Diverse Roles of Lysin-Motif (LysM) Proteins in Mediating Plant-Microbe Interactions

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Abstract

Lysin-motif (LysM) is a protein domain initially identified in a phage protein responsible for binding peptidoglycan, an important component of bacterial cell walls. LysM-containing proteins are distributed in diverse organisms, ranging from microbes to plants and animals (including human beings). Recent studies demonstrated that this group of proteins plays different roles in mediating plant-microbe interactions, leading to defense, symbiosis, or suppression of host defense. These roles are probably related to their potential ability to recognize and bind a specific signal molecule, such as chitooligosaccharides, peptidoglycan, nodulation factors (NFs), and mycorrhization factors (MFs).

Keywords: Chitooligosaccharide, LysM, plant-microbe interaction, nodulation factor, P/MAMP

Introduction

Plants are constantly under attack by a wide range of pathogenic microbes. However, very rarely are they killed by such attacks due to the fact that plants have evolved various mechanisms to defend themselves. One such mechanism is innate immunity triggered by pathogen/microbe-associated molecular patterns (P/MAMPs), the so-called P/MAMP-triggered immunity (P/MTI) [1-5]. The well-studied examples of P/MAMPs are flagellin and EF-Tu (Elongation factor-Tu) from bacteria and chitooligosaccharides from fungi [1,5,6]. These P/MAMPs are recognized by their cognate pattern recognition receptors (PRRs) to lead to plant innate immunity [5,6]. Recent studies have shown that various receptor-like kinases (RLKs) play an important role in such recognition, functioning as a receptor or as a critical component of the receptor complex [3,5-12]. Two well-studied examples are FLS2 and EFR, responsible for the recognition of flagellin and EF-Tu, respectively [12]. Both of them are leucine-rich repeat receptor-like kinases (LRR RLKs). Recently, a different group of receptor-like kinases, called LysM RLKs, was shown to be critical for perception of chitooligosaccharides [13-18], which are derived from chitin, a polymer of β-1, 4-linked N-acetylglucosamine units and a major component of fungal cell walls. This group of receptor-like kinases possesses an extracellular part that contains at least one LysM domain, instead of LRRs. The LysM domain was initially identified in a lysozyme from the Bacillus phage phi 29 [19,20], which can bind peptidoglycan, a component of bacterial cell walls [20,21]. Peptidoglycan is composed of multiple chains of the repeating *N*-acetylglucosamine and *N*-acetylmuramic acid units, cross-linked by short peptides. Subsequently, this LysM domain was found in other proteins in diverse organisms, including bacteria, fungi, plants, and animals (including human beings) [22-25]. A typical LysM domain consists of approximately 44 - 65 amino acids and forms a β-strand-α-helix-α-helix-β-strand secondary structure [23]. The structures of several LysM domains in LysM-containing proteins have been revealed and they share a conserved architecture [23,26-28].

In addition to pathogenic microbes, there are also beneficial ones in the environment that can benefit plant growth and development. For example, rhizobial bacteria (or rhizobia) can induce the

formation of nodules on legume roots to fix nitrogen from air for the host plant, and mycorrhizal fungi can induce the formation of mycorrhizae on roots of most land plants to absorb phosphate and other nutrients for the host plant [29-33]. In these cases, plants appear to be capable of recognizing and embracing these microbial symbionts by suppressing or reducing defense, probably through detecting certain special signals by the cognate recognition receptors to lead to symbiosis. Among them are nodulation factors (NFs) from rhizobial bacteria and mycorrhization factors (MFs) from mycorrhizal fungi. NFs are substituted lipochitooligosaccharides of 3 to 5 N-acetylglucosamine units [34,35]. MFs are also modified lipochitooligosaccharides of 4 or 5 N-acetylglucosamine units [36]. Of course, suppression or reduction of host defense may also come from the involved symbionts (see below for more discussion).

Notably, there are considerable structural similarities between these symbiotic signals and P/MAMPs, as well as between their corresponding receptors: NFs, MFs, chitooligosaccharides, and peptidoglycan have a similar backbone made of modified sugar units [36,37], and their corresponding receptors are all LysM proteins. Therefore, plants are capable of recognizing these symbiotic microbial partners through detecting symbiotic signals via receptor proteins similar to those involved in detecting P/MAMPs.

