Crystallography

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Structure of the N-terminal Regulatory Domain of Plant NADPH oxidase and Its Functional Implications

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Introduction

Various groups of eukaryote, such as mammals and land plants, possess enzymes dedicated to reactive oxygen species (ROS) production, and use for many biological activities. One of the most important enzymes producing ROS is NADPH oxidase family. Plant NADPH oxidase (Rboh) exists in plasma membrane, and reduce molecular oxygen to superoxide anion (O_2^-) by oxidation of cytosolic NADPH. ROS produced by Rboh plays multiple roles as signals that mediate immune response, abiotic stress, and developmental cues. Especially, importance of ROS production in immune response has been known from early on. Once an invasion of microbes is recognized, plant cell immediately generates ROS which trigger multiple immune responses. Rboh is regulated by Ca^{2+}, small GTPase [1], protein kinase, and other factors. However, details of activation mechanism have not been understood.

Structure of Rboh and fanctional implications

Rboh is composed of three distinguished segments: N-terminal regulatory region, Six transmembrane region, and C-terminal FAD/NADPH binding region. Both N-terminal region and C-terminal region exist in cytoplasm. terminal regulatory region of Rice (*Oryza sativa*) RbohB (OsRbohB) which contains Ca^{2+} binding motifs so called EF-hand [2]. It revealed that two molecules form a dimer with swapped EF-hands (Fig.1) and that OsRbohB contains two additional EF-hand-like motifs so far not predicted from sequence analysis. We show that Ca^{2+} binding to the EF-hands is necessary for the Ca^{2+} mediated conformational change. Structure based mutagenesis revealed that key residues for the interaction of OsRbohB and OsRac1 are located in the coiled-coil region created by EF-hand swapping (Fig.1). We demonstrate a direct intramolecular interaction between the N-terminal regulatory region and C-terminanl FAD/NADPH binding region. The highly conserved amino acid sequences of the N- and C-terminal regions suggest that the structural features and intramolecular interactions above mentioned might be common elements shared by Rbohs that contribute to the regulation of ROS production.

References

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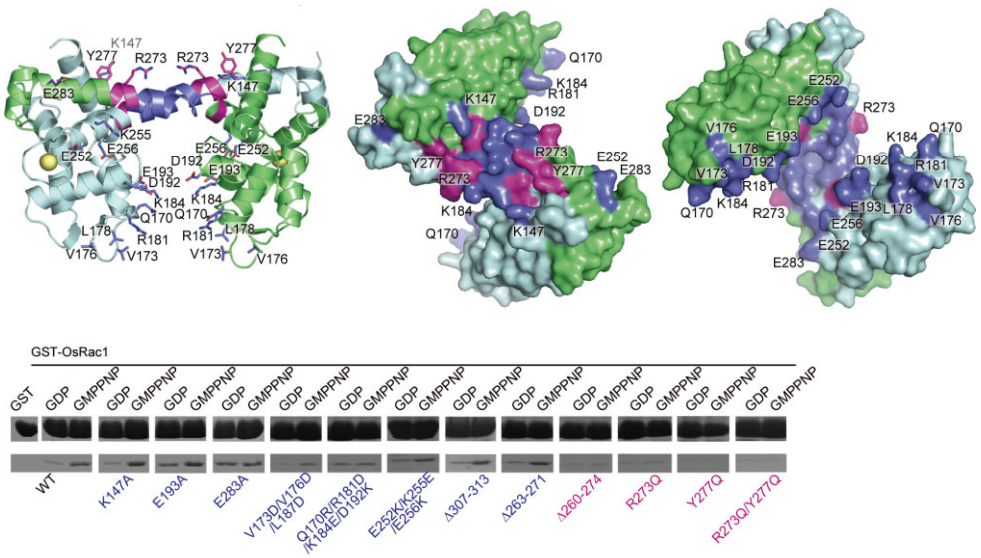

Figure 1. Crystal structure of OsRbohB and structure based mutagenesis analysis. In upper panel, structure of OsRbohB N-terminal regulatory region (amino acid residues 138–313) is shown as a ribbon model and as surface representations (two views from opposite sides). Two molecules (*green* and *cyan*) are paired by domain swapping. *In vitro* pulldown assays using OsRbohB-(138–313) mutants and GST-OsRac1 is displayed in lower panel. Mutation and deletion sites are mapped onto the structure. Residues that are necessary to maintain the full binding affinity are colored *magenta*, whereas residues exhibiting no or little effect on the binding are shown in *blue*.