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REVIEW PAPER

Peptides as triggers of plant defence

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Abstract

Plants are confronted with several biotic stresses such as microbial pathogens and other herbivores. To defend against such attackers, plants possess an array of pattern recognition receptors (PRRs) that sense the danger and consequently initiate a defence programme that prevents further damage and spreading of the pest. Characteristic pathogenic structures, so-called microbe-associated molecular patterns (MAMPs), serve as signals that allow the plant to sense invaders. Additionally, pathogens wound or damage the plant and the resulting release of damage-associated molecular patterns (DAMPs) serves as a warning signal. This review focuses on peptides that serve as triggers or amplifiers of plant defence and thus follow the definition of a MAMP or a DAMP.

Key words: Damage-associated molecular pattern, ligand–receptor interaction, microbe-associated molecular pattern, pattern recognition receptors, peptide ligand, plant defence, plant immunity.

Introduction: plant immunity

Plants as sessile organisms have to resist abiotic and biotic stresses without the option to escape. To defend against pathogen attacks, plants possess an efficient innate immune system to battle the—mostly microbial—enemies. Typical defence reactions and cellular responses are initiated right after a plant comes into contact with a pathogen and serve as useful bioassays to monitor plant defence. They can be subdivided into very early or early responses (1–30 min post-pathogen contact) and late responses (hours–days post-pathogen contact) (Boller and Felix, 2009). Very characteristic early responses are ion fluxes (Boller, 1995; Nürnberger *et al.*, 2004), the production of reactive oxygen species (ROS) (Apostol *et al.*, 1989; Apel and Hirt, 2004), ethylene production (Spanu *et al.*, 1994), mitogen-activated protein kinase (MAPK) activation (Nühse *et al.*, 2000; Asai *et al.*, 2002), and the expression of typical defence-related genes (Ramonell and Somerville, 2002; Zipfel *et al.*, 2004, 2006). Late responses include cell wall modifications such as callose deposition (Rodriguez-Galvez and Mendgen, 1995; Gomez-Gomez *et al.*, 1999) or seedling/root growth inhibition (Zipfel *et al.*, 2006). A clearly visible resistance reaction is represented by the hypersensitive response (HR) including programmed cell death (PCD) of the infected tissue. This necrosis is mediated via resistance proteins encoded by resistance genes (R-genes) and restricts the growth of the pathogen to prevent the plant from further damage (Lukasik and Takken, 2009; Takken and Tameling, 2009). In addition to the above-mentioned locally restricted responses, systemic responses can also be initiated by the plant, termed systemic acquired resistance (SAR) and induced systemic resistance (ISR) (Ross, 1961; Prime *et al.*, 2006; Spoel and Dong, 2012).

However, a successful defence is dependent on a highly sensitive and specific recognition system with the ability to sense 'danger' and consequently to switch on plant defence. Pathogens, in turn, provide signatures or characteristic 'patterns' that serve as a 'molecular identity card' and allow the plant to identify the external invader. Prominent examples for such microbe-associated molecular patterns (MAMPs) derive from typical microbial structures such as fungal chitin (Felix *et al.*, 1993), bacterial peptidoglycan (Gust *et al.*, 2007), or flagellin (Felix *et al.*, 1999) (Fig. 1). In addition, many pathogens utilize degrading and cleaving enzymes during plant infection that damage plant cells

