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Rice Serine/Threonine Kinase 1 Is Required for the Stimulation of OsNug2 GTPase Activity

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Abstract : Several GTPases are required for ribosome biogenesis and assembly. We recently characterized rice (Oryza sativa) nuclear/nucleolar GTPase 2 (OsNug2), belonging to the YlqF/YawG family of GTPases, as playing a role in pre-60S ribosomal subunit maturation. To investigate the potential factors involved in regulating the function of OsNug2, yeast two-hybrid screens were carried out using OsNug2 as bait. Rice serine/threonine kinase 1 (OsSTK1) was identified as a potential interacting protein candidate. In vitro pull down and bimolecular fluorescence complementation assays confirmed the interaction between OsNug2 and OsSTK1, and like green fluorescent protein-tagged OsNug2, green fluorescent protein-tagged OsSTK1 was targeted to the nucleus of Arabidopsis protoplasts. OsSTK1 was not found to affect the GTP-binding activity of OsNug2; however, when recombinant OsSTK1 was included in OsNug2 assay reaction mixtures, OsSTK1 increased the GTPase activity of OsNug2. To test whether OsSTK1 phosphorylates OsNug2 in vitro, a kinase assay was performed. OsSTK1 was found to have weak autophosphorylation activity and strongly phosphorylated serine 209 of OsNug2. Yeast complementation testing resulted in a GAL::OsNug2(S209N) mutant-harboring yeast strain exhibiting a growth-defective phenotype on galactose medium at 39°C, divergent from that of a yeast strain harboring GAL::OsNug2. The intrinsic GTPase activity of mutant OsNug2(S209N) was found to be similar to that of OsNug2, was not fully enhanced upon weak binding of OsSTK1. Our findings reported here indicate that OsSTK1 functions as a positive regulator protein of OsNug2 by enhancing the GTPase activity of OsNug2, and that the phosphorylation of serine 209 of OsNug2 is essential for the complete function of OsNug2 in ribosome biogenesis.

Keywords : OsSTK1, OsNug2, GTPase activity, GTP binding activity, phosphorylation
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