論文の要旨

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Identification of molecules regulating IDEF1-mediated iron deficiency responses in rice

（イネにおける IDEF1 を介した鉄欠乏応答制御分子の同定）

To acquire sparingly soluble iron (Fe) from the rhizosphere, plants transcriptionally induce genes involved in Fe acquisition in response to low Fe availability. The transcription factor IDEF1 plays an important role in regulation of Fe deficiency in graminaceous plants. In order to clarify the molecular mechanisms of Fe deficiency responses in rice, I screened, identified and characterized the candidate molecules regulating IDEF1-mediated Fe responses.

Screening of factors interacting with IDEF1 by yeast two-hybrid

I screened rice cDNA library by yeast two-hybrid to isolate the factors interacting with IDEF1. Through the screening of approximately 1.9×10^5 transformants, 16 positive clones containing partial sequences were identified to interact with IDEF1. After primary analysis, I focused on three of them for further research and designated them as IDEF1-Binding Proteins (IBPs).

Molecular characterization of IBP1

By yeast two-hybrid screening, IDEF1-binding proteins IBP1.1 and IBP1.2, which are homologous to Bowman-Birk trypsin inhibitors, were identified. Interaction between IDEF1 and IBP1.1 was confirmed by pull-down assay. Expression of *IBP1.1* and *IBP1.2* showed the induction in response to Fe deficiency and IDEF1 dependence. IBP1.1 localized to cytoplasm and nucleus when transiently expressed in onion epidermal cells. Transgenic rice plants overexpressing *IBP1.1* showed enhanced expression of an Fe(II)-nicotianamine transporter gene *OsYSL2*. IDEF1 protein was found to be degraded in a 26S proteasome-dependent manner, and this degradation was prevented by IBP1.1. Under Fe-sufficient conditions, IDEF1 is thought to be vigorously degraded through 26S proteasome-dependent pathway. Under Fe-deficient conditions, IBP1 protein is thought to accumulate and prevent the degradation of IDEF1. Accumulated IDEF1 is thought to induce the expression of downstream genes such as *OsYSL2*.

Molecular characterization of IBP2

IBP2, a pentatricopeptide repeat (PPR)-containing protein interacting with IDEF1, was identified by yeast two-hybrid screening and the interaction was confirmed by pull-down assay. *IBP2*-RNAi rice in which *IBP2* gene expression was knocked down tended to accumulate higher Fe in shoots and zinc (Zn) in roots than those in non-transformants under both Fe-sufficient and Fe-deficient conditions. It is supposed that the interaction between IDEF1 and IBP2 may relate to Fe homeostasis under both Fe-sufficient and Fe-deficient conditions.

Molecular characterization of IBP3

By yeast two-hybrid and pull-down assay, it showed IBP3, a heavy metal transport/detoxification protein domain-containing protein interacts with IDEF1. *IBP3* gene expression was repressed by Fe deficiency in rice roots. As IDEF1 binds Fe and Zn through metal binding domain and is thought to sense cellular Fe status, IBP3 may also be involved in this process. IBP3 protein had the activity of binding Fe and Zn and IBP3 may transit the bound Fe to IDEF1 through the interaction with IDEF1 under

Fe-sufficient conditions.

Conclusion

By yeast two-hybrid screening, three proteins IBP1, IBP2 and IBP3 interacting with IDEF1 were identified and the interactions were confirmed by pull-down assay. IBP1, IBP2 and IBP3 are thought to work cooperatively in different ways such as prevention of IDEF1 degradation and Fe binding, in regulating IDEF1-mediated Fe responses through the interaction with IDEF1. This research clarified not only post-translational regulation of IDEF1, but also molecular mechanisms regulating Fe-deficiency responses in rice.