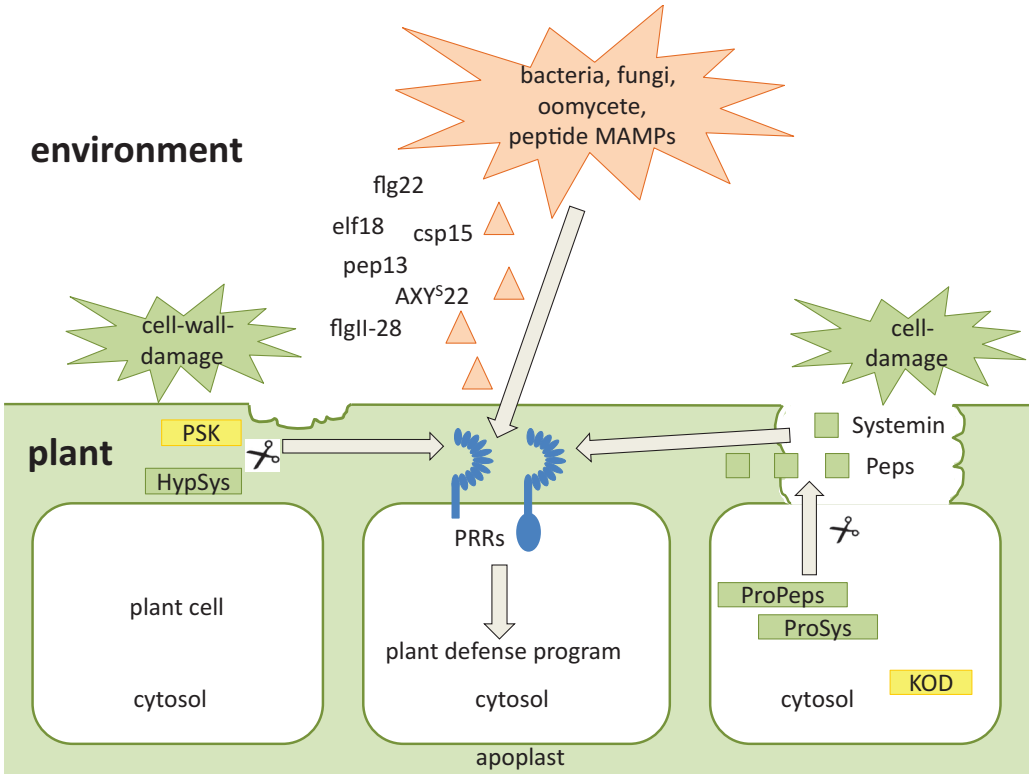

Fig. 1. Peptides in plant defence. Attacked plants can sense peptides as microbe-associated molecular patterns (MAMPs; e.g. flg22, elf18, csp15, flgII-28, pep13, axY^{S}22; for details, see Table 1) directly via pattern recognition receptors (PRRs, blue). Furthermore, after cell or cell wall damage, plants sense damage-associated molecular patterns (DAMPs) deriving from cytosolic precursor proteins after they are cleaved off (e.g. systemin from prosystemin; Peps from proPeps). Other DAMPs (HypSys, hydroxyproline-rich systemins) or DAMP candidates (PSKs, phytosulphokines), respectively, locate to the apoplast where they might become activated after plant damage. Peptides that are not yet defined as DAMPs but might be considered as DAMP candidates are indicated in yellow (PSK, KOD, kiss of death).

and generate characteristic degradation products that can serve as so-called 'damage-associated molecular patterns' (DAMPs) (Fig. 1), for example plant cell wall fragments (Ortmann *et al.*, 2006) or peptides deriving from cleaved and degraded proteins (Yamaguchi and Huffaker, 2011).

To sense these molecular patterns indicative of a pathogen attack, plants possess an array of different pattern recognition receptors (PRRs). Most of the PRRs known so far belong to the protein families of receptor-like kinases (RLKs) or receptor-like proteins (RLPs), which both locate to the plasma membrane and have an extracellular domain for highly specific ligand binding (Shiu and Bleecker, 2001, 2003; Shiu *et al.*, 2004). While RLPs only have a very short cytoplasmic tail of ~20–30 amino acid residues (Wang *et al.*, 2008), RLKs possess a cytosolic kinase domain. A further interaction of ligand-activated receptors with co-receptors, most often other RLKs, is then necessary for initialization of the cellular responses leading to a successful defence (Roux *et al.*, 2011). The most prominent co-receptor is BAK1 (BRI1-associated kinase 1) that is also an important interactor for the brassinosteroid receptor BRI1 (brassinosteroid-insensitive 1) (Li *et al.*, 2002; Chinchilla *et al.*, 2007). MAMP-triggered defence responses represent the first layer of immunity. Many microbial pathogens evolved mechanisms to suppress this host defence with so-called 'effector proteins' (effectors) that enter the plant cell. In turn, many host plants have advanced detection systems for such effectors and mount effective second layers of immunity termed effector-triggered immunity (ETI) (Spoel and Dong, 2012). This interplay between plants and pathogens follows the concept of the so-called 'zig-zag' model defined by Jones and Dangl (2006). In ETI, cytosolic NBS-LRR (nucleotide-binding site leucine-rich repeat) proteins (Meyers *et al.*, 1999) encoded by R-genes (Flor, 1956, 1971) play an important role in detecting the pathogen effectors and in further initiating a strong and fast HR to battle against the pathogens (reviewed in Maekawa *et al.*, 2011).

This review deals with molecules that indicate danger for a plant, thereby serving as initial triggers of plant defence responses. A special focus is placed on peptides that serve as MAMPs or DAMPs and their role as mediators, amplifiers, or initial triggers of plant immunity.

