Bead aggregation assay to demonstrate the clustering of postsynaptic proteins by the NGL family of cell adhesion molecules

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Method Article

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Abstract

Introduction

Bead-induced direct aggregation of neuroligin on the surface membrane of neuronal dendrites induced the clustering of neuroligin and various postsynaptic proteins \[1]. We employed this assay to demonstrate that direct aggregation of NGL, a family of cell adhesion molecules that associates with the netrin-G family of cell adhesion molecules and the postsynaptic scaffolding protein PSD-95, on the dendritic surface induces coclustering of excitatory postsynaptic proteins including PSD-95, GKAP, Shank, and NMDA glutamate receptors.

Procedure

Neuron transfection 1. Transfect cultured neurons with a form of NGL-2 in which EGFP was tagged to the N terminus \(EGFP-NGL-2) at DIV 14, and incubate for 2 days. **Preparation of EGFP antibody-coated beads** 2. Mix 5 µg of biotin-conjugated EGFP antibodies \(Rockland) and 2 µL of neutravidin-conjugated FluoSphere beads \(Molecular Probes; 1 µm diameter), and incubate for 1 h. 3. Increase the volume of the mixture to 1 mL by adding Hank's balanced salt solution \(HBSS), and centrifuge at 13,000 rpm for 1 min in a microcentrifuge. 4. Carefully remove the supernatant, and resuspend the precipitates in 100 µL of conditioned media where neurons were growing. **Bead-induced aggregation of NGL-2** 5. Place the coverslips containing transfected neurons \(DIV16) face-up on 6-well \(or larger) dishes. Save the conditioned media for Step 7. 6. Add 100 µL of the resuspended beads onto the neurons and incubate at 37 ^{o}C for 30 min. 7. Wash the neurons twice with HBSS, and place the coverslip back into the culture media. 8. After 24 h of incubation, fix and immunostain the neurons with primary antibodies against various synaptic proteins and fluorophore-conjugated secondary antibodies. **Image acquisition and quantitation** 9. Capture Z-stacked images by confocal immunofluorescence microscopy as well as DIC imaging. 10. For quantitation, manually trace the boundaries of the beads. Copy a boundary to a nearby dendritic area for normalization. 11. An average immunofluorescence intensity of a synaptic marker from a bead area was measured and normalized to that from a nearby control area.

References

1. Graf, E. R., Zhang, X., Jin, S. X., Linhoff, M. W. & Craig, A. M. Neurexins induce differentiation of GABA and glutamate postsynaptic specializations via neuroligins. _Cell_ **119**, 1013-26 \(2004).