Presence of LYM2 dependent but CERK1 independent disease resistance in *Arabidopsis*

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**Abbreviations:** ROS, reactive oxygen species

Plants have the ability to detect invading fungi through the perception of chitin fragments released from the fungal cell walls. Plant chitin receptor consists of two types of plasma membrane proteins, CEBiP and CERK1. However, the contribution of these proteins to chitin signaling is different between *Arabidopsis* and rice. In *Arabidopsis*, it seems CERK1 receptor kinase is enough for both ligand perception and signaling, whereas both CEBiP and OsCERK1 are required for chitin signaling in rice. Here we report that *Arabidopsis* CEBiP homolog, LYM2, is not involved in chitin signaling but contributes to resistance against a fungal pathogen, *Alternaria brassicicola*, indicating the presence of a novel disease resistance mechanism in *Arabidopsis*.

Detection of invading pathogens through the perception of microbe-associated molecular patterns (MAMPs) by corresponding pattern recognition receptors is an important basis of plant immune system. Chitin is a representative fungal molecular pattern and its recognition by plant chitin receptor triggers various defense responses in plants. Both of the infection experiments with the knockout mutants of plant chitin receptors, as well as the functional studies on fungal effectors that inhibit the perception of chitin oligosaccharides indicated that the chitin-triggered immunity plays an important role to protect plants from the invasion of fungal pathogens.

So far, two types of lysin motif (LysM)-containing proteins have been identified as the components of cell surface chitin receptor in plants. CEBiP, a receptor-like protein, was identified biochemically as a major chitin oligosaccharide binding protein in the plasma membrane of rice. On the other hand, CERK1, a receptor-like kinase, was identified genetically as an essential molecule for chitin signaling in *Arabidopsis*. We recently showed that the rice chitin receptor system requires both CEBiP and OsCERK1 for chitin perception and signaling, whereas *Arabidopsis* does not require CEBiP-like molecule for chitin perception and CERK1 seems sufficient both for chitin perception and membrane signaling.

There are three closely related CEBiP homologs in *Arabidopsis*, LYM1-3, LYM1 and LYM3 bind peptidoglycan and constitute peptidoglycan receptor in combination with CERK1. Although another CEBiP homolog, LYM2/AtCEBiP, specifically binds chitin oligosaccharides as similar to rice CEBiP, neither the knockout of LYM2 nor all of LYM1-3 affected chitin signaling. Interestingly, *Arabidopsis* CERK1 was shown to bind chitin but rice OsCERK1 was not. Thus, it was concluded that CERK1 serves both for chitin perception and membrane signaling in *Arabidopsis*.

If so, what can be the function of LYM2, which is biochemically very similar to rice CEBiP but does not contribute to CERK1 mediated chitin signaling? Is it a useless molecule left behind the evolution? Here we show that LYM2 does contribute to disease resistance against fungal pathogens but the mechanism seems independent of chitin signaling mediated by CERK1.

First we compared the disease resistance of a triple mutant for all three LYM proteins, *lym1/lym2/lym3*, which could respond to chitin oligosaccharide as similar to wild type Col-0 (Fig. 1 and ref. 11), against a fungal pathogen, *Alternaria brassicicola* (Fig. 2A). Interestingly, the triple mutant showed an increased susceptibility to the pathogen as similar to *cerk1* mutant. As the chitin-induced defense responses were completely suppressed in the *cerk1* mutant, the increased susceptibility of *cerk1* mutant against *A. brassicicola* can be interpreted as the results of the impairment of chitin signaling. On the other hand, as the triple...
understanding on plant immune system, especially on the significance of chitin perception as a trigger of the battle against invading fungal pathogens.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interests were disclosed.

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Note
After we completed the manuscript, Faulkner et al. reported that LYM2 contributes to the regulation of molecular flux via plasmodesmata and contributes to disease resistance against a necrotic fungus, Botrytis cinerea, independently of CERK1. This observation completely matches with our finding reported here.
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