Review

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Papain-like cysteine proteases as hubs in plant immunity

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Summary

Plants deploy a sophisticated immune system to cope with different microbial pathogens and other invaders. Recent research provides an increasing body of evidence for papain-like cysteine proteases (PLCPs) being central hubs in plant immunity. PLCPs are required for full resistance of plants to various pathogens. At the same time, PLCPs are targeted by secreted pathogen effectors to suppress immune responses. Consequently, they are subject to a co-evolutionary host–pathogen arms race. When activated, PLCPs induce a broad spectrum of defense responses including plant cell death. While the important role of PLCPs in plant immunity has become more evident, it remains largely elusive how these enzymes are activated and which signaling pathways are triggered to orchestrate different downstream responses.

I. Introduction

Plants are continuously challenged by microbes and have developed different mechanisms to defeat pathogens and other invaders. The first contact with microbes usually takes place in extracellular compartments, namely on the epidermal surface and in the apoplast, including cell walls. Processes in this extracellular battleground determine the primary outcome in the majority of plant–microbe interactions. After this first contact, different signaling pathways in the cell are activated to orchestrate downstream responses such as modulation of various enzymatic activities. In this article, we will emphasize the role of papain-like cysteine proteases (PLCPs), which control key processes at different levels of plant defense. PLCPs are prominent enzymes in the plant apoplast and belong to MEROPS (https://merops.sanger.ac.uk/) protease family C1A of clan CA, of which papain is the type member. In animals PLCPs are often called cathepsins and PLCPs in plants fall into nine subfamilies (Richau *et al.*, 2012). PLCPs are produced as pre-proproteases, containing an N-terminal signal peptide for secretion and an auto-inhibitory pro-domain that needs to be removed for protein activation, releasing a mature 25–35 kDa active protease. The protease domain contains the catalytic triad formed by the amino acids Cys, His and Asn. Some PLCPs also carry a C-terminal granulin domain with unknown function. In plants, nine PLCP subfamilies can be found (Fig. 1; Richau *et al.*, 2012). Based on recent research, we present here five observations

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demonstrating that PLCPs are essential and central hubs of plant immunity:

II. Depletion of PLCPs hampers plant immunity

Many cases of protease depletion (e.g. by knockout or RNAi) indicate important roles for PLCPs in plant immunity. Arabidopsis null mutants for the PLCP RD21 are more susceptible to the necrotrophic fungal pathogen *Botrytis cinerea* (Shindo *et al.*, 2012), although these lines were more resistant for the same pathogen in detached leaf assays (Lampl *et al.*, 2013). Silencing of *Nicotiana benthamiana C14* leads to increased susceptibility for the oomycete pathogen *Phytophthora infestans* (Kaschani *et al.*, 2010; Bozkurt *et al.*, 2011). Likewise, tomato *rcr3* null mutants have lost resistance based on the *Cf-2* resistance gene against both the fungus *Cladosporium fulvum* and the nematode *Globodera rostochiensis* (Dixon *et al.*, 2000; Lozano-Torres *et al.*, 2012). The *rcr3* null mutants are also more susceptible for *P. infestans* (Song *et al.*, 2009), even in the absence of *Cf-2* (Ilyas *et al.*, 2015). Antisense lines depleted for the Pip1 protease of tomato are hypersusceptible to *C. fulvum*, *Pseudomonas syringae* and *P. infestans* (Ilyas *et al.*, 2015). Interestingly, silencing *NbPip1* in *N. benthamiana* blocks Avr4/Cf-4 induced hypersensitive response (HR) (Xu *et al.*, 2012), whereas silencing *NbCYP1* or *NbCYP2* in *N. benthamiana* increases susceptibility to the necrotrophic fungal pathogen *Colletotrichum destructivum* (Hao *et al.*, 2006). Furthermore, Arabidopsis *rd19* null mutants are impaired in resistance to the bacterial pathogen *Ralstonia solanacearum* (Bernoux *et al.*, 2008). Resistance to herbivore attack is also tightly linked to protease expression. Most prominent example is papain from Papaya, which is present in wound-exuding latex and is activated during wounding (El Moussaoui *et al.*, 2001; Azarkan *et al.*, 2006). Papain is also