In response to plant defense, microbes (including both pathogenic and symbiotic) have evolved various ways to counter host defense [6,38-41]. Recent studies showed that microbial LysM proteins are important in this process, since these proteins are capable of competing with host receptors for their ligands (such as chitooligosaccharides) to evade or suppress defense triggered by these ligands [42-46].

Interestingly, a LysM RLK, Lyk3, in Arabidopsis, was recently shown to be involved in the suppression of plant innate immunity triggered by P/MAMPs in the presence of NFs or chitotetraose [47]. Therefore, LysM proteins appear to play different roles to benefit either the host or the microbe, or both during their interactions (**Table 1**).

Table 1 Roles of LysM proteins in plant-microbe interactions.

Function	Examples of LysM proteins	Plant species	References
Perception of Nod factors during legume-rhizobium symbiotic interactions	NFR1/LYK3, NFR5/NFP	*Lotus japonicus, Medicago truncatula*	[48-52]
Perception of Myc factors during plant-fungus symbiotic interactions	PaNFP, NFR1/LYK3, OsCERK1	*Parasponia andersonii, L. japonicus, M. truncatula, Oryza sativa*	[59-61]
Perception of chitooligosaccharides during plant-fungus pathogenic interactions	CEBiP, CERK1/LysM RLK1, OsCERK1 LYP4, LYP6	*A. thaliana, O. sativa*	[15,17,72,75,78]
Perception of peptidoglycan during plant-bacterium pathogenic interactions	LYM1, LYM3, CERK1/LysM RLK1, LYP4, LYP6, OsCERK1	*A. thaliana, O. sativa*	[13,78,81,82]
Suppression of host defense during plant-microbe interactions - from the microbe side	Ecp6, Mg3LysM, Slp1	*Cladosporium fulvum, Mycosphaerella graminicola, Magnaporthe oryzae*	[44-46]
Suppression of host defense during plant-microbe interactions - from the plant side	Lyk3	*A. thaliana*	[47]

LysM RLK protein-mediated perception of NFs during legume-rhizobium symbiotic interactions

The first concrete evidence to support the perception and signaling role for LysM RLKs came from studying legume-rhizobium symbiotic interactions. Through reverse and forward genetics, two legume LysM RLKs were shown to be critical for perceiving NFs during legume-rhizobium interactions. For example, in legume *Lotus japonicus*, two LysM RLKs, NFR1 and NFR5, were shown to be indispensable for the earliest physiological and cellular responses to the NFs derived from its cognate rhizobium *Mesorhizobium loti* and for the establishment of the symbiotic relationship between these two organisms [48,49]; and their orthologs, LYK3 and NFP, in legume *Medicago truncatula* also played similar roles in the symbiosis [50-52]. Subsequently, LysM RLKs from other legumes were also shown to be important for such symbiotic interactions [53-55]. Further studies have demonstrated that these LysM RLKs can bind their cognate NFs [56-58]. Therefore, these LysM RLKs play a critical role in perceiving their cognate NFs during legume-rhizobial symbiotic interactions, either as a receptor or as a critical component in the receptor complex.

LysM RLK protein-mediated perception of MFs during plant-fungus symbiotic interactions

Both MFs and their receptors have been very evasive. So far only a few LysM RLKs have been implicated in perceiving MFs. For example, one single LysM RLK, PaNFP, was shown to be responsible for both nodulation and mycorrhization in the nonlegume plant *Parasponia andersonii* [59], and is therefore a good candidate receptor for both NFs and MFs in this plant. Recently, the rice CERK1 (chitin elicitor receptor kinase 1), a LysM RLK important in chitin-triggered immunity (see the next section for more details), and the legume *NFR1/LYK3,* a LysM RLK important in NF-triggered symbiosis, were also shown to be critical in the mycorrhizal symbiosis [60-61]. Interestingly, the legume NFR5/NFP, another LysM RLK critical for NF-triggered symbiosis, is not required for the establishment of the mycorrhizal interaction in legumes [51,61,62], although it may play a role in host responses to MFs [36,63]. Consistent with this, the recently identified potential MFs from the mycorrhizal fungus, *Glomus intraradices*, which can form mycorrhizae with legumes and many other plants, are structurally very similar to NFs, i.e., they are also modified lipochitooligosaccharides [36,37]. Therefore, the afore-mentioned LysM RLKs should play a critical role in perceiving MFs, either as a receptor and as an important component in the receptor complex.

