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Manuscript Title:

Purification of Endogenous Drosophila TRP Channels

Date of Revision:

November 11, 2021

Abstract:

Drosophila phototransduction is one of the fastest known G-protein-coupled signaling pathways. To ensure the specificity and efficiency of this cascade, the Ca2+-permeable cation channel transient receptor potential (TRP) binds tightly to scaffold protein inactivation-no-after-potential D (INAD) and forms a large signaling protein complex with eye-specific protein kinase C (ePKC) and phospholipase Cβ/No receptor potential A (PLCβ/NORPA). However, the biochemical properties of the Drosophila TRP channel remain unclear. Based on the assembling mechanisms of INAD protein complex, a modified “affinity purification plus competition” strategy was developed to purify the endogenous TRP channel. First, the purified his-tagged NORPA 863-1095 fragment was bound to Ni-beads and used as the bait to pull down the endogenous INAD protein complex from Drosophila head homogenates. Then, excessive purified GST-tagged TRP 1261-1275 peptide was added to the Ni-beads slurry to compete the TRP channel. Finally, the TRP channel in the supernatant was separated from the excessive TRP 1261-1275 peptide...

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