LysM Effectors: Secreted Proteins Supporting Fungal Life

Anja Kombrink, Bart P. H. J. Thomma*
Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands

Introduction

Fungi occupy a plethora of niches and play essential roles in diverse environments through decomposition of organic material as saprophytes or through establishment of symbiotic relationships with plants and animals that range from mutually beneficial to pathogenic. During colonization of their niches, fungi secrete proteins that include carbohydrate-degrading enzymes to feed on complex molecules and effectors that mediate the establishment of interactions with host organisms [1]. Although effectors are typically thought to be species- or even lineage-specific, some effectors are widespread among pathogens, such as the necrosis- and ethylene-inducing-like proteins (NLPs) that are widely spread in bacteria, fungi, and oomycetes [2,3]. Several studies have shown that NLPs contribute to pathogen virulence through phytotoxic activity, but more recent work has revealed that some NLPs act in processes other than pathogenicity, such as fungal growth and sporulation [4]. A more recently identified class of conserved effectors are LysM effectors: fungal effectors that carry no recognizable protein domains other than lysin motifs (LysMs) [5]. Intriguingly, like NLPs, LysM effectors occur in both pathogenic and nonpathogenic fungi.

Plant Pathogen LysM Effectors: Virulence Factors through Interactions with Chitin

Microbial pathogens carry conserved structures, termed microbe-associated molecular patterns (MAMPs), that are recognized by host cell surface receptors and trigger an immune response [6,7]. Chitin, a major constituent of fungal cell walls, is a well-described MAMP, and several plasma membrane–localized chitin receptors have been identified in plants that all contain extracellular LysMs, well-known carbohydrate-binding protein domains [8–10]. To overcome host immunity, genuine pathogens secrete effector molecules that manipulate host physiology, including immune responses, to support host colonization [2,7]. Likewise, other microbes that establish intimate relationships with host plants, such as mutualistic symbiotic microbes and endophytes, secrete effectors to bring about their association.

The fungal tomato leaf mould pathogen Cladosporium fulvum secretes the LysM-containing effector Ecp6 that binds chitin with high specificity [11,12]. Ecp6 does not protect fungal hyphae against the hydrolytic activity of tomato chitinases, a function that was previously assigned to C. fulvum effector Avr4 that contains an invertebrate chitin-binding domain [13,14]. Consequently, it was speculated that Ecp6 interferes with chitin detection by the host [5]. Indeed, Ecp6 was demonstrated to perturb chitin-induced immunity, and it was proposed that Ecp6 functions by sequestration of cell wall–derived chitin fragments that would otherwise be perceived by host immune receptors [12] (Figure 1). The crystal structure of Ecp6 showed that two LysM domains (LysM1 and LysM3) collectively bind a single chitin molecule [15] (Figure 1). This ligand-induced composite binding groove is deeply buried in the effector and displays ultra-high (picomolar) chitin-binding affinity, which is significantly higher than that of plant immune receptors [15]. Through analysis of a crystal structure of the Arabidopsis chitin elicitor receptor kinase (AtCERK1) it was previously demonstrated that only one of the three LysM domains in this immune receptor binds chitin [16]. Moreover, the structural orientation of the three LysM domains in AtCERK1 does not permit intramolecular LysM dimerization as observed in Ecp6 [15,16]. Interestingly, the singular LysM domain of Ecp6 that is not involved in the intramolecular composite binding site (LysM2) also contains a functional chitin-binding site (Figure 1), and has the capacity to perturb chitin-induced immunity [12,15]. Since the chitin-binding affinity of this singular LysM domain is significantly lower than that of the composite binding site, it is unlikely to deregulate chitin-induced immunity merely by chitin oligosaccharide sequestration. As it has been suggested that chitin-induced immune receptor dimerization is required for the activation of immune signalling, LysM2 may perturb chitin-induced immunity through interference with this dimerization [15,16] (Figure 1, Figure 2). Since LysM effectors produced by the wheat blast fungus Magnaporthe oryzae, Mg3LysM and Slp1 respectively, similarly suppress chitin-triggered immunity, it seems that deregulation of chitin-triggered immunity is an important function of LysM effectors [17,18]. Nevertheless, functional analysis of M. graminicola LysM effectors has revealed that they may have additional functions during host colonization [10,17]. Fungal cell wall chitin is a target of plant chitinases that act in fungal immunity; exochitinases release chitin oligosaccharide MAMPs from fungal cell walls that can induce host immune responses, which include the secretion of endochitinases that cause hyphal lysis [8,19]. Interestingly, M. graminicola Mg1LysM and Mg3LysM prevent hyphal lysis by plant chitinases, whereas Ecp6 and Slp1 do not have this capacity [12,17,18] (Figure 2). Thus, functional diversification of LysM effectors during host colonization has occurred in plant pathogens.