It is worth pointing out that short chitooligosaccharides (chitotetraose and chitopentaose) can mimic arbuscular mycorrhizal germinated spore exudates (GSEs)-elicited Ca^{2+} spiking. The spiking response is also dependent on the common SYM signaling pathway (DMI1/DMI2), but not on NFR5/NFP [62]. However, MFs are less efficient elicitors of Ca^{2+} spiking in *M. truncatula* root organ cultures. The authors suggested that these unmodified chitooligosaccharides secreted by AM fungi are probably perceived by another LysM RLK protein instead of *NFR1/LYK3 or* NFR5/NFP, and contribute to the activation of a SYM-dependent signaling pathway that is important for mycorrhizal fungal root colonization [62].

Therefore, plants, especially legumes, appear to be able to distinguish between MFs, NFs, and chitooligosaccharides to induce appropriate responses, although certain ambiguities exist (Please see the following sections for more details).

LysM protein-mediated perception of chitooligosaccharides during plant-fungus pathogenic interactions

It has long been speculated that when a fungus comes in contact with a plant, plant cells can secrete chitinases to degrade chitin in the fungal cell wall to directly inhibit infection; meanwhile, the released chitin fragments or chitooligosaccharides will further serve as an elicitor to be perceived by a plant receptor to activate plant innate immunity [64-67]. Indeed, plants overexpressing chitinases were more resistant to fungal pathogens, especially in combination with a glucanase [68-70]. Furthermore, plants pretreated with chitooligosaccharides were more resistant to fungal pathogens than untreated plants [71]. The protein important in perceiving chitooligosaccharides was first identified in rice. It turned out to be a LysM protein, named CEBiP (Chitin elicitor-binding protein) [72]. Notably, this LysM protein does not

have a kinase domain. Subsequently, similar proteins involved in perceiving chitooligosaccharides were also identified in other plants [73,74]. Approximately one year later, a LysM protein with an intracellular kinase domain, called CERK1/LysM RLK1, was also revealed to be important in the perception of chitooligosaccharides in Arabidopsis [15,17]. Mutations in this CERK1/LysM RLK1 gene virtually blocked all the chitin-triggered defense responses, such as induction of reactive oxygen species (ROS), activation of MAPKs, and induction of defense genes, and meanwhile also led to enhanced susceptibility to fungal pathogens [15,17]. This protein binds chitooligosaccharides [14,16,28]. Therefore, this CERK1/LysMRLK1 is likely the receptor or a critical component in a receptor complex for chitooligosaccharides in Arabidopsis. Subsequently, similar LysM RLKs involved in chitooligosaccharide perception were also revealed in other plants, such as in rice and tomatoes [74-76].

Recent studies suggest that multiple LysM proteins are probably involved in forming a receptor complex for perceiving chitooligosaccharides as well as peptidoglycan [13,16,77,78] (Please see the following section for more information).

It is important to point out that perception of short chitooligosaccharides, such as chitotetraose, may be through different LysM RLK proteins, and may lead to suppression of plant innate immunity triggered by P/MAMPs in Arabidopsis [47], or activation of the AM-related signaling pathway in *M. truncatula* [62].

LysM protein-mediated perception of peptidoglycan during plant-bacterium pathogenic interactions

The first evidence that LysM proteins are likely involved in defense against bacterial pathogens came from the following observation: the mutation in the Arabidopsis CERK1/LysM RLK1 not only enhanced susceptibility to fungal pathogens, but also to the bacterial pathogen *Pseudomonas syringe* pv. *tomato* DC3000 [79,80]. This LysM RLK protein was targeted by the bacterial effector AvrPtoB for degradation [79,80]. A similar phenomenon was also subsequently observed in tomatoes [76]. The studies suggest that those LysM proteins involved in chitooligosaccharide perception may also be involved in detecting a bacterial signal, such as peptidoglycan, to activate defense against bacterial pathogens. Indeed, recent studies demonstrated that multiple LysM proteins likely form a receptor complex to mediate perception of peptidoglycan as well as chitooligosaccharides in plants [13,78,81,82]. The recognition of multiple signals by one receptor or a receptor complex may be an economical way for plants to regulate their interactions with diverse microbes, such as bacterial and fungal pathogens.