Peptide MAMPs as molecular signatures of plant pathogens

To date, only a few peptide MAMPs and their corresponding receptors have been identified. For some MAMPs, however, the plant receptor is as yet unknown. One of the first identified peptides is the 13 amino acid residue peptide Pep13,

representing an epitope of transglutaminases (TGases) present in different species of the oomycete *Phytophthora*. The 13 amino acid motif 'vwnqpvrgfkvye' (Table 1) was first identified from *Phytophthora sojae* and is highly conserved among TGases of many *Phytophthora* strains. Via an as yet unidentified receptor, the peptide induces the expression of defence-related genes already at concentrations of ~1 nM specifically in parsley (*Petroselinum crispum*) and potato (*Solanum tuberosum*) (Brunner *et al.*, 2002). Another example of a peptide MAMP, representing the 15 amino acid peptide epitope of the RNP-1 (RNA binding) motif of the bacterial cold shock protein, csp15 (Table 1), has been identified as a very potent elicitor, triggering defence responses with an EC_{50} of ~0.1 nM (Felix and Boller, 2003). The PRR with a high sensitivity and specificity for csp15 has not yet been identified. Interestingly, the receptor for csp15 is most probably present only in solanaceous plants since only in this plant family have responses to the cold shock protein been observed (Felix and Boller, 2003).

Bacteria of the genus *Xanthomonas* cause a lot of severe plant diseases with tremendous damage among crops, and thus are of special scientific interest to identify components of successful plant defence. To date, besides many *Xanthomonas* spp. effectors (Jia *et al.*, 2000), a few peptide MAMPs have also been identified which initiate plant defence responses. The 133 amino acid type three secreted elicitor protein HpaG of *Xanthomonas axonopodis* pv. *glycines* has been found to trigger a HR and innate immune responses in tobacco leaves. As the critical and fully active peptide epitope, a 23 amino acid fragment from the N-terminus of the protein seems to be sufficient (Table 1) (Kim *et al.*, 2003, 2004). Later, it was also shown that fragments of a *Xanthomonas oryzae* pv. *oryzicola* HpaG homologue stimulated rice growth and resulted in higher levels of resistance to *X. oryzae* pv. *oryzae* and *Magnaporthe grisea* pathogens, which cause bacterial leaf blight and rice blast, respectively (Chen *et al.*, 2008).

A well-known example for a *Xanthomonas* MAMP is AXY^S22, a sulphated peptide that derives from the *X. oryzae* protein AX21. The 17 amino acid long peptide of the AX21 N-terminal part is highly conserved among different *Xanthomonas* species and is only active when carrying a sulphated residue (Lee *et al.*, 2009). However, the perception of the synthetic peptide AXY^S22 was active only with an EC_{50} of ≥10 μM, indicating that alternative and probably better ligands for the corresponding receptor might be present in *Xanthomonas* spp. The leucine-rich repeat receptor-like kinase (LRR-RLK) XA21, the receptor for AX21, was identified long before its predicted ligand and was already cloned in 1995 (Song *et al.*, 1995). XA21 confers resistance against the rice pathogen *X. oryzae* also after transfer to other plants. By triggering a broad set of typical defence responses, including necrotic lesions that restrict pathogen growth, XA21 protects against rice blast (Song *et al.*, 1995).

The most thoroughly studied proteinaceous MAMPs, or peptide epitopes derived therefrom, are the bacterial flagellin

Table 1. *Overview of peptide MAMPs*

Peptide	Origin	Amino acid sequence	Perception		Related publication
csp22	Bacteria	AVGTVKWFNAEKGFGFITPDDG	Unknown		Felix and Boller (2003)
Pep13	*Phytophtora sojae*	VWNQPVRGFKVYE	Unknown		Brunner *et al.* (2002)
HaX23	*Xanthomonas axonopodis* pv. *glycines*	NQGISEKQLDQLLTQLIMALLQQ	Unknown		Kim *et al.* (2003)
axY^S22	*Xanthomonas oryzae* pv. *oryzae*	AENLSY^{S}NFVEGDYVRTP	XA21		Lee *et al.* (2009)
elf18	*Escherichia coli*	SKEKFERTKPHVNVGTIG	EFR		Kunze *et al.* (2004)
elf12		SKEKFERTKPHV	EFR		
flgII-28	*Pseudomonas syringae* pv. *tomato*	ESTNILQRMRELAVQSRNDSNSATDRDA	Unknown		Cai *et al.* (2011)
flg22	*Pseudomonas aeruginosa*	QRLSTGSRINSAKDDAAGLQIA	FLS2		Felix *et al.* (1999)
flg22	*Xanthomonas campestris* pv. *campestris*	QQLS**S**G**K**RI**T**SA**SV**DAAGL**AI**S	Inactive MAMP, unknown		Sun *et al.* (2006)
$flg15^{mel}$	*Rhizobium meliloti*	RV**GG**A**A**D**N**AA**YWS**IA	Inactive MAMP, unknown		Felix *et al.* (1999)
$flg22^{Atum}$	*Agrobacterium tumefaciens*	**D**RI**SS**G**LKVG**SA**S**D**N**AA**YWS**IA	Inactive MAMP, unknown		Felix *et al.* (1999)
$flg22^{Rsol}$	*Ralstonia solanacearum*	QR**L**STGLR**V**NSA**Q**DD**S**A**AYA**A**S**	AtFLS2[a]	SlFLS2	Bauer *et al.* (2001)
flg22-AYA	Synthetic	QRLSTGSRINSAKDDAA**AYA**IA	AtFLS2[a]	SlFLS2	
flg22-Δ2	Truncated flg22	QRLSTGSRINSAKDDAAGLQ	AtFLS2[a]	SlFLS2	
flg15	Truncated flg22	RINSAKDDAAGLQIA	AtFLS2[b]	SlFLS2	Mueller *et al.* (2012)
flg15Δ7	Truncated flg22	RINSAKDD	AtFLS2[c]	SlFLS2[a]	

