptor-like kinases (RLKs). They also might participate in receptor–coreceptor extracellular networks of RLKs, although no PERK interacting RLK or any coreceptor has been identified as yet. Their signaling activities and downstream signaling components are just beginning to be deciphered, and KIPK1, KIPK2, KCBP and RNS2 have been found to bind their kinase domain. Reactive oxygen species (ROS) and hormonal pathways, notably abscisic acid (ABA), have been reported to participate in PERK signaling. Other complex signaling components might intervene, such as microtubules or the cytoskeleton. Finally, transcriptional reprogramming leading to adaptive responses should occur through diverse transcription factors (TFs). Hatched elements are putative, while full elements are based on the literature. CW, cell wall; JA, jasmonic acid; NO, nitric oxide; NSP, nuclear shuttle protein; PM, plasma membrane; SA, salicylic acid; WRKY, WRKY transcription factor; MYB, MYB transcription factor.

seem more prone to be altered by mechanical cues and are accessible to some plant receptors, they may constitute the best target to sense cell wall alteration (Bacete *et al.*, 2018; Vaahtera *et al.*, 2019). Thus, PERKs might also indirectly sense cell wall modifications such as damage or enzymatic degradation in a similar manner to WAK1, which binds pectin fragments or oligo-galacturonides that are induced in the context of pathogen attack (Brutus *et al.*, 2010; Gramegna *et al.*, 2016). Another study showed that a fraction of the

oral secretion of the herbivore *Spodoptera litura* containing a mixture of polysaccharides elicits soybean defense through GmHAK2, a PERK member from soybean, although the direct binding was not demonstrated (Uemura *et al.*, 2020). The authors suggest that GmHAK2 might be a coreceptor, not directly perceiving the cell wall damage signals but instead transducing the signal inside the cell to other partners. Indeed, RLKs often work in association with coreceptors to achieve their biological function. For instance, ligand perception by FLAGELLIN SENSING 2 (FLS2) and ELONGATION FACTOR-Tu RECEPTOR (EFR) induces a conformational change leading to their association with the coreceptor BRI1-ASSOCIATED KINASE (BAK1), which is crucial for downstream signaling (Chinchilla *et al.*, 2007; Heese *et al.*, 2007; Roux *et al.*, 2011). Yet so far, no PERK interacting RLK or any coreceptor has been identified.

Perspectives: how to better understand the roles of this RLK 'forgotten' subfamily?

A role for PERKs in the plant immune system?

As mentioned earlier, PERKs can exert a crucial role in diverse aspects of the plant life, including different developmental stages and, in certain cases, stress responses. However, only a few of them have been functionally defined, even in Arabidopsis which contains a small family of 15 PERKs. Some functional data have been accumulated for eight of them (Table 1), and we still have a very partial view of their roles in the plant. They have been mainly considered as regulators of plant growth and development (Borassi *et al.*, 2016). However, as already mentioned, PERKs might sense cell wall changes and generate an adapted response to maintain cell wall integrity (Jose *et al.*, 2020). Interestingly, plant cell wall alterations have been demonstrated to have an effect on disease resistance and to be perceived by sensors, activating the immune functions (Bacete *et al.*, 2018). This suggests a role for PERKs, as found for most RLKs (Jose *et al.*, 2020; Ou *et al.*, 2020), in responses to biotic and abiotic stresses. Surprisingly, only two PERKs have been associated with plant defense in Arabidopsis (Florentino *et al.*, 2006).

Most single mutants for the 15 *AtPERKs* do not exhibit any detectable phenotype, possibly because of functional redundancy (Borassi *et al.*, 2016). Another possible explanation is that these mutants have not been tested in response to various external stimuli, and that PERKs might exert functions in plant defense. A systematic analysis of their expression profiles in response to various pathogens or environmental constraints (high or low temperature, drought, nutrient supply, etc.), coupled with single and multiple mutant phenotyping, should shine some light on their possible functions. In the same line, exploitation of genome-wide analyses and coexpression networks previously generated (Qanmber *et al.*, 2019; Chen *et al.*, 2020) will also help to elucidate their functions. For these approaches, a possible research focus might consider, more particularly, interactions with micro- or macroorganisms that exert a specific impact on plant cell wall integrity during the infection process, such as nematodes, insects, pathogenic fungi or even other plants. Thus, PERKs represent promising candidates as immune sensors and, if we consider the immune system in its full complexity, they should be an important component of the whole system (Fig. 4). Indeed, RLKs have emerged as essential players in danger sensing and defense signaling, and more generally in the complex network of immune responses, including those initiated at either the extracellular or intracellular levels (Zhou & Zhang, 2020).