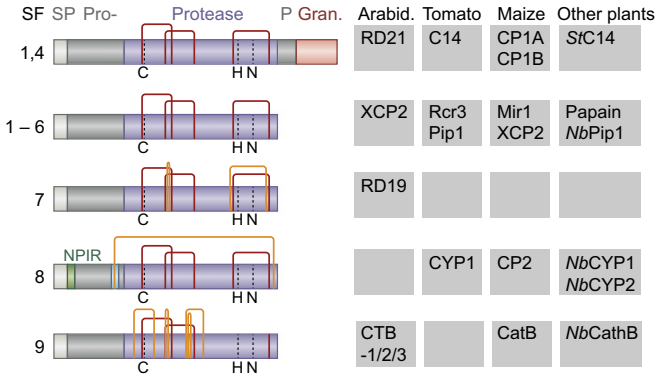

Fig. 1 Schematic representation of papain-like cysteine proteases (PLCPs) found in different plants. In general, PLCPs contain a signal peptide (SP, light grey), an auto-inhibitory pro-domain (Pro-, grey) and a protease domain (purple). The mature protease domain holds the catalytic triad Cys-His-Asn. Some members of subfamily 1 and subfamily 4 also have at the C- terminus a proline rich domain (P, grey) and a granulin domain (Gran., pink). Subfamily 8 proteases have at the N-terminus a vacuolar targeting signal (NPIR, green) and a minichain that remains after cleavage of the pro-domain (light blue). Subfamily 9 proteases contain the C-terminus motif ECGIE (red). Disulphide bridges common to most PLCPs (red thin lines) and, subfamily specific disulphide bridges (orange thin lines) are indicated. SF, subfamily classification; Arabid., Arabidopsis. Examples of known and characterized PLCPs described in plants (right, grey boxes).

responsible for the strong toxicity of papaya leaves to insects (Konno *et al.*, 2004). In maize leaves, Mir1 accumulates at wounding sites and confers enhanced resistance against caterpillars by degrading the peritrophic matrix of the insect gut (Pechan *et al.*, 2000, 2002). Accumulation of Mir1 also enhances resistance to root-feeding herbivores (Gill *et al.*, 2011) and Mir1 itself acts as an ethylene-dependent, long-distance transport signal that confers resistance to corn leaf aphids (Louis *et al.*, 2015). In summary, PLCPs are found to be required for plant defense to various kinds of biotic stresses in unrelated species.

III. PLCPs are common targets of pathogen effectors

PLCPs representing different subfamilies are targeted by a variety of unrelated pathogen-derived effectors (Table 1). C14 of tomato and potato is inhibited by the cystatin-like effectors EpiC1 and EpiC2B, which are secreted by *P. infestans* (Kaschani *et al.*, 2010). The C14 protease of tomato is also targeted by the *P. infestans* effector AvrBlb2, which prevents C14 secretion into the apoplast presumably by blocking its function in defense (Bozkurt *et al.*, 2011). Closely related to C14 are maize proteases CP1A and CP1B, which are inhibited by the Pit2 effector from the fungal pathogen *Ustilago maydis* (Mueller *et al.*, 2013). Pit2 also suppresses the activity of maize proteases XCP2 and CP2, respectively (Mueller et al., 2013). Likewise, tomato CYP1, is targeted and inhibited by the RNA-silencing suppressor V2 from the tomato yellow leaf curl geminivirus (Bar-Ziv *et al.*, 2012, 2015). A striking example for a PLCP being targeted by unrelated plant pathogens is tomato Rcr3. At first, it was found to be required for fungal resistance (Krüger *et al.*, 2002). The fungal pathogen *C. fulvum* secretes the effector Avr2, which inhibits Rcr3 (Rooney *et al.*, 2005). In addition, Rcr3 is inhibited by EpiC1 and EpiC2B from *P. infestans* (Song *et al.*, 2009) as well as by Gr-VAP1, an allergen-like effector secreted by the nematode *G. rostochiensis* (Lozano-Torres *et al.*, 2012). Notably, Avr2, EpiC1/2B and Gr-VAP1, although all inhibiting Rcr3, are unrelated proteins. A PLCP closely related to Rcr3 is tomato Pip1, which is also inhibited by EpiC2B (Tian *et al.*, 2007) and Avr2 (Shabab *et al.*, 2008). Another example is Arabidopsis RD19, which is re-localized to the host cell nucleus by the bacterial type III effector PopP2 from *R. solanacearum* (Bernoux *et al.*, 2008). In summary, a growing body of literature demonstrates that evolutionarily unrelated plant pathogens including fungi, oomycete, nematodes, bacteria and viruses actively interfere with the activity and subcellular location of plant PLCPs.

IV. PLCPs induce defense responses and cell death

One of the first indications that apoplastic cysteine proteases may act in immune signalling was the finding that E-64, a well-known inhibitor of cysteine proteases, can delay hypersensitive response in the cowpea–cowpea rust fungus system (D'Silva *et al.*, 1998). Later on, it was discovered that *N. benthamiana* Cathepsin B (*Nb*CathB) is required for the hypersensitive response and disease resistance induced by nonhost bacterial pathogens (Gilroy *et al.*, 2007; McLellan *et al.*, 2009). Furthermore, Arabidopsis Catheptin B

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Table 1 Plant papain-like cysteine proteases (PLCPs) involved in biotic interactions