Listed are peptides that have been identified as active MAMPs or as their related inactive forms (= antagonist), respectively. Species indicated as 'origin' represent the organism where the MAMP was first identified.
Amino acid residues indicated in bold are different from the originally conserved flg22 sequence.
EFR, elongation factor-Tu receptor; FLS2, flagellin sensing 2; At, *Arabidopsis thaliana*; Sl, *Solanum lycopersicum.*
[a] Peptide acts as antagonist only.
[b] Peptide acts as a weak agonist.
[c] Peptide is inactive as an agonist or antagonist.

and elongation factor Tu (EF-Tu). The receptors have been identified for both MAMPs: the LRR-RLKs EFR (EF-Tu receptor) (Zipfel *et al.*, 2006) and FLS2 (Flagellin sensing 2) (Gomez-Gomez and Boller, 2000; Gomez-Gomez *et al.*, 2001). While EFR seems to be unique for *Arabidopsis thaliana* and a few other closely related Brassicaceae, FLS2 seems to be more ubiquitous, and a perception system for flagellin is present in most higher plants (Albert *et al.*, 2010).

EF-Tu was initially identified as an elicitor from *Escherichia coli* bacteria lacking flagellin (*E. coli* flic$^-$) and represents one of the most abundant and conserved proteins among bacteria (Kunze *et al.*, 2004). The active epitope, the peptide elf18, was assigned to the N-terminus of EF-Tu and comprises 18 amino acids (Table 1). Interestingly, the corresponding receptor EFR (Zipfel *et al.*, 2006) shares similarity with XA21 based on the kinase domain, the number of 21 LRRs, and a characteristic small island domain of six amino acid residues between LRR 10 and 11 (Boller and Felix, 2009). Small gene families encoding such XA21-/EFR-like proteins can be found in all plant genomes sequenced so far. Comparing only the kinase domains of all XA21-/EFR-like proteins, a high sequence similarity can be observed and thus suggests an involvement of these proteins probably in similar cellular response programmes. This seems different for the ectodomains of the XA21-/EFR-like proteins: the sequences look very different from each other. Probably, the ligand specificities of other members belonging to this receptor clade are distinct and independent of elf18 or AXY^{S}22 perception. Thus, EFR or XA21 seem to be unique to Brassicaceae or rice, respectively, and other members of the XA21-/EFR-like proteins seem to be potential MAMP receptors for so far unknown ligands (Boller and Felix, 2009).

FLS2, the receptor for bacterial flagellin (Gomez-Gomez *et al.*, 2001), belongs to the same superfamily of proteins, the LRR-RLK XII family (Shiu and Bleecker, 2001; Shiu *et al.*, 2004), such as EFR, the XA21-/EFR-like proteins, and a few other related LRR-RLKs (11 receptors in total). It differs from EFR mainly in size and number of LRRs—28 instead of 21—and of course in its ligand specificity. The corresponding ligand and shortest active peptide is flg22, a 22 amino acid sequence that derives from the most conserved part from the N-terminal region of flagellin (Felix *et al.*, 1999). The sequence of this classically and often used flg22 is based on that of flagellin from *Pseudomonas aeruginosa* (Table 1). Filaments of a bacterial flagellum are tubular structures made of protofilaments, which are arrays of up to hundreds of flagellin molecules (O'Brien and Bennett, 1972). Interestingly, the flg22 epitope is buried in the interior of the flagellum and is neither solvent exposed nor accessible for the FLS2 receptor (Samatey *et al.*, 2001; Yonekura *et al.*, 2003). Flagellin monomers might leak into the bacterial environment during construction of the flagella (Komoriya *et al.*, 1999) or could be perceived after the flagella collapse.

