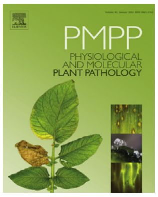
Physiological and Molecular Plant Pathology 95 (2016) 60–65

Contents lists available at ScienceDirect

Physiological and Molecular Plant Pathology

journal homepage: www.elsevier.com/locate/pmpp



Molecular mechanisms of chitin recognition and immune signaling by LysM-receptors☆

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ARTICLE INFO

Article history:
Received 15 December 2015
Received in revised form
4 February 2016
Accepted 4 February 2016
Available online 8 February 2016

Keywords:
MAMP
LysM-receptor
Chitin
OsCEBiP
OsCERK1

ABSTRACT

Plants have the ability to recognize microbe-associated molecular patterns (MAMPs) and initiate various defense responses. Chitin is a representative fungal MAMP that triggers defense signaling in a wide range of plant species. In rice, OsCEBiP and OsCERK1 form a receptor complex and play critical roles in chitin-triggered defense signaling. Recently, we found the formation of a unique sandwich-type dimer of OsCEBiP plays an important role for activation of chitin signaling. We now understand why *N*-acetyl groups and the longer chitin-oligosaccharides are required for receptor binding. We also found OsCERK1 is a bifunctional molecule acting in defense and also in AM symbiosis.

Plants have the ability to detect potential pathogens through the recognition of microbe-associated molecular patterns (MAMPs; also known as pathogen-associated molecular patterns, PAMPs), which are typical molecular signatures for whole classes of microbes [1–5]. This pattern-triggered immunity (PTI) is the first barrier to the invasion of pathogens and plays a major role in the basal resistance of plants. It is also well known that this defense is strikingly similar to the innate immunity of animals [3,6,7].

MAMP molecules, such as flagellin, EF-Tu, peptidoglycan, LPS, chitin and β-glucan have been isolated from the cell walls and secreted materials of bacteria and fungi. Concerning the receptors for these MAMPs, FLS2 and EFR, which recognize bacterial flagellin and EF-Tu, respectively, are the best characterized examples [8,9]. Although for most crops, the most serious pathogens are fungi, receptors for fungal MAMPs are poorly understood.

Chitin (β-1,4-linked polymer of *N*-acetylglucosamine) is a common component of the cell walls of various fungi. Fragments of chitin, *N*-acetylchitooligosaccharides, have been shown to act as potent MAMP signals in various plant systems [10,11]. For example, they induce lignification in wheat, ion flux and protein phosphorylation in cultured tomato cells, chitinase activity in melon, and gene expression of β-glucanase in cultured barley cells [12–14]. It has also been shown that among MAMPs, chitin is perceived by a particularly wide range of plants. Interestingly, chitin and chitin derivatives are not only recognized by plants, but also stimulate innate immune responses in mammals [15,16]. In suspension-cultured rice cells, chitin oligosaccharides also induced various defense-related cellular responses, such as rapid and transient membrane depolarization, generation of reactive oxygen species (ROS), induction of defense genes (β-glucanase and chitinase), and biosynthesis of jasmonic acid and phytoalexins [10]. These elicitor activities are strictly dependent on the structure of *N*-acetylchitooligosaccharides. That is, *N*-acetylchitoheptaose and octaose showed the highest activity, whereas *N*-acetylchitooligosaccharides shorter than the hexamer and deacetylated forms (chitooligosaccharides) showed much lower activity [17]. These results strongly indicated that the elicitor signal is mediated by a specific receptor that is likely to be localized in the plasma membrane of rice.

1. OsCEBiP is the major receptor for chitin elicitor binding in rice

Two plasma membrane proteins required for chitin perception and signaling, CEBiP (Chitin elicitor binding protein) and CERK1 (Chitin elicitor receptor kinase) were identified from rice and *Arabidopsis thaliana*, respectively [18–21]. The ectodomains of these molecules have a lysin motif (LysM), which is known to bind

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☆ This article is part of a special issue entitled "The U.S.-Japan Scientific Seminar: Molecular Contact Points in Host-Pathogen Co-evolution".

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http://dx.doi.org/10.1016/j.pmpp.2016.02.003
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bacterial peptidoglycan and fungal chitin [22,23].)-anchored glycoprotein, showed high-affinity binding to elicitor-active chitin oligosaccharides but did not contain an intracellular domain for defense signaling. OsCERK1, a LysM receptor-like kinase (RLK) with one transmembrane domain, was identified as a partner molecule of OsCEBiP in rice [20]. Cell lines with loss-of-function mutations of *OsCEBiP* or *OsCERK1* generated by homologous recombination lost chitin-induced responses [24,25], confirming a requirement for these molecules in chitin signaling, and suggesting formation of a OsCEBiP-OsCERK1 receptor complex. OsCERK1 was found not to have chitin binding activity by affinity labeling with biotinylated chitin octamer (GN8-Bio) as well as by precipitation experiments with colloidal-chitin [26]. On the other hand, affinity labeling experiments showed that the binding protein for GN8-Bio was completely absent from the microsomal membranes of *cebip* knockout cell lines, confirming that CEBiP is the major chitin receptor in rice [24].