Citation: Kombrink A, Thomma BPHJ (2013) LysM Effectors: Secreted Proteins Supporting Fungal Life. PLoS Pathog 9(12): e1003769. doi:10.1371/journal.ppat.1003769

Editor: Joseph Heitman, Duke University Medical Center, United States of America

Published December 12, 2013

Copyright: © 2013 Kombrink, Thomma. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: The authors acknowledge support by the Research Council for Earth and Life sciences (ALW) of the Netherlands Organization for Scientific Research (NWO). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: bart.thomma@wur.nl
LysM Effectors as Virulence Factors of Mammalian Pathogens?

Genome mining revealed that LysM effectors are not confined to plant pathogens as, for instance, genomes of most (opportunist) fungal pathogens of mammals contain LysM effector genes as well [5]. For instance, in the dermatophyte Trichophyton rubrum, causal agent of athlete’s foot, as well as in related dermatophyte species, the gene family encoding LysM effectors appears to be expanded [20]. Similar to plants, mammals do not synthesize chitin but can respond to chitin with an immune response, which includes the production of chitinases [21]. These observations tempt speculation that fungal pathogens of mammals secrete LysM effectors to deal with host immunity in a similar fashion as plant pathogens [10]. Furthermore, allergies such as asthma are associated with fungal infections, although the underlying mechanisms presently remain unclear [22]. Emerging evidence suggests an important role for host chitinases that might mediate host responses to chitin and its derivatives [22], which may again be influenced by fungal LysM effectors. However, the fact that chitin is not universally recognized as a MAMP in mammalian systems argues against the hypothesis that fungal mammalian pathogens secrete LysM effectors to establish infection [23]. Furthermore, the genome of the human pathogenic yeast Candida albicans, as well as of most other Candida spp. that occur as opportunistic human pathogens, appears to lack LysM effector genes [5]. Similarly, in the genome of the skin-associated fungus Malassezia globosa that is responsible for the onset of dandruff and other skin disorders, and the fungus Pneumocystis jiroveci that causes pneumonia among immunocompromised hosts, no LysM effector genes are found [24,25]. Since many mammalian pathogens show a low degree of host adaptation and lack host specificity, it has been suggested that infection by fungal mammalian pathogens does not require effector activity [1]. In contrast to plant pathogens, most fungal pathogens of mammals spend a considerable amount of their life cycle free-living in the environment and only infect mammalian hosts in an opportunistic manner. Thus, mammalian fungal pathogens may use their LysM effector homologues in processes other than host colonization, such as survival in the environment. The aforementioned absence of LysM effector genes in Candida albicans, Malassezia globosa, and Pneumocystis jiroveci, which are among the few fungal species that are commensals of humans and animals and that do not occur free-living in the environment, seems to support this hypothesis [5,24,25].

LysM Effectors of Saprophytes: Diverse Possibilities

Considering that LysM effectors are ubiquitous in fungi, it could be argued that they might act in general physiological processes, such as cell wall modification. Fungi secrete lytic enzymes that break chitin polymers and in this manner maintain cell wall flexibility to allow hyphal growth, branching, morphogenesis, and spore germination. Recently, a Trichoderma atroviride LysM effector was found to be coexpressed with an adjacent chitinase gene [26]. Since addition of the purified LysM effector to T. atroviride inhibited spore germination in vitro, a role in hyphal growth was proposed for this LysM effector. However, further experimental evidence that includes targeted deletion of the LysM effector gene in T. atroviride is required to support such a role. Their occurrence in saprophytes may furthermore suggest that LysM effectors contribute to growth in any fungal niche, as likely other microbes are encountered that compete for the same niche or may act as mycoparasites. In this respect, several hypotheses can be envisaged. Extrapolating the findings for LysM effectors of plant pathogens, LysM effectors may protect fungi against chitinases and other hydrolytic enzymes produced by mycoparasites. Moreover, sequestration of cell wall-derived chitin oligosaccharides may be relevant if mycoparasites would be attracted by gradients of such fragments (Figure 2). One step further, LysM effectors may also have functions that are not associated with chitin binding. Originally, LysMs were identified in bacterial lysozymes (hence the name of the domain) that bind and hydrolyse peptidoglycan, a chitin-related glycan and a major component of bacterial cell walls [27]. LysMs occur in various peptidoglycan-binding proteins, and thus it is conceivable that some LysM effectors bind peptidoglycan as well. Such LysM effectors may help fungi to affect bacterial competitors in their niches, for instance because they immobilize them in a similar fashion as antibodies do [28] (Figure 2).