PLCP	Species	SF	Function/phenotype	References
RD21	Arabidopsis	1	Knockout (KO)-lines susceptible to *Botrytis cinerea*	Shindo *et al.* (2012)
			KO-lines resistant to *B. cinerea* in detached leaves and *Sclerotinia sclerotiorum*. Inhibited by AtSerpin1	Lampl *et al.* (2013)
Mir1	Maize	1	Accumulates at wounding sites	Pechan *et al.* (2000, 2002)
			Enhanced resistance against caterpillars/root-feeding herbivores	Gill *et al.* (2011)
			Acts as ethylene signal conferring resistance to aphids	Louis *et al.* (2015)
C14	Potato	1	Inhibited by *Phytophthora infestans* effectors EPIC1 and EPIC2B. Protease under diversifying selection.	Kaschani *et al.* (2010); Kaschani & Van der Hoorn (2011)
	Tomato		Targeted by *P. infestans* effectors EPIC1, EPIC2B and AvrBlb2	Kaschani *et al.* (2010); Bozkurt *et al.* (2011)
Papain	Papaya	3	Activated during wounding	Azarkan *et al.* (2006)
			Involved in defense against polyphagous pests	Konno *et al.* (2004)
XCP2	Arabidopsis	3	Increases susceptibility to *Ralstonia solanacearum*	Zhang *et al.* (2014)
	Maize		Inhibited by *Ustilago maydis* effector Pit2 and maize cystatin CC9	Mueller *et al.* (2013); Van der Linde *et al.* (2012a,b)
C14	*Nicotiana benthamiana*	4	Silenced plants resistant to *P. infestans*	Kaschani *et al.* (2010); Bozkurt *et al.* (2011)
CP1A/ CP1B	Maize	4	Inhibited by *U. maydis* effector Pit2 and maize cystatin CC9	Mueller *et al.* (2013); Van der Linde *et al.* (2012a,b)
Rcr3	Tomato	6	Resistance to *Cladosporium fulvum*, *Globodera rostochiensis* and *P. infestans*	Dixon *et al.* (2000); Lozano-Torres *et al.* (2012); Song *et al.* (2009)
			Required for the function of Cf2 conferring fungal resistance	Krüger *et al.* (2002)
			Inhibited by effectors Avr2, EPIC1, EPIC2B and GrVAP1	Rooney *et al.* (2005); Song *et al.* (2009); Lozano-Torres *et al.* (2012)
Pip1	Tomato	6	Mutants are hypersusceptible to *C. fulvum*, *P. infestans* and *Pseudomonas syringae*	Ilyas *et al.* (2015)
			Inhibited by *P. infestans* EPIC2B and *C. fulvum* Avr2	Tian *et al.* (2007); Shabab *et al.* (2008)
	N. benthamiana		Silencing blocks HR induced by Avr4/Cf4 recognition	Xu *et al.* (2012)
RD19	Arabidopsis	7	Mutants are impaired in resistance to *R. solanacearum*	Bernoux *et al.* (2008)
			Targeted by PopP2 from *R. solanacearum*	Bernoux *et al.* (2008)
CYP1/ CYP2	*N. benthamiana*	8	Silencing enhanced susceptibility to *Colletotrichum destructivum*	Hao *et al.* (2006)
CYP1	Tomato	8	Inhibited by V2 from tomato yellow leaf curl geminivirus	Bar-Ziv *et al.* (2012, 2015)
CP2	Maize	8	Inhibited by *U. maydis* effector Pit2 and maize cystatin CC9	Mueller *et al.* (2013); Van der Linde *et al.* (2012a,b)
CathB	Arabidopsis	9	Required for hypersensitive response (HR) induced by nonhost bacterial pathogens	Gilroy *et al.* (2007); McLellan *et al.* (2009)
			Mutants show reduced programmed cell death during abiotic stress	Ge *et al.* (2016)

SF, phylogenetic classification of PLCPs into subfamilies according to Richau *et al.* (2012).

(*ctb*) mutants show reduced programmed cell death (PCD) induced by abiotic stresses (Ge *et al.*, 2016). Arabidopsis RD21 has been identified as a 'pro-death' signal activated during elicitation of cell death. The serpin protease inhibitor, AtSerpin1, exhibits a pro-survival function by covalently inhibiting RD21 and causing a change in compartmentalization (Lampl *et al.*, 2013). Gene expression analyses on barley have shown upregulation of PLCPs during senescence, a form of PCD, for almost all members of different subfamilies (Diaz-Mendoza *et al.*, 2014) but a role during disease resistance still remains to be elucidated.