In some solanaceous plants such as tomato, a second 28 amino acid peptide epitope of flagellin termed flgII-28 was shown to induce oxidative burst and other defence-related responses (Cai *et al.*, 2011). The flgII-28 sequence, ESTNILQRMRELAVQSRNDSNSATDRDA (Table 1), derives from the most common lineage (T_1) of *P. syringae* pv. tomato (Pto), and was the most active peptide compared with other flgII-28 sequences originating from other related Pto strains.

flg22: conservation and camouflage

Bacterial flagellin, especially the highly conserved 22 amino acid sequence that is recognized via FLS2, is under strong selective pressure since it is indispensable for functional flagella and bacterial mobility (Haefele and Lindow, 1987). However, some plant pathogenic bacteria have flg22 epitopes with exceptionally deviating sequences that lead to less efficient perception by the plant defence systems. Examples of bacteria with altered flg22 sequences include *Agrobacterium tumefaciens* (Felix *et al.*, 1999; Albert *et al.*, 2010), *Ralstonia solanacearum* (Pfund *et al.*, 2004), or a specific *Xanthomonas campestris* pv. *campestris* strain where a particular valine/aspartate polymorphism determines whether the flagellin can be detected via FLS2 in *A. thaliana* or not (Sun *et al.*, 2006) (Table 1). This sort of camouflage also occurs in the case of the symbiotic interaction partner *Rhizobium meliloti* (Table 1), that might avoid plant defence responses and enables a profitable interaction for both the plant and the bacterium (Felix *et al.*, 1999).

However, while some of the above-mentioned flg22 peptides are not sensed via FLS2 in *A. thaliana*, other plants are able to perceive such altered sequences. For example, the orthologous flagellin receptor of tomato, SlFLS2 (*Solanum lycopersicum* FLS2), is able to perceive different flg22-derived peptides with distinct sensitivity compared with AtFLS2 of *Arabidopsis* (Felix *et al.*, 1999; Bauer *et al.*, 2001; Robatzek *et al.*, 2007). These features were used in a recent work to map important interaction sites of flg22 and the FLS2 LRR domain (Mueller *et al.*, 2012). An exciting finding is that the flg22 peptide from *R. solanacearum*, flg22^{Rsol}, which is not sensed at all via AtFLS2 (Pfund *et al.*, 2004), acts as an antagonist on SlFLS2—indicating a strategy of this Solanaceae pathogen to suppress innate immunity in tomato. Interestingly, a synthetic peptide consisting of the classical conserved flg22 sequence except the last five amino acid residues that derive from flg22^{Rsol}—the peptide flg22-AYA (Table 1)—acts as an agonist via SlFLS2 but is an antagonist for AtFLS2 (Mueller *et al.*, 2012). This means that SlFLS2 is tolerating the C-terminal end of flg22^{Rsol}, and a further identification of pairing amino acid residues on the receptor and ligand might consequently help to construct an FLS2 receptor that could sense flg22^{Rsol} and thus lead to a molecular weapon against *R. solanacearum*.

Peptide–receptor interaction follows the address–message concept

To date, shortened peptides represent one of the best tools to study the ligand–receptor interaction and support the 'address–message' hypothesis, originally proposed for receptor activation by neuropeptides (Schwyzer, 1980). According

to this hypothesis, ligand binding to a receptor occurs in at least two steps: first the peptide binds to its address part and subsequently activates the receptor in a second interacting step with its message part (summarized in Albert *et al.*, 2010). This hypothesis is supported by the finding that several of the peptides which are shortened from one side are still able to bind to their receptors but block them as antagonists. For the EFR ligand elf18, the N-terminal part with the first 12 amino acid residues acts as the address part, since the elf12 peptide (Table 1) still binds to EFR as an antagonist (Kunze *et al.*, 2004) but is unable to induce cellular defence responses. Starting from elf18, a stepwise reduction of two amino acid residues increases the EC_{50} of the elf peptides until a total loss of function using the 12 amino acid long elf12 which still binds to EFR but with an increased K_d above 100 nM.

In the case of the interaction between flg22 and FLS2, a truncation of the last two amino acid residues, isoleucine and alanine, is already sufficient to convert flg22 into an antagonist, termed flg22-Δ2 (Bauer *et al.*, 2001). However, regarding the interaction with SlFLS2, flg22-Δ2 is still active as an agonist and only peptides with four or more amino acid residues removed from the C-terminus lack agonist activity and exhibit antagonist activity (Table 1) (Mueller *et al.*, 2012). Besides elf18 and flg22, the address–message concept has also been observed for other peptide ligands such as fungal glycopeptide elicitors (Basse and Boller, 1992; Basse *et al.*, 1993), the wound peptide hormone systemin (Pearce *et al.*, 1993), or the bacterial cold shock protein (Felix and Boller, 2003) (summarized in Albert *et al.*, 2010).