Concluding Remarks

Fungal LysM effectors are versatile proteins that occur in fungal species with extremely divergent lifestyles. Conceivably, LysM effectors function in various ecological niches. In addition, even LysM effectors of plant pathogens that function in the same niche (the plant host) and that bind the same substrate (chitin) were demonstrated to have distinct roles in promoting fungal virulence [10,17]. Furthermore, pathogens interact with other microbes, both in the free-living stage and during colonization of their hosts where they may encounter opportunistic pathogens, commensals, and endophytes. In this respect it is interesting to note that strains of the vascular wilt pathogen Verticillium dahliae have a significantly expanded LysM effector family of six to seven members [29,30]. However, functional analysis has revealed that only one of these LysM effectors is induced in planta and contributes to pathogenicity, while the role of the others still remains obscure [30]. V. dahliae is known to survive as a resting structure in the soil for decades in the absence of suitable host plants, and it is tempting to speculate...
that its LysM effectors contribute to persistence of these structures through protection against microbial activity. Therefore, the study of LysM effectors of fungi that thrive in a variety of niches will reveal additional LysM effector functions that are relevant for pathogenic fungi as well.

Acknowledgments

The authors thank Dr. Andrea Sánchez-Vallet for the preparation of Figure 1.

References

1. Lowe RGT, Howlett BJ (2012) Indifferent, affectionate, or deceitful: lifestyles and secretomes of fungi. PLoS Pathog. 8: e1002515. doi:10.1371/journal.ppat.1002515.
2. de Jonge R, Bolton MD, Thomma BPHJ (2011) How filamentous pathogens co-opt plants: the ins and outs of fungal effectors. Curr Opin Plant Biol 14: 1–7.
3. Gijzen M, Nürnberger T (2006) Nep1-like proteins from plant pathogens: recruitment and diversification of the NPP1 domain across taxa. Phytochemistry 67: 1800–1807.
4. Santhanam P, van Esse HIP, Albert I, Faino L, Nürnberger T, et al. (2013) Evidence for functional diversification within a fungal NEP1-like protein family. Mol Plant Microbe Interact 26: 278–286.
5. de Jonge R, Thomma BPHJ (2009) Fungal LysM effectors – extinguishers of host immunity? Trends Microbiol 17: 151–157.
6. Nürnberger T, Brunner F (2002) Innate immunity in plants and animals: emerging parallels between the recognition of general elicitors and pathogen-associated molecular patterns. Curr Opin Plant Biol 5: 318–324.
7. Thomma BPHJ, Nürnberg T, Joosten MHAJ (2011) Of PAMPs and effectors: the blurred PTI-ETI dichotomy. Plant Cell 23: 4–15.
8. Felix G, Regenass M, Boller T (1993) Specific perception of subnanomolar concentrations of chitin fragments by tomato cells: induction of extracellular alkalization, changes in protein phosphorylation, and establishment of a refractory state. Plant J 4: 307–316.
9. Kaku H, Nishizawa Y, Ishii-Minami N, Akimoto-Tomiyama C, Dohmae N, et al. (2006) Plant cells recognize chitin fragments for defence signalling through a plasma membrane receptor. Proc Natl Acad Sci U S A 103: 11086–11091.
10. Kombrink A, Sanchez-Vallet A, Thomma BPHJ (2011) The role of chitin detection in plant-pathogen interactions. Microbes Infect 13: 1168–1176.
11. Bolton MD, van Esse HP, Vossen JH, de Jonge R, Stergiopoulos I, et al. (2008) The novel \textit{Cladosporium fulvum} lysis motif effector Ecp6 is a virulence factor with orthologues in other fungal species. Mol Microbiol 69: 119–136.