LysM protein-mediated suppression of host defense during plant-microbe interactions - from the microbe side

Although plants have evolved mechanisms to detect and recognize P/MAMPs to trigger innate immunity against potential pathogens, microbes have correspondingly evolved ways to counter such defense mechanisms during co-evolution (or arms race) between plants and their pathogens as well as symbionts [6,38-41]. These counter-defense mechanisms have been extensively studied in the interactions between Arabidopsis and *P. syringae* pv. *tomato* DC3000. In this pathosystem, various effector proteins from the bacterium have been shown to transport into the host cells to modify host defense mechanisms to actively suppress defense [1,12,64,82-85]. So far not much is known about the mechanisms used by fungi to suppress host defense. However, a few recent studies showed that fungi can employ LysM proteins to sequester chitooligosaccharides from detection or recognition by host receptors to get around defense triggered by chitooligosaccharides [42-46]. This is probably a common strategy employed by fungal pathogens to suppress host defense triggered by chitooligosaccharides, because LysM effectors are widely distributed and conserved in the fungal kingdom [43]. Of course, fungi may also employ other mechanisms to counter host defense triggered by chitooligosaccharides and other elicitors.

LysM proteins exist not only in fungi, but also in bacteria. I speculate that certain bacterial LysM proteins may be involved in suppressing peptidoglycan-triggered defense, by protecting and/or sequestering the peptidoglycan signal from detection by the host receptor. Of course, some other

mechanisms may also be employed by bacterial pathogens to counter defense triggered by peptidoglycan. Indeed, as mentioned before, the bacterial effector protein AvrPtoB targets a LysM RLK, which is involved in chitooligosaccharide and/or peptidoglycan perception, for degradation to benefit bacterial infection in plants [76,79,80].

LysM proteins exist not only in pathogenic fungi and bacteria, but also in beneficial fungi and bacteria, which can form a symbiotic relationship with their host plants. For example, the genome of the mycorrhizal fungus *Laccaria bicolor*, which can form symbiosis with many land plants, encodes approximately 30 LysM proteins [86], and the genome of the rhizobial bacterium *Bradyrhizobium japonicum*, which can form symbiosis with soybean, encodes approximately 4 LysM proteins [87]. Like their pathogenic counterparts, these beneficial microbes also contain chitin and peptidoglycan, respectively. In order for symbioses to occur, defense against these beneficial microbes has to be prevented or minimized. The detailed mechanisms for preventing or minimizing host defense triggered by chitooligosaccharides and/or peptidoglycan associated with symbiotic microbes are not well understood. However, we speculate that LysM proteins may be part of the solution: Microbial LysM proteins may bind to chitooligosaccharides and/or peptidoglycan to sequester these signals from being detected by host PRRs. Since symbiotic signals (NFs and MFs) are also structurally similar to chitooligosaccharides, it is speculated that these signals may also be bound by LysM proteins (derived from microbes and/or hosts) to somehow regulate symbiosis. Hopefully, further research will confirm this speculation. Additionally, suppression of host immunity triggered by these P/MAMPs may also be mediated by symbiotic signals, such as NFs. Future studies will reveal more mechanisms for beneficial microbes to modulate host defense. Possibly, some of them will be similar to, but others will be different from those used by pathogenic microbes.

LysM protein-mediated suppression of host defense during plant-microbe interactions - from the plant side

A recent study by Liang and colleagues demonstrated that suppression of host defense can also come from host LysM proteins [47]. The defense responses triggered by P/MAMPs, such as flagellin, EF-Tu and chitooctaose, can be suppressed by the application of NFs or chitotetraose, and such suppression requires another LysM RLK, called Lyk3 in Arabidopsis [47]. Interestingly, this Arabidopsis Lyk3 protein is also a member of the LysM RLK family [25], which contains other LysM RLKs involved in the perception of chitooligosaccharides, peptidoglycan, NFs, and MFs. The finding suggests that a legume LysM RLK similar to the Arabidopsis Lyk3 may play a similar role in the NF-mediated suppression of plant innate immunity, which is triggered by P/MAMPs present in rhizobial bacteria, to contribute to the establishment of symbiosis. A similar mechanism may also occur during the establishment of mycorrhizal symbiosis.