Besides the contribution of PLCPs in PCD, direct evidence for the importance of apoplastic cysteine proteases during defence responses came from the finding that salicylic acid (SA) treatment activates PLCPs in maize, and that PLCPs themselves activate SA-related gene expression (Van der Linde *et al.*, 2012a,b). Remarkably, inhibition of maize apoplastic cysteine proteases by the endogenous cystatin CC9 is essential to suppress host immunity during infection with the biotrophic pathogen *U. maydis* (Van der Linde *et al.*, 2012a,b). Furthermore, Arabidopsis PIRIN2, a member of the cupin protein subfamily, stabilizes the protease XCP2 and increases susceptibility to the vascular pathogen *R. solanacearum* (Zhang *et al.*, 2014). Recently, a 9-lipoxygenase-derived cyclopentanone in maize, 10-oxo-11-phytoenoic acid (10-OPEA), was found to act as a potent cell death signal in multiple organs present during biotic stresses and developmental conditions (Christensen *et al.*, 2015, 2016). The cell death inducing activity of 10-OPEA was characterized by ion leakage and apoptotic-like DNA fragmentation in maize treated leaves (Christensen *et al.*, 2015). Interestingly, the cell-death inducing activity of 10-OPEA requires induction of PLCPs. Consequently, maize plants overexpressing the cystatin CC9 were partially insensitive to 10-OPEA providing further evidence for the importance of PLCPs during immunity.

V. PLCPs can act as co-receptors

Tomato Rcr3 is required for the function of the receptor-like protein Cf-2, which confers resistance against *C. fulvum* secreting

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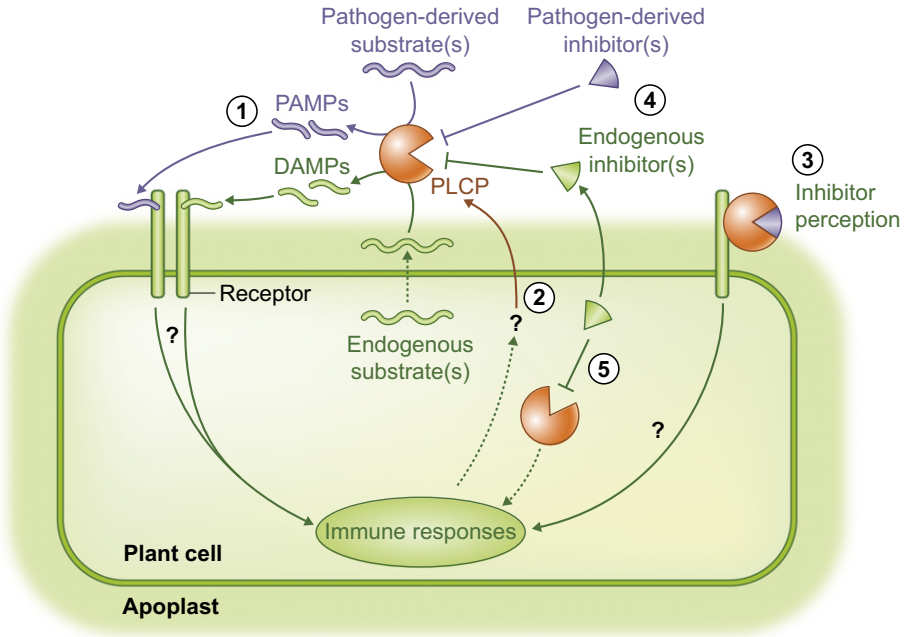

Fig. 2 Tentative model summarizing known and hypothetical functions of papain-like cysteine proteases (PLCPs) during plant immune-signaling. (1) PLCPs might release damage associated molecular patterns (DAMPs) or pathogen associated molecular patterns (PAMPS) that are recognized by receptors activating signaling cascades and consequently immune responses. (2) Likewise, induction of defense responses, for example by salicylic acid (SA) signaling, may lead to an activation of PLCPs, establishing a feedback loop. (3) PLCPs act as co-receptors and 'decoys' that evolve during an evolutionary arms race to avoid pathogen colonization. (4) To overcome immunity, pathogens produce effector molecules inhibiting PLCP activity. Because PLCPs are mainly activated by post-transcriptional processing, endogenous inhibitors such as cystatins or serpins may control the outcome in different signaling pathways leading to activation or deactivation of immune responses including programmed cell death (PCD) (5).

Avr2 (Krüger *et al.*, 2002). Avr2 binds to and inhibits Rcr3 and this complex is sensed by Cf-2, consistent with the Guard and Decoy Models (Rooney *et al.*, 2005; Van der Hoorn & Kamoun, 2008). Remarkably, Rcr3 is also required for the perception of nematode effector Gr-VAP1, which also inhibits Rcr3 and triggers immune responses in the presence of Cf-2 (Lozano-Torres *et al.*, 2012). Interestingly, VAP proteins from different nematodes can suppress PCD mediated by surface-localized immune receptors in Arabidopsis (Lozano-Torres *et al.*, 2014). The molecular mechanism of Avr2/Gr-VAP1 perception is not yet understood fully, but one emerging hypothesis is that Rcr3 is constitutively bound to Cf-2 protein, acting as a co-receptor to perceive the presence of protease inhibitors (Ilyas *et al.*, 2015). Rcr3 of cultivated tomato ($Rcr3^{lyc}$) triggers auto-necrosis in combination with Cf-2, which originates from *S. pimpinellifolium* (Krüger *et al.*, 2002). However, the allelic $Rcr3^{pim}$ protein suppresses this necrotic response in the $Rcr3^{pim}$/$Rcr3^{lyc}$ hybrid, suggesting that $Rcr3^{pim}$ protein can outcompete $Rcr3^{lyc}$ and consistent with the pre-existing co-receptor model (Ilyas *et al.*, 2015). These data illustrate that PLCPs can operate as co-receptors, sensing perturbations of receptor proteins thus activating defense responses.