12. de Jonge R, van Esse HP, Kombrink A, Shinaya T, Desaki Y, et al. (2010) Conserved fungal LysM effector Ecp6 prevents chitin-triggered immunity in plants. Science 329: 953–955.
13. van den Burg HA, Harrison SJ, Joosten MHAJ, Vervoort J, de Wit PJGM (2006) \textit{Cladosporium fulvum} Avr4 protects fungal cell wall against hydrolysis by plant chitinases accumulating during infection. Mol Plant Microbe Interact 19: 1420–1430.
14. van Esse HP, Bolton MD, Stergiopoulos I, de Wit PJGM, Thomma BPHJ (2007) The chitin-binding \textit{Cladosporium fulvum} effector protein Avr4 is a virulence factor. Mol Plant Microbe Interact 20: 1092–1101.
15. Sánchez-Vallet A, Saleem-Batcha R, Kombrink A, Hansen G, Valkenburg D-J, et al. (2013) Fungal effector Ecp6 outcompetes host immune receptor for chitin binding through intrachain LysM dimerization. eLife 2: e00790.
16. Liu T, Liu Z, Song C, Hu Y, Han Z, et al. (2012) Chitin-induced dimerization activates a plant immune receptor. Science 336: 1160–1164.
17. Marshall R, Kombrink A, Motteram J, Loza-Reyes E, Lucas J, et al. (2011) Analysis of two in planta expressed LysM effector homologues from the fungus \textit{Mycophanarella graminicola} reveals novel functional properties and varying contributions to virulence on wheat. Plant Physiol 156: 756–769.
18. Menkul T A, Kombrink A, Shinaya T, Ryder LS, Oomoa I, et al. (2012) Effectors-mediated suppression of chitin-triggered immunity by \textit{Magnaporthe oryzae} is necessary for rice blast disease. Plant Cell 24: 322–335.
19. Schuhbauer A, Mauch F, Vogeli U, Boller T (1996) Plant chitinases are potent inhibitors of fungal growth. Nature 324: 363–367.
20. Martínez DA, Oliver BG, Graüer Y, Goldberg JM, Li W, et al. (2013) Comparative genome analysis of \textit{Triticophyton rhaemum} and related dermatophytes reveals candidate genes involved in infection. MBio 3: e00239–12.
21. Lee CG, Da Silva CA, Lee J-Y, Hart D, Elias JA (2008) Chitin regulation of immune responses: an old molecule with new roles. Curr Opin Immunol 20: 684–689.
22. Goldman DL, Vicencio AG (2012) The chitin connection. MBio 3: e00056–12.
23. Mora-Montes HM, Netea MG, Ferwerda G, Lenardon MD, Brown GD, et al. (2011) Recognition and blocking of innate immunity cells by \textit{Candida albicans} chitin. Infect Immun 79: 1961–1970.
24. Xu J, Saunders CW, Hu P, Grant RA, Bockhout T, et al. (2007) Danduff-associated Malassezia genomes reveal convergent and divergent virulence traits shared with plant and human fungal pathogens. Proc Natl Acad Sci U S A 104: 18730–18735.
25. Casé OH, Pagni M, Hauer PM (2012) De novo assembly of the \textit{Pneumocystis jirovecii} genome from a single bronchoalveolar lavage fluid specimen from a patient. MBio 4: e00429–12.
26. Seidl-Seiboth V, Zach S, Frischmann A, Spaduti O, Dietsch G, et al. (2013) Spore germination of \textit{Triticophyton atrorubens} is inhibited by its LysM protein TAL6. FEBS J 280: 1226–1236.
27. Buist G, Steen A, Kok J, Kuipers OP (2008) LysM, a widely distributed protein motif for binding to (peptido)glycans. Mol Microbiol 68: 838–847.
28. Yang J, Wang W, Wei X, Qiu L, Wang L, et al. (2010) Peptidoglycan recognition protein of \textit{Chlamydia farreri} (CfPGRP-S1) mediates innate defenses against bacterial infection. Dev Comp Immunol 34: 1300–1307.
29. Klostermann SJ, Subbarao KV, Kang S, Veronese P, Gold SE, et al. (2011) Comparative genomics yields insights into niche adaptation of plant vascular wilt pathogens. PLoS Pathog 7: e1002137. doi:10.1371/journal.ppat.1002137.
30. de Jonge R, Bolton MD, Kombrink A, van den Berg GC M, Yadeta KA, et al. (2013) Extensive chromosomal reshuffling drives evolution of virulence in an asexual pathogen. Genome Res 23: 1271–1282.