Organization and functions of PERKs at the cell surface: part of the immune receptor network?

A critical step to better understanding of PERK function and mode of action will be to identify their ligand(s) as well as their interactors. If we consider the features of the PERK ECD, it is likely that the ligands are related to the cell wall. One way to demonstrate their docking/anchoring in intact cell wall components would be to monitor the behavior of fluorescent PERKs with live-imaging while biomechanical stimuli are applied (Robinson *et al.*, 2017). Several components of the cell wall or by-products might also be tested using a chimeric receptor between PERK ECDs and pattern recognition receptors (PRRs) whose defense activation would be recorded. This approach has already been used successfully to show that WAK1 ECD fused to EFR was able to perceive oligogalacturonides (Brutus *et al.*, 2010). Progress in computational modeling of protein structure prediction represents a great opportunity to perform a wide screen of ECD–ligand interactions before molecular validation (Del Hierro *et al.*, 2021; Tunyasuvunakool *et al.*, 2021). Yet at this stage, we cannot exclude the possibility that PERKs could also perceive peptides or any other molecules from the abiotic or biotic environment (Fig. 4).

Many RLKs require one or several coreceptors to fulfill their function (Gou & Li, 2020; DeFalco & Zipfel, 2021; Lee *et al.*, 2021). For instance, the most versatile coreceptor is BAK1, which interacts with multiple RLKs perceiving various ligands and that are involved in plant development and plant defense. PERKs probably function similarly with other cell surface proteins, the question being whether they play the role of the receptor or coreceptor. A homodimerization of GmHAK2, a PERK of soybean that triggers defense signaling in response to a herbivore, was recently shown (Uemura *et al.*, 2020). A yeast two-hybrid assay was performed using the kinase domain of a few PERKs as bait to identify downstream components (Humphrey *et al.*, 2015). However, identification of PERK coreceptors requires the use of the ECD as bait, whatever the approach used for this search.

Signaling from PERKs to downstream components and adaptive responses?

During the past two decades, considerable progress has been made in deciphering the early signaling events underlying RLK functions, notably those with ECDs associated with the cell wall (Fig. 1). WAKs, CrRLK1Ls, LRR and lectin RLKs have been shown to play a role in cell wall sensing and signaling (Rui & Dinnemy, 2019). They act as sensors of diverse ligands using their ECD, possibly associated with cell wall components, and as signal transducers via activation of their kinase domain (Kohorn & Kohorn, 2012). The

kinase module then activates or inactivates downstream components to initiate a signaling pathway, ultimately modulating transcription factors or other downstream components of the adaptive response (Hohman *et al.*, 2017). Even if there are only fragmentary data available on these functions for PERKs, they might be activated by cell wall components and/or other ligands, and initiate a downstream signaling cascade. Future efforts are needed to decipher the complex signaling network responsible for the initiation of the signaling cascade, because multiple signaling components might be recruited, as for other cell wall-associated RLKs, such as kinases/phosphatases, NO, mRNA local translation and microtubule cytoskeleton; and hormones need to be explored, such as auxin, brassinosteroid (BR), ABA, ethylene and jasmonic acid signaling, as shown for CrRLK1Ls (Deslauriers & Larsen, 2010; Yu *et al.*, 2012; Chen *et al.*, 2016; Zhu *et al.*, 2020).

Finally, numerous questions remain regarding not only the identification of PERK downstream signaling components (with or without *a priori*), but also the way they are organized, modulated and propagated by PERKs to control diverse responses related to either developmental or stress responses. A combination of transcriptomics (coexpression networks) and interactomics (protein–protein interaction networks) will undoubtedly shed some light on these cascades, either on direct targets of PERKs or on more downstream components. Systems biology should then help to integrate these data for functional validation and possible use in predicting new regulatory components of these signaling networks (Delplace *et al.*, 2020). Such analysis should also help us to understand how the specificity of the signaling is achieved when PERKs are involved in the regulation of diverse processes.

Beyond the issues discussed, there are still many unanswered questions about PERKs. For example, the transcriptional and/or post-transcriptional regulation of PERKs in response to developmental or environmental changes is poorly documented. The natural variation associated with these genes, not only in Arabidopsis but also in various species, should be explored systematically, as proposed more widely for RLKs (Dievart *et al.*, 2020). This type of analysis might produce some interesting findings, offering researchers a better understanding of their functions in plants and of their adaptive value, and potential strategies to improve agronomic traits by modulating PERK signaling.

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Author contributions

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Maximum likelihood tree of the PERK family.

Table S1 List of species used in this study.

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