DAMPs and endogenous peptides as defence triggers

Many microbial pathogens or insects use lytic enzymes to breach the barriers of plant tissues and to gain access to the host cell. Consequently, degradation products might serve as endogenous elicitors or 'damage-associated molecular patterns' (DAMPs) (Darvill and Albersheim, 1984; Lotze *et al.*, 2007) which can be perceived by mechanisms comparable with those in the case of MAMP-triggered immunity (Boller and Felix, 2009). Known DAMPs are, for example, cutin monomers (Schweizer *et al.*, 1996) that could stimulate plant defence responses in cucumber hypocotyls or in plant cell cultures (Kauss *et al.*, 1999). Also cell wall fragments such as oligogalacturonides or cellulose fragments are potent triggers of plant defence and are generated during pathogen or herbivore attacks (reviewed by Nühse, 2012).

Proteinaceous DAMPs are probably equal in their mode of action, but are of different origin according to the cellular compartments. Thus, a subdivision into three major groups makes sense and might help to keep track of the class of endogenous peptide elicitors (Yamaguchi and Huffaker, 2011): (i) peptides derived from cytosolic precursor proteins; (ii) peptides which originated from extracellular, secreted precursors; and (iii) peptides that resulted from degradation of proteins with distinct primary functions.

Peptide DAMPs from the cytosol

Systemin was the first isolated peptide with hormone characteristics and with a clear role in plant defence and wound-related responses (Pearce *et al.*, 1991). Systemin derives from the precursor protein prosystemin which mainly accumulates in the cytosol of vascular phloem parenchyma cells. Immediately after wounding, it is cleaved to its active form, the 18 amino acid peptide systemin (McGurl and Ryan, 1992). Systemin induces jasmonic acid (JA) biosynthesis in the neighbouring cells, leading to induction of proteinase inhibitors, anti-nutritive proteins, and plant volatiles to deter plant herbivores (Orozco-Cardenas *et al.*, 1993; Degenhardt *et al.*, 2010).

In the model plant *Arabidopsis*, the first isolated proteinaceous DAMP was AtPep1, which is a representative of a protein family comprising seven homologues in *A. thaliana*. All of them seem to be active as elicitors and can activate extracellular alkalinization and the expression of defence-related genes (Huffaker and Ryan, 2007). AtPep1 (as the best studied example) is a 23 amino acid peptide that derives from a 92 amino acid cytosolic protein precursor (PROPEP) and binds to the receptors AtPEPR1 and AtPEPR2, respectively (Huffaker *et al.*, 2006; Yamaguchi *et al.*, 2006; Krol *et al.*, 2010). AtPep homologues have also been identified in maize, of which ZmPep1 was shown to regulate maize disease resistance responses. Another Pep homologue in maize, ZmPep3, triggers JA and ethylene biosynthesis and induces gene expression as well as the production of volatiles known to be involved in anti-herbivore defence (Huffaker *et al.*, 2011, 2013). Although Peps are species specific, genes encoding similar peptide sequences are predicted in many other plants, and a role for such Peps as general defence regulators seems likely (Huffaker *et al.*, 2006; Yamaguchi and Huffaker, 2011).

The 'kiss of death' is a 25 amino acid peptide that has been identified in *A. thaliana* as an important early regulator of PCD during embryogenesis and root hair development (Blanvillain *et al.*, 2011). Interestingly, this peptide or its activity as a PCD trigger seems to be regulated by gene expression and not via a cleave-off from a precursor protein, since the corresponding gene only encodes the active 25 amino acid peptide. What might argue for this peptide being a potential DAMP is its gene expression due to biotic and abiotic stresses (Blanvillain *et al.*, 2011).

Secreted endogenous peptide DAMPs

Hydroxyproline-rich systemins (HypSys) are peptides closely related to systemin, but with a secretion signal, that were identified in members of the Solanaceae and in sweet potato (*Ipomoea batatas*; Convolvulaceae) (Pearce *et al.*, 2007; Chen *et al.*, 2008; Bhattacharya *et al.*, 2013). Interestingly, two distinct HypSys peptides with a size of 18 amino acids were isolated from tobacco leaves which originate from one single precursor protein (NtHypSys) encoded by one single gene (Pearce *et al.*, 2001*a*). The HypSys precursor proteins, comparably with systemin, seem to accumulate in the phloem parenchyma cells and the corresponding genes are activated

upon wounding (Narvaez-Vasquez *et al.*, 2005). HypSys peptides are important amplifiers and triggers of plant immunity, especially during herbivore attack, but also during interaction with other plant pathogens (Heiling *et al.*, 2010; Bhattacharya *et al.*, 2013).

