Cytoplasmic interaction of LysM receptors contributes to the formation of symbiotic receptor complex

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Abstract Receptor complex formation at the cell surface is a key step to initiate downstream signaling but the contribution of this process for the regulation of the direction of downstream responses is not well understood. In the plant-microbe interactions, while CERK1, an Arabidopsis LysM-RLK, mediates chitin-induced immune responses, NFR1, a Lotus homolog of CERK1, regulates the symbiotic process with rhizobial bacteria through the recognition of Nod factors. Concerning the mechanistic insight of the regulation of such apparently opposite biological responses by the structurally related RLKs, Nakagawa et al. previously showed that the addition of YAQ sequence, conserved in NFR1 and other symbiotic LysM-RLKs, to the kinase domain of CERK1 switched downstream responses from defense to symbiosis using a set of chimeric receptors, NFR1-CERK1s. These results indicated that such a subtle difference in the cytoplasmic domain of LysM-RLKs could determine the direction of host responses from defense to symbiosis. On the other hand, it is still not understood how such structural differences in the cytoplasmic domains determine the direction of host responses. We here analyzed the interaction between chimeric NFR1s and NFR5, a partner receptor of NFR1, by co-immunoprecipitation (Co-IP) of these proteins transiently expressed in Nicotiana benthamiana. These results indicated that the cytoplasmic interaction between the LysM-RLKs is important for the symbiotic receptor complex formation and the YAQ containing region of NFR1 contributes to trigger symbiotic signaling through the successful formation of NFR1/NFR5 complex.

Key words: CERK1, chimeric receptor, NFR1, plant immunity, symbiosis.

Plants have the ability to respond to environmental inputs through the recognition of various signal molecules by cell surface receptors. In the case of plant-microbe interactions, pattern recognition receptors (PRRs), which recognize molecular signatures common to a group of microbes, play important roles for the detection of invading microbes and triggering immune responses (Boller and Felix 2009). Receptor-like kinases/proteins (RLKs/RLPs) containing Lysin Motif (LysM) ectodomains are a typical PRR and involved in the recognition of carbohydrate ligands such as peptidoglycan, chitin and lipopolysaccharides (Desaki et al. 2018).

Interestingly, LysM-RLKs are also known to be involved in the symbiotic responses for rhizobial and mycorrhizal symbionts (Shinya et al. 2015). For example, while CERK1, an Arabidopsis LysM-RLK, is required for chitin-induced immune responses (Miya et al. 2007), a Lotus japonicus homolog of CERK1, NFR1, is essential for symbiotic responses triggered by Nod factors, lipochitooligosaccharides secreted by symbiotic rhizobial bacteria (Radutoiu et al. 2003). NFR1 forms a receptor complex with another LysM-RLK NFR5, which carries an inactive kinase domain, for the recognition of the Nod factor and activation of the symbiotic responses (Madsen et al. 2011).

Concerning to the question how such structurally related RLKs can regulate apparently opposite biological responses, defense and symbiosis, Nakagawa et al. previously showed that a conserved amino acid sequence, YAQ, in the kinase domains of NFR1 and orthologs plays important roles in switching downstream responses from defense to symbiosis using chimeric receptors (Nakagawa et al. 2011). They showed, while the transgenic plants expressing NFR1-CERK1 chimera in the nfr1 background failed to form symbiotic nodules,

Abbreviations: PRR, pattern recognition receptor; CERK1, chitin elicitor Receptor like kinase 1; NFR1, nod factor receptor 1; NFR5, nod factor receptor 5; MYR1, myc factor receptor 1; LysM, Lysin Motif; RLK, receptor like kinase; RLP, receptor like protein; AA, amino acid.

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those expressing the same chimera but carrying the YAQ sequence in the kinase domain, significantly recovered the symbiotic ability, showing the importance of this sequence for the initiation of rhizobial symbiosis. The conclusion has been further extended that the LysM-RLKs carrying the YAQ, or an analogous sequence YAR, are also involved in mycorrhizal symbiosis, as evidenced for OsCERK1 in rice (Miyata et al. 2014).

While these experiments clearly showed the importance of the YAQ-containing kinase domain for symbiotic signaling, it is still not understood “how” such structural differences in the cytoplasmic domains determine the direction of host responses to symbiosis. To obtain further insight into the contribution of cytoplasmic domains in the initiation of symbiotic responses, we constructed several types of NFR1-CERK1 chimera (Figure 1A) and evaluated their ability to form a receptor complex with NFR5 by co-immunoprecipitation (Co-IP) of these proteins transiently expressed in Nicotiana benthamiana. The plasmids for transient expression in N. benthamiana were constructed as follows and finally inserted into pGWBI4 (3xHA) or pGWBI5 (4xMyc) (Suzuki et al. 2019). pENTR/D-TOPO entry vector inserted with NFR1, NFR1-CERK1, NFR1-CERK1(31AA) and NFR5 were generated as reported previously (Nakagawa et al. 2011; Suzuki et al. 2018). N. benthamiana was grown under a photoperiod of 14 h of light (20°C) and 10 h of darkness (22°C) (Suzuki et al. 2018). Agrobacterium tumefaciens C58C1 carrying the corresponding construct was infiltrated into N. benthamiana leaves using a syringe. As the co-expression of NFR1 and NFR5 in N. benthamiana was known to induce cell death at the infiltrated region (Madsen et al. 2011; Pietraszewska-Bogiel et al.