Interestingly, the chitin receptor systems in rice and *Arabidopsis* are substantially different (Fig. 1A). In the case of *Arabidopsis*, the AtCERK1 receptor kinase seems to function as a ligand binding protein and a signaling molecule with kinase activity. AtCERK1 was shown to bind chitin and chitin oligosaccharides and does not require the closest homolog of CEBiP in *Arabidopsis*, LYM2 (AtCEBiP), which also has high affinity binding activity for chitin oligosaccharides, for chitin signaling [26]. Faulkner *et al.* later indicated that the plasmodesmata-localised LYM2 is involved in chitin-induced plasmodesmata closure, which seems to be important for chitin-triggered immunity [27].

They also showed that LYM2 contributes to disease resistance of *Arabidopsis*, independently of chitin signaling mediated by AtCERK1 [27,28]. These results indicated that ligand-induced dimerization of CERK1 is directly linked to downstream signaling in *Arabidopsis*. On the other hand, Cao et al. recently reported that the other *Arabidopsis* LysM RLK, AtLYK5, is the major chitin receptor in *Arabidopsis* and forms a receptor complex with AtCERK1 to initiate chitin signaling [29]. The chitin receptor system in *Arabidopsis* seems to be more complicated than expected and should further be clarified in future studies.

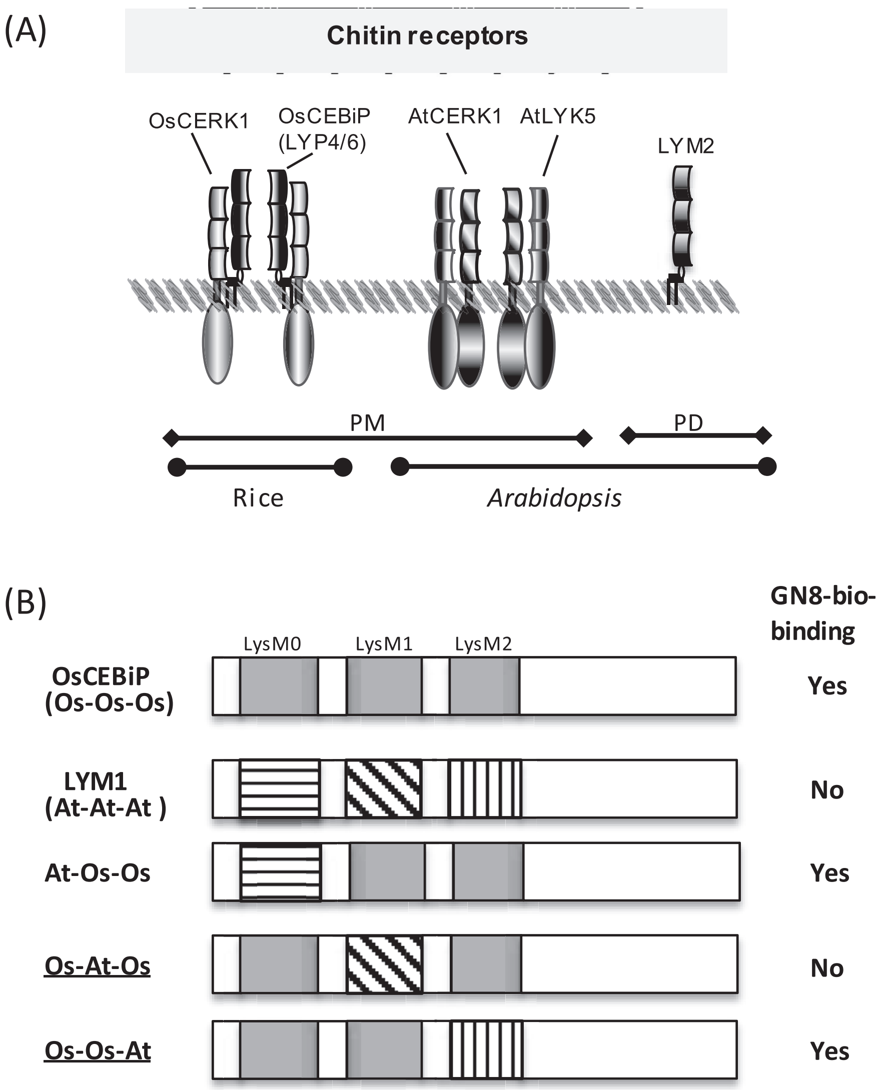

Fig. 1. LysM receptors and domain for chitin perception. (A) Chitin recognition receptors in rice (*Oryza sativa*) and Arabidopsis (*Arabidopsis thaliana*) [40]. PM: Plasma membrane. PD: Plasmodesumata. (B) Characterization of LysM domains of OsCEBiP with respect to chitin binding. The ectodomain of OsCEBiP consisting of three LysM (Os–Os–Os) has a high binding ability for biotinylated (GlcNAc)8 (GN8-Bio), whereas LYM1(At-At-At) identified from Arabidopsis does not have affinity for chitin.