For another class of peptides, the phytosulphokines (PSKs), a dual role might be proposed since these peptides were originally identified as regulators of developmental processes. PSKs are five amino acid long sulphated peptides, deriving and processed from a secreted 80 amino acid precursor protein (Srivastava *et al.*, 2008; Komori *et al.*, 2009). PSKs were originally discovered in the supernatant of *Asparagus* cell cultures (Matsubayashi and Sakagami, 1996) and were shown to promote developmental processes, such as somatic embryogenesis (Hanai *et al.*, 2000; Igasaki *et al.*, 2003), tracheary element differentiation (Matsubayashi *et al.*, 1999; Motose *et al.*, 2009), and adventitious root formation (Amano *et al.*, 2007). Another PSK-related peptide purified from cell cultures, the 18 amino acid glycosylated and sulphated peptide PSY1, plays overlapping roles in regulating developmental processes. In *A. thaliana*, PSKs as well as PSY1 bind to the closely related LRR-RLKs, PSKR1/2 and PSY1R, respectively (Matsubayashi *et al.*, 2002). A role for these receptors and the corresponding PSKs in plant defence has been demonstrated recently (Igarashi *et al.*, 2012; Mosher *et al.*, 2013). Interestingly, PSK receptor mutant plants exhibit enhanced defence gene expression and increased resistance to the biotrophic bacterium *P. syringae* pv. *tomato* (Pto) DC3000 (Igarashi *et al.*, 2012; Mosher *et al.*, 2013), whereas they were more susceptible to the necrotrophic fungus *Alternaria brassicicola* (Mosher *et al.*, 2013). These findings in *pskr* mutants correlated with an increase in salicylate levels and a repression of JA-related genes. As a conclusion, the authors suggest sulphated peptide signalling as a mediating process that shifts the balance of defence signalling towards JA responses (Mosher and Kemmerling, 2013; Mosher *et al.*, 2013). Thus, PSKs and PSY1 must be considered as phytohormones rather than being classical DAMPs.

Peptides that result from degradation of proteins with distinct primary function

A family of peptides termed inceptins originates from the chloroplastic ATP synthase and was first identified as defence triggers from cowpea (*Vigna ungiculata*). There, inceptin was shown to elicit plant defence responses such as the production of salicylic acid, JA, terpene volatiles, and other metabolites with defensive roles. Remarkably, the active 13 amino acid peptide is processed from the ATP synthase γ-subunit in the gut of fall armyworm larvae and is active on *V. unguiculata* plants as an immune stimulant upon release (Schmelz *et al.*, 2006). Inceptin-like sequences are ubiquitous in all plant chloroplastic ATP synthase γ-subunits, but the stimulatory effect is merely specific for legumes in the *Phaseolus* and *Vigna* genera (Schmelz *et al.*, 2007).

In soybean (*Glycine max*), a 12 amino acid peptide has been identified that seems to be unique and is embedded within the sequence of a subtilisin-like protein. This peptide, named GmSubPep, triggers extracellular alakalinization and induces the expression of defence- and stress-related genes (Pearce *et al.*, 2010). Two other peptides, GmPep914 and GmPep890, were also identified in *G. max*, and corresponding encoding genes seem to be present only in Fabales and the closely related Cucurbitales. Both peptides are eight amino acids in size and derive from the C-terminal end of ~50-amino acid precursor proteins. They were found to induce characteristically extracellular alkalinization and the expression of typical defence marker genes (Yamaguchi *et al.*, 2011). The corresponding genes of these peptides are strongly expressed in the roots and are inducible by defence/stress-related phytohormones. While the primary function of the subtilisin-like protein seems clear or at least predictable, for the latter two peptides or the protein precursors no 'primary function' was assigned and a clear subcellular localization could not be predicted *in silico* (TargetP 1.1 server, Technical University of Denmark). Probably, these peptides function as DAMPs or local wound signals, comparable with systemin or AtPEPs, and would also fit in the category of 'peptide DAMPs from the cytosol'.

