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**Rice aquaporin PIP1;3 interact with *Xanthomonas oryzae* pathovar *oryzae* translocator Hpa1 and the identification of determinant domain****Yiqun Hu and Hansong Dong**

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Varieties of Gram-negative bacterial pathogens infect their eukaryotic hosts by deploying the type III translocon to deliver effector proteins into the cytosol of eukaryotic cells. The translocon is hypothetically assembled by bacterial translocators in associations with the assumed receptors situated on eukaryotic plasma membranes. By carrying out SUB-Y2H assays with multiple controls, we clearly detected a direct interaction between Hpa1 and OsPIP1;3, a rice aquaporin, plasma membrane intrinsic protein. The interaction was confirmed by co-immunoprecipitation (Co-IP). Furthermore, the OsPIP1;3-Hpa1 interaction was monitored by YFP bimolecular fluorescence complementation (BiFC). In rice protoplasts, the OsPIP1;3-Hpa1 interaction was localized to PMs, where the BiFC signal was colocalized with the PM marker, FM4-64. In tobacco *Nicotiana benthamiana* leaves, Hpa1 was localized to PMs and interacted with the introduced OsPIP1;3. These findings indicate that Hpa1 and OsPIP1;3 directly interact with each other at plant PMs. The primary roles of PIPs in substrate transport across PMs are executed based on their topological structures. In the current model, PIPs consist of six  $\alpha$ -helical trans-membrane (TM) domains (TM1-TM6) that are tilted along the plane of the PM and are linked one to the other by five connecting loops (LA-LE). Hpa1 interacts with OsPIP1;3, but not with OsPIP1;1 and OsPIP1;2. Therefore, either OsPIP1;1 or OsPIP1;2 is pertinent for site and fragment substitutions with OsPIP1;3 to look for Hpa1-interacting motifs in the OsPIP1;3 sequence. With this idea, we initially performed a series of site-directed mutations within LA, LC and LE at amino acid residues that are different between OsPIP1;3 and OsPIP1;2 between OsPIP1;3 and OsPIP1;1. Unfortunately, none of site substitutions affected OsPIP1;3 interaction with Hpa1. Then, we turned to create substitutive proteins by switching each of the six extramembrane-related regions between OsPIP1;3 and OsPIP1;1 and we find LE of OsPIP1;3 determines its interaction with Hpa1.

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