COMMUNICATIVE & INTEGRATIVE BIOLOGY
2018, e1381806 (4 pages)
https://doi.org/10.1080/19420889.2017.1381806

Taylor & Francis
Taylor & Francis Group

ARTICLE ADDENDUM

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.

ARTICLE HISTORY
Received 19 July 2017
Revised 12 September 2017
Accepted 13 September 2017

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.[1-3] Moreover, synapses are dynamic structures which undergo morphological and functional changes through the membrane and cytoskeletal remodeling.[4] 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 its ability to link membrane dynamics with cytoskeletal changes has augmented our appreciation of these processes [5]. 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.[6] 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.[7-10] Actin is also enriched in the postsynaptic terminals, where it anchors receptors by interacting with a number of scaffolding proteins including Spectrin.[11] 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 pre- and postsynaptic compartments of hippocampal neurons and the RNAi- mediated depletion of these proteins results in altered synapse number.[12,13]

Among various membrane and cytoskeleton remodelers, BAR-domain super family proteins are considered to be one of the major key players in this process.[14,15] BAR-domain containing proteins may regulate actin cytoskeleton and hence, possibly modulate a variety of cellular processes, including synaptic development, synapse

CONTACT Vimlesh Kumar vimlesh@iiserb.ac.in
Addendum to: Mallik B, Dwivedi MK, Mushtaq Z, Kumari M, Verma PK, Kumar V. Regulation of neuromuscular junction organization by Rab2 and its effector ICA69 in Drosophila. Development 2017; 144:2032-44. doi:10.1242/dev.145920