Dual roles played by LysM RLKs during plant-microbe interactions

As partially mentioned before, some LysM proteins possess multiple roles during plant-microbe interactions. Notably, the LysM RLK in the nonlegume plant *P. andersonii* is very likely responsible for the perception of both NFs and MFs derived from its mycorrhizal fungus and rhizobial bacterium, respectively [59]. Since it is the only LysM RLK in the plant, it will not be surprising if this protein is also involved in perceiving chitooligosaccharides and/or peptidoglycan. The *M. truncatula* LysM RLK protein, NFP, is not only important for both NF and MF perception, but is also capable of perceiving pathogenic signals to lead to plant immunity against a fungal pathogen [88,89]. The rice CERK1 protein is involved in both pathogenic and symbiotic interactions [60,61], and the Arabidopsis CERK1/LysM RLK1 protein appears to be involved in the perception of both chitooligosaccharide and peptidoglycan signals [13]. Additionally, the rice LysM LYP4 and LYP6 proteins, which do not have an intracellular kinase domain, also play dual roles in peptidoglycan and chitooligosaccharide perception in rice innate immunity [78]. Such dual and ambiguous roles in perceiving structurally similar signals (NFs, MFs,

chitooligosaccharides, and peptidoglycan) to lead to specific downstream events are probably fulfilled in collaboration with other LysM proteins.

Conclusions and perspective

LysM proteins are widely distributed in plants and microbes. Recent studies strongly support that some of them are capable of recognizing N-acetylglucosamine-containing signals: chitooligosaccharides, peptidoglycan, NFs, and MFs, to mediate plant-microbe interactions. On the one hand, they are used as a defense mechanism by plants to defend against potential infection by pathogens. On the other hand, they are used by microbes as a counter-defense mechanism to suppress plant defense. Furthermore, some LysM proteins appear to benefit both sides in the case of symbiotic plant-microbe interactions. Those roles are not necessarily clear-cut, because a number of LysM proteins tend to be involved in mediating plant interactions with multiple types of microbes.

Clearly, recent advances have significantly improved our understanding of this group of proteins and their roles in mediating plant-microbe interactions, either symbiotic or antagonistic. However, there are still more questions than answers. For example, are LysM proteins the only proteins involved in recognizing N-acetylglucosamine-containing signals? Have plants (at least some plant species) evolved other mechanisms to perceive these signals? Another relevant question is: Are the mechanisms perceiving N-acetylglucosamine-containing signals conserved in plants? Although current data suggest that specificities do exist between certain LysM proteins and their ligands, the question is: How are such specificities achieved? Our previous study showed the mutations in NF receptors did not affect chitooligosaccharide signaling in legume *L. japonicus*, suggesting that in legumes they have different perception systems for NFs and chitooligosaccharides, respectively [17]. But certain ambiguities also exist for LysM proteins, i.e., these LysM proteins can recognize more than one signal. For example, the only LysM RLK protein in *P. andersonii* [59] appears to recognize both NF and MF to mediate the symbiotic interactions of this plant with both the rhizobial bacterium and the mycorrhizal fungus. The Arabidopsis CERK1/LysM RLK1 is involved in detecting and recognizing both chitooligosaccharides and peptidoglycan [13]. So now the question is: What is the evolutionary relationship between the mechanisms perceiving these structurally related signals? It would be very interesting to see how different or similar the chitooligosaccharide, peptidoglycan, NF, and MF receptors are in the same legume plant, and whether/how downstream signaling pathways interact with each other. So far only a handful of LysM proteins have been shown to be involved in plant-microbe interactions. Since each plant and microbial genome generally encodes multiple LysM proteins, do all these proteins function in plant-microbe interactions? Or, do some of them play other roles, such as in growth and development and other stress responses? Indeed, recent studies suggest LysM RLKs may also be involved in abiotic-stress signaling [90,91]. Additionally, we observed that the mutation in one LysM RLK in Arabidopsis appeared to affect flowering time (our unpublished data), suggesting that this protein may somehow be involved in plant growth and development. Considering their important roles in mediating plant-microbe interactions, either beneficial or antagonistic, how can we manipulate these LysM proteins to benefit the beneficial, and meanwhile inhibit antagonistic plant-microbe interactions? Surely, more work is needed before we can answer these questions and fully understand the functions of these proteins. It can be foreseen that revealing more of such proteins during plant-microbe interactions and in other biological processes, and structures of these proteins, especially in combination with their cognate ligands will significantly enhance our understanding of the functions of these proteins and plant-microbe interactions. Such advances will eventually allow us to engineer LysM proteins to favor plant defense [92] and plant-microbe symbiotic interactions.

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