Cloning and characterization of GUKHolder, a novel synaptically expressed protein that interacts with Discs -Large and SCRIBBLE at the Drosophila neuromuscular junction

L. Sian Gramates, *University of Massachusetts Amherst*

Abstract

Synaptic transmission between a neuron and its target is crucially dependent upon the precise spatial arrangement of proteins in the pre- and postsynaptic apparatus. PDZ domain-containing proteins such as the *Drosophila* tumor suppressor Discs-Large (DLG) play critical roles in synapse maturation by regulating the assembly of synaptic protein complexes. ^ DLG is composed of a number of modular domains, including three PDZ-domains, an SH3 domain and an enzymatically inactive Guanylate Kinase-like (GUK) domain. Previous studies have shown that the PDZ domains of DLG mediate clustering of Shaker K^+ channels and of the cell adhesion molecule Fasciclin II. However, the function of the GUK domain has been unclear. ^ To understand the role of the GUK domain, we carried out a yeast-two hybrid screen for interacting partners of the DLG GUK domain. This screen lead to the identification of a novel synapse-associated protein, GUKHolder (GUKH). ^ GUKH is a 1044 amino acid protein with a molecular weight of 110 kDa. Its sequence includes a GUK-holding domain, a region homologous to the C-terminal of the long isoform of Kelch, a WH1-like domain, and a PDZ-domain binding motif. These latter two features suggest that GUKH may interact not only with DLG, but also with other proteins, including proteins containing PDZ domains. ^ GUKH is expressed at the larval neuromuscular junction and at epithelial cell borders in partial colocalization with DLG. Further, DLG can be co-immunoprecipitated with GUKH from *Drosophila* extracts, indicating an *in vivo* interaction between the two proteins. GUKH has also been shown to interact directly with SCRIBBLE (SCRIB), another synaptically expressed PDZ-domain protein known to have a genetic interaction with *dlg* in epithelial tissues. Synaptic SCRIB immunoreactivity is mislocalized in both *gukh* and *dlg* mutants. *gukh, scrib*, and *dlg* mutants all exhibit synaptic bouton defects at the ultrastructural level. ^ These data indicate that all three proteins are required for proper synapse maturation, and support a model that the three proteins exist in a tripartite complex, with GUKH forming a link between the other two proteins, and further, between the protein scaffolds organized by the two proteins. ^

Subject Area

Molecular biology|Neurosciences|Genetics

Recommended Citation