VI. Natural variation in PLCPs is caused by arms races and host adaptation

Antagonistic protease-inhibitor interactions cause an arms race that has left its traces in the natural variation of proteases. This was first observed for Rcr3 and Pip1 (Shabab *et al.*, 2008). Natural variation of Rcr3 in wild tomato species resides on the surface of Rcr3, surrounding the active site, and likely represents the footprints of pathogen-derived inhibitors. Indeed, the variant N194D residue in Rcr3 locates close the catalytic Cys and reduces its interaction with Avr2 (Shabab *et al.*, 2008). Interestingly, N194D is also the only variant residue that exclusively prevents inhibition by Avr2 in natural Rcr3 variants (Hörger *et al.*, 2012). The N194D mutation also abolished HR-inducing activity in plants carrying *Cf-2* resistance genes. Other variant residues affect the strength of the HR response, presumably because of the interaction of Rcr3 with Cf-2 (Hörger *et al.*, 2012). Natural variation within Rcr3 also affects its interaction with Gr-VAP1 of the nematode *G. rostochiensis*, which interacts with $Rcr3^{pim}$ but not $Rcr3^{lyc}$, even though these proteases only differ in a few amino acids (Lozano-Torres *et al.*, 2012). The other apoplastic proteases of tomato do not accumulate many variant residues on the surface, consistent with not being targeted by pathogen-derived inhibitors (Shabab *et al.*, 2008). This includes C14 of wild tomato, which is inhibited by cystatin-like EpiC of *P. infestans*. However, *P. infestans* has coevolved with wild potato, and C14 in wild potato carries variant residues at its surface, illustrating that traces of arms races can be found only in coevolving host–pathogen interactions (Kaschani & Van der Hoorn, 2011). Interestingly, the cystatin-like *Pm*EpiC inhibitor of *P. mirabilis*, which has jumped onto a different host plant only recently, carries an adaptation that facilitates inhibition of the proteases of the new host, but causes reduced affinity to the proteases of the presumed former host (Dong *et al.*, 2014). Taken together,

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PLCP inhibitor arms races strengthen the notion that PLCPs are an important part of extracellular defense.

VII. Conclusion: how do PLCPs activate immunity?

In light of the increasing evidence of papain-like cysteine proteases (PLCPs) being crucial components of plant immunity, one of the most intriguing questions is how their activity actually results in defense stimulation (Fig. 2). Interestingly, their capability to induce immune responses is not restricted to plants, which may suggest activation of highly conserved pathways in the innate immune system. Known plant-derived allergens are cysteine proteases, such as the ragweed (*Ambrosia artemisiifolia*) allergen Amba11 (Bouley *et al.*, 2015), papain or bromelain (Stewart & Thompson, 1996). For Papain it has been found that its proteolytic activity is required for triggering immune responses including MAPK signaling in human cells (Rosenstein *et al.*, 2014). Besides the well-known mechanism that proteases break down the barrier in lungs against allergens, a recently discovered mechanism of PLCPs to induce immune responses is the activation of protease-activated G-protein coupled receptors (Reddy *et al.*, 2015). Interestingly, not only endogenous Cathepsin S, but also the plant-derived proteases papain and mucunan (from tropical bean) were found to induce protease-activated receptors in mammals (Reddy *et al.*, 2015). Controlled proteolysis of receptor proteins, also referred to as ectodomain shedding, is well known in animal systems but little known in plants. First evidence for this mechanism comes from the *Arabidopsis thaliana* chitin receptor CERK1, yet the protease involved in this process remains elusive (Petutschnig *et al.*, 2014). Besides activation of receptors, proteases can release small peptides that are perceived as DAMPs to induce immunity. A fascinating mechanism was found for a soybean subtilisin-like protease, which releases an embedded cryptic 12-aa signal that triggers defense gene activation (Pearce *et al.*, 2010). However, for PLCPs this kind of mechanism has not been identified so far. It is challenging to deepen our understanding of the involvement of PLCPs in plant immunity because many open questions still have to be addressed. For example, to which extent is proteolytic activity of PLCPs required for triggering plant immunity? How is activation of PLCPs orchestrated? Salicylic acid treatment in maize triggers activation of PLCPs but there is still the possibility that it acts as a feedback loop because PLCPs themselves induce *PR*-gene expression (Van der Linde *et al.*, 2012a). Additionally, the substrates of PLCPs are still unknown. Plants contain a plethora of PLCPs localized in different compartments, but how is specificity achieved? Interestingly, E-64d has been used extensively to suppress autophagy but also apoplastic cysteine proteases. Is it a strategy that pathogens like *P. infestans* deploy to prevent secretion of PLCPs into the apoplast by AvrBlb2 (Bozkurt *et al.*, 2011), or to antagonize host autophagy cargo receptors to counteract host defenses (Dagdas *et al.*, 2016)? Furthermore, activation of PLCPs in the cell might induce a massive proteolytic activity provoking clearance of cell contents and cell death. In this case PLCPs may need little specificity whilst still releasing signaling molecules. In light of all the different immune responses involving PLCP activity, it will be a striking challenge to elucidate how target-specificity of PLCPs is regulated and how they discriminate between pathogen and host proteins.

