Regulation of actin-Spectrin cytoskeleton by ICA69 at the Drosophila neuromuscular junction

Bhagaban Mallik and Vimlesh Kumar

Laboratory of Neurogenetics, Department of Biological Sciences, Indian Institute of Science Education and Research (IISER) Bhopal, Madhya Pradesh, India

ABSTRACT

Bin-Amphiphysin-Rvs (BAR) domain containing proteins with their membrane deforming properties have emerged as key players in shaping up neuronal morphology and regulating cytoskeletal dynamics. However, the in vivo contexts in which BAR-domain proteins integrate membrane dynamics with cytoskeletal rearrangements remain poorly understood. Recently, we identified islet cell autoantigen 69 kDa as one of the N-BAR-domain containing proteins which regulate synaptic development and organization at the Drosophila neuromuscular junction. ICA69 genetically functions downstream of Rab2 to regulate synapse morphology. We found that ICA69 alters Spectrin level at the Drosophila NMJ, and redistributes actin regulatory proteins in cultured cells suggesting that ICA69 may regulate NMJ organization by regulating actin-Spectrin cytoskeleton. We propose a model in which ICA69 genetically interact with components of actin regulatory proteins for cytoskeleton dynamics to regulate NMJ development and synapse organization.

KEYWORDS

Actin; Spectrin; Arp2/3; Drosophila; ICA69; N-BAR; Neuromuscular junction; Wasp

Synapses are asymmetric structures where neurotransmitters are released from the presynaptic neuron and activate neurotransmitter receptors on the postsynaptic membrane. Thus, proper functioning of the nervous system critically relies on the development and establishment of a precise and selective pattern of synaptic connections. Moreover, synapses are dynamic structures which undergo morphological and functional changes through the membrane and cytoskeletal remodeling. The dynamic nature of cell membrane combined with a reorganization of underlying cytoskeleton plays a central role in the establishment and shaping up of the neuronal membrane, both during structural and functional plasticity in the neurons. The identification of BAR-domain containing proteins and their ability to link membrane dynamics with cytoskeletal changes has augmented our appreciation of these processes. However, mechanisms that underlie regulation of the synaptic cytoskeleton and membrane dynamics by BAR-family proteins remain poorly understood. Moreover, little is known about how actin-regulatory proteins associate with BAR-domain containing proteins to modulate synaptic actin and membrane dynamics. We recently identified Drosophila ICA69 (dICA69), a BAR-domain containing protein that regulates synaptic actin-Spectrin cytoskeleton at the neuromuscular junction synapses. Here we discuss a possible model by which dICA69 may regulate cytoskeletal rearrangement to regulate NMJ organization.

Actin cytoskeleton contributes towards organizing the scaffolding components to facilitate trafficking of synaptic proteins. At the presynapses, the role of actin has also been implicated in maintaining and regulating vesicle pools as well as activation of silent synapse. Actin is also enriched in the postsynaptic terminals, where it anchors receptors by interacting with a number of scaffolding proteins including Spectrin. In particular, actin cytoskeleton machinery is involved in regulation of postsynaptic receptor targeting/retention at the synapses. The requirement of actin dynamics at synapses is also strengthened from the observations that actin regulatory proteins such as Arp2/3 and N-Wasp are located in presynaptic and postsynaptic compartments of hippocampal neurons and the RNAi-mediatesed depletion of these proteins results in altered synapse number.

Among various membrane and cytoskeleton remodelers, BAR-domain super family proteins are considered to be one of the major key players in this process. BAR-domain containing proteins may regulate actin cytoskeleton and hence, possibly modulate a variety of cellular processes, including synaptic development, synapse...
organization and vesicle trafficking in neurons. The BAR domain proteins, dRich and dCIP4 are involved in localization of actin regulatory protein Wasp at the postsynaptic compartment.16,17 Interestingly nervous wreck (Nwk), another BAR domain containing protein has been shown to interact with Wasp and regulates presynaptic cytoskeleton.18 However, the underlying mechanisms by which the BAR-domain proteins regulate pre or post-synaptic cytoskeleton remains to be investigated.