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

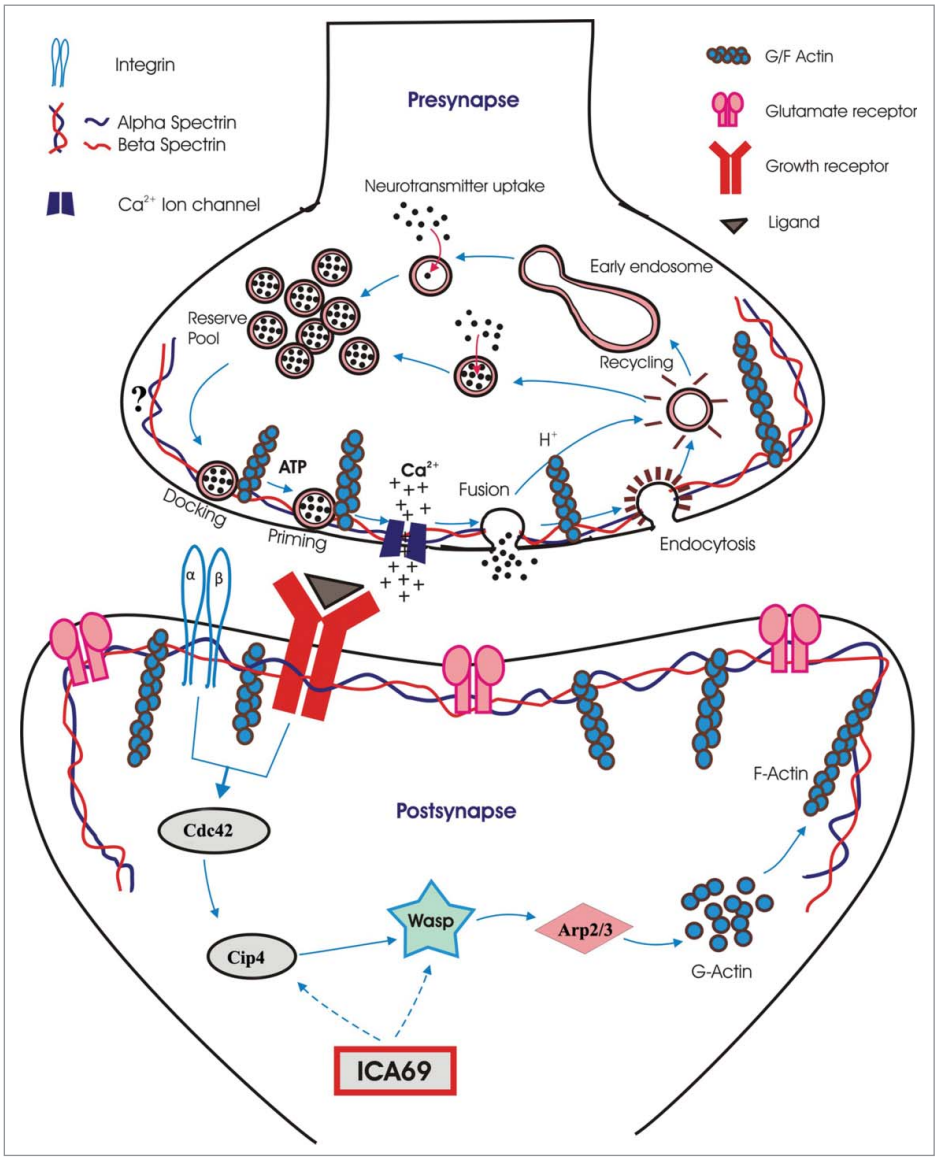

Figure 1. A model depicting NMJ structural organization mediated by dICA69 through its interaction with the actin regulatory proteins in the Cdc42-Cip4-Wasp pathway. Cdc42 activates localization of dCIP4 which in turn control the levels of Wasp at the postsynapse.[17] The Cdc42-Cip4-Wasp pathway negatively 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.

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.[21] 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 β*IV-spectrin* mutants in mice, indicating that Spectrin cytoskeleton is essential for the stabilization of the integral membrane clusters.[22,23] 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*.[24,25] 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.[26] Thus, actin-Spectrin cytoskeleton plays crucial roles in NMJ organization, and ICA69 appear 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.[26] However, whether *dlg* mutants show altered actin-Spectrin network need to be investigated. Similarly, the distribution and organization of Dlg and Spectrin in *cip4* mutants also remain unknown. Interestingly, *Ica69* mutant showed normal distribution of presynaptic vesicle associated proteins and postsynaptic Dlg.[19] 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.

Acknowledgments

We are grateful to Saumitra Dey Chaudhury for useful comments on this manuscript and Amrutha Valasakumar for help with the model.

Funding

B. M. thanks the University Grants Commission, Government of India for a graduate fellowship. V. K. thanks Department of Biotechnology (BT/PR-15163/GBD/27/349/2011), Government of India for funding.

References

1. Menon KP, Carrillo RA, Zinn K. Development and plasticity of the Drosophila larval neuromuscular junction. Wiley Interdiscip Rev Dev Biol. 2013;2:647-70. doi:10.1002/wdev.108. PMID:24014452
2. Ryan TJ, Grant SG. The origin and evolution of synapses. Nat Rev Neurosci. 2009;10:701-12. doi:10.1038/nrn2717. PMID:19738623
3. Yogev S, Shen K. Cellular and molecular mechanisms of synaptic specificity. Annu Rev Cell Dev Biol. 2014;30:417-37. doi:10.1146/annurev-cellbio-100913-012953. PMID:25150010
4. Ojelade SA, Acevedo SF, Rothenfluh A. The role of the actin cytoskeleton in regulating Drosophila behavior. Rev Neurosci. 2013;24:471-84. doi:10.1515/revneuro-2013-0017. PMID:24077615
5. Itoh T, Erdmann KS, Roux A, Habermann B, Werner H, De Camilli P. Dynamin and the actin cytoskeleton cooperatively regulate plasma membrane invagination by BAR and F-BAR proteins. Dev Cell. 2005;9:791-804. doi:10.1016/j.devcel.2005.11.005. PMID:16326391
6. Rocca DL, Martin S, Jenkins EL, Hanley JG. Inhibition of Arp2/3-mediated actin polymerization by PICK1 regulates neuronal morphology and AMPA receptor endocytosis. Nat Cell Biol. 2008;10:259-71. doi:10.1038/ncb1688. PMID:18297063