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References

Azarkan M, Dibiani R, Baulard C, Baeyens-Volant D. 2006. Effects of mechanical wounding on *Carica papaya* cysteine endopeptidases accumulation and activity. *International Journal of Biological Macromolecules* **38**: 216–224.

Bar-Ziv A, Levy Y, Citovsky V, Gafni Y. 2015. The Tomato yellow leaf curl virus (TYLCV) V2 protein inhibits enzymatic activity of the host papain-like cysteine protease CYP1. *Biochemical and Biophysical Research Communications* **460**: 525–529.

Bar-Ziv A, Levy Y, Hak H, Mett A, Belausov E, Citovsky V, Gafni Y. 2012. The Tomato yellow leaf curl virus (TYLCV) V2 protein interacts with the host papain-like cysteine protease CYP1. *Plant Signaling & Behavior* **7**: 983–989.

Bernoux M, Timmers T, Jauneau A, de Briere C, Wit PJ, Marco Y, Deslandes L. 2008. RD19, an *Arabidopsis* cysteine protease required for RRS1-R-mediated resistance, is relocalized to the nucleus by the *Ralstonia solanacearum* PopP2 effector. *Plant Cell* **20**: 2252–2264.

Bouley J, Groeme R, Le Mignon M, Jain K, Chabre H, Bordas-Le Floch V, Couret MN, Bussières L, Lautrette A, Naveau M *et al.* 2015. Identification of the cysteine protease Amb a 11 as a novel major allergen from short ragweed. *Journal of Allergy and Clinical Immunology* **136**: 1055–1064.

Bozkurt T, Schornack S, Win J, Shindo T, Ilyas M, Oliva R, Cano LM, Jones AME, Huitema E, Van der Hoorn RAL. 2011. *Phytophthora infestans* effector AVRblb2 prevents secretion of a plant immune protease at the haustorial interface. *Proceedings of the National Academy of Sciences, USA* **108**: 20832–20837.

Christensen SA, Huffaker A, Hunter CT, Alborn HT, Schmelz EA. 2016. A maize death acid, 10-oxo-11-phytoenoic acid, is the predominant cyclopentenone signal present during multiple stress and developmental conditions. *Plant Signaling & Behavior* **11**: e1120395.

Christensen SA, Huffaker A, Kaplan F, Sims J, Ziemann S, Doehlemann G, Ji L, Schmitz RJ, Kolomiets MV, Alborn HT *et al.* 2015. Maize death acids, 9-lipoxygenase derived cyclopentenones and derivatives, display activity as cytotoxic phytoalexins and transcriptional mediators. *Proceedings of the National Academy of Sciences, USA* **112**: 11407–11412.

Dagdas YF, Belhaj K, Maqbool A, Chaparro-Garcia A, Pandey P, Petre B, Tabassum N, Cruz-Mireles N, Hughes RK, Sklenar J *et al.* 2016. An effector of the Irish potato famine pathogen antagonizes a host autophagy cargo receptor. *eLife* **5**: e10856.

Diaz-Mendoza M, Arroyo-Velasco B, Gonzalez-Melendi P, Martinez M, Diaz I. 2014. C1A cysteine protease–cystatin interactions in leaf senescence. *Journal of Experimental Botany* **65**: 3825–3833.

Dixon M, Golstein C, Thomas CM, van der Biezen EA, Jones JDG. 2000. Genetic complexity of pathogen perception by plants: the example of *Rcr3*, a tomato gene required specifically by *Cf-2*. *Proceedings of the National Academy of Sciences, USA* **97**: 8807–8814.

Dong S, Stam R, Cano LM, Song J, Sklenar J, Yoshida K, Bozkurt TO, Oliva R, Liu Z, Tian M *et al.* 2014. Effector specialization in a lineage of the Irish potato famine pathogen. *Science* **343**: 552–555.

D'Silva I, Guy GMichèle C. 1998. Activation of cysteine proteases in cowpea plants during the hypersensitive response – a form of programmed cell death. *Experimental Cell Research* **245**: 389–399.

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El Moussaoui A, Nijs M, Paul C, Wintjens R, Vincentelli J, Azarkan M, Looze Y. 2001. Revisiting the enzymes stored in the laticifers of *Carica papaya* in the context of their possible participation in the plant defence mechanism. *Cellular and Molecular Life Sciences* **58**: 556–570.