Figure 1. Evaluation of the interaction between chimeric NFR1s and NFR5 in N. benthamiana. (A) LysM-RLK constructs used for Co-IP experiments. (B) Evaluation of the interaction between NFR1-CERK1(31AA)-4Myc and NFR5-3HA by Co-IP experiments. Similar results were obtained in 3 independent experiments.
N. benthamiana leaves were collected before the visible cell death (about 24 h after the infiltration). A microsomal membrane fraction was prepared from the leaves and solubilized with phosphate-buffered saline (PBS) containing 0.5% TritonX-100, 62.5 mM sucrose, 1 mM phenylmethanesulfonyl fluoride (PMSF) and Phosphatase Inhibitor Cocktail Solution II (Wako Pure Chemical Industries, Ltd.) (Antolin-Llovera et al. 2014; Suzuki et al. 2016). Immunoprecipitation using a solubilized protein fraction was performed with Monoclonal Anti-HA-Agarose beads (Sigma-Aldrich) according to the manufacturer’s protocol. The solubilized protein fractions were incubated overnight with Anti-HA-Agarose beads at 4°C and the beads were washed five times with 0.01% TritonX-100 in PBS. Immunoprecipitates were eluted with a buffer of SDS buffer (IP: α-HA) and the proteins were analyzed using western blot with anti-HA antibody (WB: α-HA) or anti-Myc antibody (WB: α-Myc).

The results of these experiments showed that the 4xMyc-tagged NFR1 (NFR1-4Myc), a positive control, was co-precipitated with the 3xHA-tagged NFR5 (NFR5-3HA) as expected (Figure 1B, left; Madsen et al. 2011). On the other hand, the amount of 4xMyc-tagged chimeric NFR1-CERK1-4Myc co-precipitated with the NFR5-3HA was much less (about 30%) compared to that of the NFR1-4Myc, indicating the replacement of the cytoplasmic domain of the NFR1 with that of the CERK1 significantly decreased the interaction between the NFR1-CERK1-4Myc chimera and the NFR5-3HA (Figure 1B, middle). Interestingly, the amount of 4xMyc-tagged chimeric NFR1-CERK1(31AA) (NFR1-CERK1(31AA)-4Myc), which contained 31 amino acid sequence of the NFR1 including the activation loop and YAQ motif, co-precipitated with the NFR5-3HA (i.e., the ratio of co-precipitated NFR1-CERK1(31AA)-4Myc and NFR5-3HA) increased more than twice compared to the NFR1-CERK1-4Myc (Figure 1B, right), indicating the addition of the 31AA at least partly recovered the ability to interact with the NFR5-3HA. Previously, Nakagawa et al. suggested that the YAQ motif is primarily important to confer symbiotic activity upon the NFR1-CERK1 chimera, and the other region in the 31AA contributes to increase the efficiency on symbiotic signaling based on the hairy-root transformation experiments (Nakagawa et al. 2011). Present results coincided with the above notion and further indicated that the YAQ-containing cytoplasmic domains of the NFR1/NFR5-CERK1(31AA) chimera plays a crucial role for the formation of receptor complex with the NFR5 and thus for the initiation of symbiotic signaling. He et al. also indicated recently that the OsCERK1, which contains the YAQ-analogous YAR motif in the cytoplasmic domain and mediates mycorrhizal symbiosis in rice (Miyata et al. 2014), forms a complex with the rice ortholog of the NFR5, OsMYR1, through the interaction of cytoplasmic domains (He et al. 2019).

Biochemical basis of the function of the 31AA region other than YAQ motif is still not well understood. Interestingly, in vitro phosphorylation pattern of this region in NFR1 (Madsen et al. 2011) and CERK1 (Suzuki et al. 2016) is significantly different and whether such a difference in the phosphorylation pattern relates to the function of this region in the symbiotic receptor complex formation is an intriguing question and should further be evaluated in future study.

We indicated in the present study that the cytoplasmic interaction between the LysM-RLKs is important for the symbiotic receptor complex formation and the YAQ containing region of the NFR1 contributes to the formation of such a receptor complex. Structural studies on the cytoplasmic domains of related LysM-RLKs, e.g. CERK1, NFR1, NFR5, should contribute to understand the contribution of the YAQ containing region for the formation of the receptor complex formation and the initiation of symbiotic signaling.

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