Drosophila ICA69 orchestrates cytoskeletal dynamics during synapse development and/or organization at the Drosophila NMJ.19 We found that Drosophila ICA69 is highly enriched at the NMJ and is located in the same micro domain as actin-Spectrin. The hemizygous mutant of dica69 shows a reduced level of α-Spectrin staining at the synapses. Over-expression of dICA69 in the Drosophila S2R+ cell causes induction of filopodia and redistribution of the positive regulators of actin polymerization at the site of filopodia. Interestingly, other actin remodeling Drosophila mutants like wasp, nwk, arp3 and cip4 also exhibit NMJ morphological defects.17,18,20 While these actin remodeling mutants exhibit synaptic

![Figure 1. A model depicting NMJ structural organization mediated by dICA69 through its interaction with the actin regulatory proteins in the Cdc42-Cip4-Wasp pathway. Cdc42 activates localization of dCIP4 which in turn control the levels of Wasp at the postsynapse.17 The Cdc42-Cip4-Wasp pathway negatively regulates Drosophila NMJ development. Hence, ICA69 could be a positive regulator of this pathway at the postsynapse. Wasp machinery interacts with the Arp2/3 complex for the polymerization and organization of the actin-Spectrin cytoskeleton.27 dICA69 may associate with any of the components of this pathway to regulate the underlying cytoskeleton. Analysis of loss-of function alleles of ICA69 and genetic epistatic interactions with the components of actin-Spectrin cytoskeleton will help test this model.](image-url)
overgrowth, the Ica69 mutants show synaptic undergrowth in Drosophila. One of the modes of regulation of the cytoskeleton is guided by the activity of Cdc42-Cip4-Wasp pathway, and it is possible that ICA69 might be associated with this pathway to regulate NMJ structural organization. Our data suggest that dIca69 may be a positive regulator of the Cdc42-Cip4-Wasp pathway. Genetic analysis of this pathway will help test this hypothesis. Moreover, it remains to be determined whether and how ICA69 mediates its influence on the stability or localization of postsynaptic actin-Spectrin scaffolds.

We envisage the role of dICA69 as one of the regulators of the postsynaptic actin-Spectrin network. The postsynaptic Spectrin is composed of hetero-tetramers of two α- and two β-Spectrin subunits, together with chains of actin filaments, that from an actin-Spectrin scaffold which parallels the plasma membrane. Earlier studies have indicated that Spectrin cytoskeleton is linked to the plasma-membrane through its interactions with either Ankyrin, integral membrane proteins or phospholipids. The Spectrin and Ankyrin network is required for the proper establishment of cell adhesion molecules into distinct microdomains. The organization of the membrane domains is severely impaired in βIV-spectrin mutants in mice, indicating that Spectrin cytoskeleton is essential for the stabilization of the integral membrane clusters. Spectrin is located both in the peripheral and central nervous system and has been postulated for clustering of the neurotransmitter receptors and neuronal growth in C. elegans. In Drosophila, α- and β-Spectrin is located at the NMJ, and mutants of α- and β-Spectrin have severe NMJ growth defects in late embryonic/early larval stages. Thus, actin-Spectrin cytoskeleton plays crucial roles in NMJ organization, and ICA69 appears to be one of the key players in the process.

In α-spectrin mutants, the localization of the pre-synaptic vesicle proteins and a postsynaptic component such as Disc-large (Dlg) is altered. However, whether dlg mutants show altered actin-Spectrin network need to be investigated. Similarly, the distribution and organization ofDlg and Spectrin in cip4 mutants also remain unknown. Interestingly, Ica69 mutant showed normal distribution of presynaptic vesicle associated proteins and postsynaptic Dlg. Surprisingly, we found that ~50% reduction in ICA69 levels does not have a significant effect on the distribution or localization of scaffolding protein, Dlg at the NMJ. One possibility is that ICA69 does not regulate Dlg levels. Another possibility could be ~50% ICA69 levels may be sufficient to maintain normal synaptic Dlg levels. It would be interesting to further address the relationship between ICA69 and Dlg in Ica69 null mutants.

To summarize, we propose a model (Fig. 1) that circumscribes the role of dICA69 in the context of cytoskeletal regulation. Firstly, ICA69 is highly enriched at the NMJ and localized to the same micro domain as Spectrin. Secondly, Ica69 mutants show reduced Spectrin levels at the NMJ. Finally, overexpression of dICA69 redistributes regulators of the actin cytoskeleton at the site of filopodia. These evidences led us to hypothesize that ICA69 might genetically interact with the components in the Cdc42-Cip4-Wasp pathway to regulate the cytoskeleton for the NMJ structural organization. Therefore, our model places ICA69 as a major player in the control of cytoskeletal remodeling that coordinate NMJ development and organization.

Disclosure of potential conflicts of interest
No potential conflicts of interest were disclosed.

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