IRBIT, a Novel Inositol 1,4,5-Trisphosphate(IP3) Receptor-binding Protein, Is Released from the IP3 Receptor upon IP3 Binding to the Receptor

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The inositol 1,4,5-trisphosphate (IP3) receptors (IP3Rs) are IP3-gated Ca2+ channels on intracellular Ca2+ stores. Herein, we report a novel protein, termed IRBIT (IP3R binding protein released with inositol1,4,5-trisphosphate), which interacts with type 1 IP3R (IP3R1) and was released upon IP3 binding to IP3R1. IRBIT was purified from a high salt extract of crude rat brain microsomes with IP3 elution using an affinity column with the huge immobilized N-terminal cytoplasmic region of IP3R1 (residues 1-2217). IRBIT, consisting of 530 amino acids, has a domain homologous to *S*-adenosylhomocysteine hydrolase in the C-terminal and in the N-terminal, a 104 amino acid appendage containing multiple potential phosphorylation sites. *In vitro* binding experiments showed the N-terminal region of IRBIT to be essential for interaction, and the IRBIT binding region of IP3R1 was mapped to the IP3 binding core. IP3 dissociated IRBIT from IP3R1 with an EC50 of ~0.5 μM, *i.e.* it was 50 times more potent than other inositol polyphosphates. Moreover, alkaline phosphatase treatment abolished the interaction, suggesting that the interaction was dualistically regulated by IP3 and phosphorylation. Immunohistochemical studies and co-immunoprecipitation assays showed the relevance of the interaction in a physiological context. These results suggest that IRBIT is released from activated IP3R, raising the possibility that IRBIT acts as a signaling molecule downstream from IP3R.

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