Ge Y, Cai Y, Bonneau L, Rotari V, Danon A, McKenzie EA, McLellan H, Mach L, Gallois P. 2016. Inhibition of cathepsin B by caspase-3 inhibitors blocks programmed cell death in *Arabidopsis*. *Cell Death and Differentiation*. doi: 10.1038/cdd.2016.34.

Gill TA, Sandoya G, Williams P, Luthe DS. 2011. Belowground resistance to western corn rootworm in lepidopteran-resistant maize genotypes. *Journal of Economic Entomology* **104**: 299–307.

Gilroy EM, Hein I, van der Hoorn R, Boevink PC, Venter E, McLellan H, Kaffarnik F, Hrubikova K, Shaw J, Holeva M *et al.* 2007. Involvement of cathepsin B in the plant disease resistance hypersensitive response. *Plant Journal* **52**: 1–13.

Hao L, Hsiang T, Goodwin PH. 2006. Role of two cysteine proteinases in the susceptible response of *Nicotiana benthamiana* to infection by *Colletotrichum destructivum* and the hypersensitive response to *Pseudomonas syringae* pv. *tomato*. *Plant Science* **170**: 1001–1009.

Hörger AC, Ilyas M, Stephan W, Tellier A, Van der Hoorn RAL, Rose LE. 2012. Balancing selection at the tomato *RCR3* guardee gene family maintains variation in strength of pathogen defense. *PLoS Genetics* **8**: e1002813.

Ilyas M, Hörger AC, Bozkurt TO, Van den Burg HA, Kaschani F, Kaiser M, Belhaj K, Smoker M, Joosten MHAJ, Kamoun S *et al.* 2015. Functional divergence of two secreted immune proteases of tomato. *Current Biology* **25**: 2300–2306.

Kaschani F, Shabab M, Bozkurt T, Shindo T, Schornack S, Gu C, Ilyas M, Win J, Kamoun S, Van der Hoorn RAL. 2010. An effector-targeted protease contributes to defense against *Phytophthora infestans* and is under diversifying selection in natural hosts. *Plant Physiology* **154**: 1794–1804.

Kaschani F, Van der Hoorn RAL. 2011. A model of the C14-EPIC complex indicates hotspots for a protease-inhibitor arms race in the oomycete–potato interaction. *Plant Signaling & Behavior* **6**: 1–4.

Konno K, Hirayama C, Nakamura M, Tateishi K, Tamura Y, Hattori M, Kohno K. 2004. Papain protects papaya trees from herbivorous insects: role of cysteine proteases in latex. *Plant Journal* **37**: 370–378.

Krüger J, Thomas CM, Golstein C, Dixon MS, Smoker M, Tang S, Mulder L, Jones JDG. 2002. A tomato cysteine protease required for *Cf-2* -dependent disease resistance and suppression of autonecrosis. *Science* **296**: 744–747.

Lampl N, Alkan N, Davydov O, Fluhr R. 2013. Set-point control of RD21 protease activity by AtSerpin1 controls cell death in Arabidopsis. *Plant Journal* **74**: 498–510.

Louis J, Basu S, Varsani S, Castano-Duque L, Jiang V, Williams WP, Felton GW, Luthe DS. 2015. Ethylene contributes to *maize insect resistance1*-mediated maize defense against the phloem sap-sucking corn leaf aphid. *Plant Physiology* **169**: 313–324.

Lozano-Torres JL, Wilbers RHP, Gawronski P, Boshoven JC, Finkers-Tomczak A, Cordewener JHG, America AHP, Overmars HA, Van 't Klooster JW, Baranowski L *et al.* 2012. Dual disease resistance mediated by immune receptor Cf-2 in tomato require a common virulence target of a fungus and a nematode. *Proceedings of the National Academy of Sciences, USA* **109**: 10119–10124.

Lozano-Torres JL, Wilbers RHP, Warmerdam S, Finkers-Tomczak A, Diaz-Granados A, van Schiak CC, Helder J, Bakker J, Goverse A, Schots A *et al.* 2014. Apoplastic venom allergen-like proteins of cyst nematodes modulate the activation of basal plant innate immunity by cell surface receptors. *PLoS Pathogens* **10**: e1004569.

McLellan H, Gilroy EM, Yun B-W, Birch PRJ, Loake GJ. 2009. Functional redundancy in the *Arabidopsis Cathepsin B* gene family contributes to basal defence, the hypersensitive response and senescence. *New Phytologist* **183**: 408–418.

Mueller AN, Ziemann S, Treitschke S, Aßmann D, Doehlemann G. 2013. Compatibility in the *Ustilago maydis*–maize interaction requires inhibition of host cysteine proteases by the fungal effector Pit2. *PLoS Pathogens* **9**: e1003177.

Pearce G, Yamaguchi Y, Barona G, Ryan CA. 2010. A subtilisin-like protein from soybean contains an embedded, cryptic signal that activates defense-related genes. *Proceedings of the National Academy of Sciences, USA* **107**: 14 921–14 925.