Final discussion

Peptides serve as common inducers or amplifiers of plant defence responses—no matter whether they are derived from the attacked plant itself (DAMPs) or originate from the pathogen (MAMPs). Which peptides, however, are more important for plant defence, MAMPs or DAMPs? Regarding the initial contact with a pathogen, it is of great advantage for the plant that the pathogen becomes perceptible. This allows an immediate response of the attacked cells and, indeed, cellular reactions can be detected in the plant in the first minute, right after MAMP–receptor interaction. Thus, MAMPs mainly serve as initial triggers of plant defence. DAMPs might come into play later and might be considered as inducers of a second wave of plant defence, leading to a prolongation or amplification of the cellular defence response. The plant consequently gets 'endogenous support' to fight against pests more successfully. In addition, DAMPs might be important warning signals for the cells of surrounding tissues that have not yet come directly into contact with the pathogen. These cells become well prepared to strike first, before the pathogen attacks, and might help the plant prevent further spread of the pest. Compared with the pathogen-specific MAMPs, the advantage of endogenous DAMPs is that they are already present in the plant or are produced by the plant itself. Thus DAMPs might serve as a more general signal for danger, no matter whether the plant is affected by a microbial pathogen, a herbivore, or if it is just mechanically wounded. If so, DAMPs function as initial triggers and not as secondary amplifiers of the plant defence.

From an evolutionary point of view, DAMPs might have occurred as defence triggers long before MAMPs. Probably, it was just a 'new specificity' that randomly appeared in the ectodomain of already existing DAMP receptors, while the signalling pathway that is initiated by the intracellular kinase

domain of the receptor remained untouched. For instance, in the model plant *A. thaliana*, only one signalling pathway exists that becomes activated by the DAMP AtPEP1 and by the MAMP flg22. The corresponding receptors for these peptides, AtPEPR1/2 and AtFLS2, both interact with the same co-receptor AtBAK1 (BRI1-associated kinase1) (Schulze *et al.*, 2010) and consequently activate the same downstream signalling such as the MAPK cascade or oxidative burst, or induce the expression of defence-related marker genes (Huffaker *et al.*, 2006; Huffaker and Ryan, 2007; Schulze *et al.*, 2010). DAMP/MAMP signalling itself might have evolved from a developmental pathway that controls cellular responses leading to PCD or senescence (Thomas, 2013), that can be observed, for example, in tracheary element development (Schuetz *et al.*, 2013) or pollen rejection in self-incompatibility (Schopfer *et al.*, 1999; Tantikanjana *et al.*, 2010). These developmental programmes often share certain steps with the defence-related DAMP/MAMP signalling cascade. A cross-over between the DAMP signalling pathway and developmental pathways seems to be gradual and could eventually be observed by the example of the endogenous plant peptides termed as RALFs (rapid alkalinization factors). RALFs are ~50 amino acid long peptides derived from secreted pre-proproteins and can be found in many plants, including dicots, monocots, and gymnosperms (Pearce *et al.*, 2001*b*). Members of these RALFs play a role in brassinosteroid-related developmental processes (Srivastava *et al.*, 2009), in root development (Wu *et al.*, 2007), or in pollen tube growth (Covey *et al.*, 2010). Interestingly, these peptides partially induce signalling outputs characteristically observed in plant defence signalling such as extracellular alkalinization (Pearce *et al.*, 2001*b*) or root growth inhibition (reviewed in Bedinger *et al.*, 2010).

Besides cellular signalling that shares parallels between development, DAMP- and MAMP-related cellular signalling, no general principles of peptide–receptor interaction can be pointed out. Comparisons of peptide sequences do not lead to any conclusion since the amino acid composition seems to occur randomly and no relationship between the sequences of, for example, flg22, elf18, AtPEP1, and csp15 can be found. Additionally, the typical length of peptides that activate plant defence responses varies from between five and up to 28 amino acid residues. Hence, a perception of different peptides must act via distinct receptors. This finding is not surprising, since—at least in the case of MAMPs—successful immunity relies on the perception of various pathogenic components or patterns via distinct perception systems. However, one commonality of peptide recognition might be given by a conserved structural organization of the corresponding receptors that have been identified so far: only LRR-RLKs or LRR-RLPs seem to sense peptides, and, for the herein described 12 peptide elicitors (seven MAMPs and five potential DAMPs), five corresponding receptors (homologues and paralogues excluded) have been identified all belonging to the class of LRR proteins. Moreover, peptides involved in developmental signalling are perceived by LRR-RLKs or RLPs as well. Thus, the structural organization of LRR proteins might be the prerequisite for a protein–protein or protein–peptide interaction since it provides a potentially endless variable surface due to the structural organization in stacked LxxLxxLxLxx motifs (L=leucine; x=variable amino acid residue) (Albert *et al.*, 2010; Hothorn *et al.*, 2011). Nevertheless, some receptors—and also new peptide ligands—still remain to be identified, and receptor candidates with other recognition domains besides LRRs should be considered.

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