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Grant Title: Molecular mechanisms of defense response induced by chitin oligosaccharides

Abstract

Perception of microbe-associated molecular patterns (MAMPs) through the corresponding pattern recognition receptors (PRRs) triggers various defense responses in plants. This MAMP-triggered immunity plays a major role in the basal resistance of plants against various pathogens. To clarify the molecular basis of the specific recognition of chitin oligosaccharides by the corresponding rice PRR, CEBiP, as well as the formation and activation of the receptor complex, we performed molecular biology, biochemical and biophysical, and computational studies. Deletion as well as domain swapping experiments showed that the central LysM region in the ectodomain of CEBiP is essential for the binding of chitin oligosaccharides. Epitope mapping by STD NMR spectroscopy indicated the preferential binding of longer chain chitin oligosaccharides such as heptamer-octamer to CEBiP and also the importance of N-acetyl groups for the binding. Molecular modeling studies clarified the molecular interaction between CEBiP and chitin oligosaccharides and indicated the importance of Ile122 in the central LysM region for ligand binding, a notion supported by the site directed mutagenesis. In addition, light scattering experiments proved that $(GlcNAc)_8$ induced the dimerization of the ectodomain of CEBiP but not of its mutant at Ile122 *in vitro* (*1*). Based on these results, we proposed a mechanism of ligand-induced activation of a receptor complex, involving both CEBiP and OsCERK1 (*1*). This model was further supported by the observation that both dimerization and $(GlcNAc)_8$ induced ROS generation were inhibited by a unique oligosaccharide, $(GlcN\beta1,4GlcNAc)_4$, which carries N-acetyl groups only on one side of the molecule (Fig1B).

Reference

1. Hayafune, M., Berisio, R., Marchetti, R., Silipo, A., Kayama, M., Desaki, Y., Arima, S., Squeglia, F., Ruggiero, A., Tokuyasu, K., Molinaro, A., Kaku, H., and Shibuya, N. (2014) Chitin-induced activation of immune signaling by the rice receptor CEBiP relies on a unique sandwich-type dimerization, *Proceedings of the National Academy of Sciences of the United States of America 111*, E404-413.

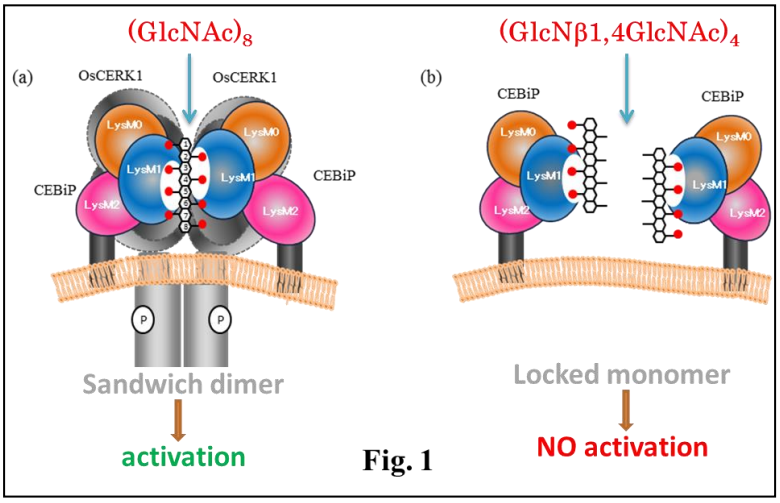

Fig. 1