Pechan T, Cohen A, Williams WP, Luthe DS. 2002. Insect feeding mobilizes a unique plant defense protease that disrupts the peritrophic matrix of caterpillars. *Proceedings of the National Academy of Sciences, USA* **99**: 13 319–13 323.

Pechan T, Ye L, Chang Y, Mitra A, Lin L, Davis FM, Williams WP, Luthe DS. 2000. A unique 33-kD cysteine proteinase accumulates in response to larval feeding in maize genotypes resistant to fall army- worm and other Lepidoptera. *Plant Cell* **12**: 1031–1040.

Petutschnig EK, Stolze M, Lipka U, Kopischke M, Horlacher J, Valerius O, Rozhon W, Gust AA, Kemmerling B, Poppenberger B *et al.* 2014. A novel Arabidopsis CHITIN ELICITOR RECEPTOR KINASE 1 (CERK1) mutant with enhanced pathogen-induced cell death and altered receptor processing. *New Phytologist* **204**: 955–967.

Reddy V, Sun S, Azimi E, Elmariah SB, Dong X, Lerner EA. 2015. Redefining the concept of protease-activated receptors: cathepsin S evokes itch via activation of Mrgprs. *Nature Communications* **6**: 1–10.

Richau K, Kaschani F, Verdoes M, Pansuriya TC, Niessen S, Stüber K, Overkleeft HS, Bogyo M, Van der Hoorn RAL. 2012. Subclassification and biochemical analysis of plant papain-like cysteine proteases displays subfamily-specific characteristics. *Plant Physiology* **158**: 1583–1599.

Rooney H, Van't Klooster J, Van der Hoorn RAL, Joosten MHAJ, Jones JDG, De Wit PJGM. 2005. *Cladosporium* Avr2 inhibits tomato Rcr3 protease required for *Cf-2*-dependent disease resistance. *Science* **308**: 1783–1789.

Rosenstein RK, Bezbradica JS, Yu S, Medzhitov R. 2014. Signaling pathways activated by a protease allergen in basophils. *Proceedings of the National Academy of Sciences, USA* **111**: E4963–E4971.

Shabab M, Shindo T, Gu C, Kaschani F, Pansuriya T, Chintha R, Harzen A, Colby T, Kamoun S, Van der Hoorn RAL. 2008. Fungal effector protein AVR2 targets diversifying defence-related Cys proteases of tomato. *Plant Cell* **20**: 1169–1183.

Shindo T, Misas-Villamil JC, Hörger A, Song J, Van der Hoorn RAL. 2012. A role in immunity for Arabidopsis cysteine protease RD21, the ortholog of the tomato immune protease C14. *PLoS ONE* **7**: e29317.

Song J, Win J, Tian M, Schornack S, Kaschani F, Muhammad I, Van der Hoorn RAL, Kamoun S. 2009. Apoplastic effectors secreted by two unrelated eukaryotic plant pathogens target the tomato defense protease Rcr3. *Proceedings of the National Academy of Sciences, USA* **106**: 1654–1659.

Stewart GA, Thompson PJ. 1996. The biochemistry of common aeroallergens. *Clinical & Experimental Allergy* **26**: 1020–1044.

Tian M, Win J, Song J, Van der Hoorn RAL, Van der Knaap E, Kamoun S. 2007. A *Phytophthora infestans* cystatin-like protein interacts with and inhibits a tomato papain-like apoplastic protease. *Plant Physiology* **143**: 364–377.

Van der Hoorn RAL, Kamoun S. 2008. From guard to decoy: a new model for perception of plant pathogen effectors. *Plant Cell* **20**: 2009–2017.

Van der Linde K, Hemetsberger C, Kastner C, Kaschani F, van der Hoorn RA, Kumlehn J, Doehlemann G. 2012a. A maize cystatin suppresses host immunity by inhibiting apoplastic cysteine proteases. *Plant Cell* **24**: 1285–1300.

Van der Linde K, Mueller AN, Hemetsberger C, Kaschani F, Van der Hoorn RAL, Doehlemann G. 2012b. The maize cystatin CC9 interacts with apoplastic cysteine proteases. *Plant Signaling & Behavior* **7**: 1397–1401.

Xu QF, Cheng WS, Li SS, Li W, Zhang ZX, Xu YP, Zhou XP, Cai XZ. 2012. Identification of genes required for *Cf*-dependent hypersensitive cell death by combined proteomic and RNA interfering analyses. *Journal of Experimental Botany* **63**: 2421–2435.

Zhang B, Tremousaygue D, Denancé N, van Esse HP, Hörger AC, Dabos P, Goffner D, Thomma BPHJ, van der Hoorn RAL, Tuominen H. 2014. PIRIN2 stabilizes cysteine protease XCP2 and increases susceptibility to the vascular pathogen *Ralstonia solanacearum* in Arabidopsis. *Plant Journal* **79**: 1009–1019.

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