7. Dillon C, Goda Y. The actin cytoskeleton: integrating form and function at the synapse. Annu Rev Neurosci. 2005;28:25-55. doi:10.1146/annurev.neuro.28.061604.135757. PMID:16029114
8. Hilfiker S, Pieribone VA, Czernik AJ, Kao HT, Augustine GJ, Greengard P. Synapsins as regulators of neurotransmitter release. Philos Trans R Soc Lond B Biol Sci 1999;354:269-79. doi:10.1098/rstb.1999.0378. PMID:10212475
9. Evergren E, Benfenati F, Shupliakov O. The synapsin cycle: a view from the synaptic endocytic zone. J Neurosci Res. 2007;85:2648-56. doi:10.1002/jnr.21176. PMID:17455288
10. Shen W, Wu B, Zhang Z, Dou Y, Rao ZR, Chen YR, Duan S. Activity-induced rapid synaptic maturation mediated by presynaptic cdc42 signaling. Neuron. 2006;50:401-14. doi:10.1016/j.neuron.2006.03.017. PMID:16675395
11. Kuriu T, Inoue A, Bito H, Sobue K, Okabe S. Differential control of postsynaptic density scaffolds via actin-dependent and -independent mechanisms. J Neurosci. 2006;26:7693-706. doi:10.1523/JNEUROSCI.0522-06.2006. PMID:16855097
12. Wegner AM, Nebhan CA, Hu L, Majumdar D, Meier KM, Weaver AM, Webb DJ. N-wasp and the arp2/3 complex are critical regulators of actin in the development of dendritic spines and synapses. J Biol Chem. 2008;283:15912-20. doi:10.1074/jbc.M801555200. PMID:18430734
13. Koch N, Kobler O, Thomas U, Qualmann B, Kessels MM. Terminal axonal arborization and synaptic bouton formation critically rely on abp1 and the arp2/3 complex. PLoS One. 2014;9:e97692. doi:10.1371/journal.pone.0097692. PMID:24841972
14. Frost A, Perera R, Roux A, Spasov K, Destaing O, Egelman EH, De Camilli P, Unger VM. Structural basis of membrane invagination by F-BAR domains. Cell. 2008;132:807-17. doi:10.1016/j.cell.2007.12.041. PMID:18329367
15. Ukken FP, Bruckner JJ, Weir KL, Hope SJ, Sison SL, Birschbach RM, Hicks L, Taylor KL, Dent EW, Gonsalvez GB, et al. BAR-SH3 sorting nexins are conserved interacting proteins of Nervous wreck that organize synapses and promote neurotransmission. J Cell Sci. 2016;129:166-77. doi:10.1242/jcs.178699. PMID:26567222
16. Nahm M, Long AA, Paik SK, Kim S, Bae YC, Broadie K, Lee S. The Cdc42-selective GAP rich regulates postsynaptic development and retrograde BMP transsynaptic signaling. J Cell Biol. 2010;191:661-75. doi:10.1083/jcb.201007086. PMID:21041451
17. Nahm M, Kim S, Paik SK, Lee M, Lee S, Lee ZH, Kim J, Lee D, Bae YC, Lee S. dCIP4 (Drosophila Cdc42-interacting protein 4) restrains synaptic growth by inhibiting the secretion of the retrograde Glass bottom boat signal. J Neurosci. 2010;30:8138-50. doi:10.1523/JNEUROSCI.0256-10.2010. PMID:20554864
18. Coyle IP, Koh YH, Lee WC, Slind J, Fergestad T, Littleton JT, Ganetzky B. Nervous wreck, an SH3 adaptor protein that interacts with Wsp, regulates synaptic growth in Drosophila. Neuron. 2004;41:521-34. doi:10.1016/S0896-6273(04)00016-9. PMID:14980202
19. Mallik B, Dwivedi MK, Mushtaq Z, Kumari M, Verma PK, Kumar V. Regulation of neuromuscular junction organization by Rab2 and its effector ICA69 in Drosophila. Development. 2017;144:2032-44. doi:10.1242/dev.145920. PMID:28455372
20. Khuong TM, Habets RL, Slabbaert JR, Verstreken P. WASP is activated by phosphatidylinositol-4,5-bisphosphate to restrict synapse growth in a pathway parallel to bone morphogenetic protein signaling. Proc Natl Acad Sci U S A. 2010;107:17379-84. doi:10.1073/pnas.1001794107. PMID:20844206
21. Bennett V, Baines AJ. Spectrin and ankyrin-based pathways: metazoan inventions for integrating cells into tissues. Physiol Rev. 2001;81:1353-92. PMID:11427698
22. Jenkins SM, Bennett V. Ankyrin-G coordinates assembly of the spectrin-based membrane skeleton, voltage-gated sodium channels, and L1 CAMs at Purkinje neuron initial segments. J Cell Biol. 2001;155:739-46. doi:10.1083/jcb.200109026. PMID:11724816
23. Komada M, Soriano P. [Beta]IV-spectrin regulates sodium channel clustering through ankyrin-G at axon initial segments and nodes of Ranvier. J Cell Biol. 2002;156:337-48. doi:10.1083/jcb.200110003. PMID:11807096
24. Hammarlund M, Davis WS, Jorgensen EM. Mutations in beta-spectrin disrupt axon outgrowth and sarcomere structure. J Cell Biol. 2000;149:931-42. doi:10.1083/jcb.149.4.931. PMID:10811832
25. Moorthy S, Chen L, Bennett V. Caenorhabditis elegans beta-G spectrin is dispensable for establishment of epithelial polarity, but essential for muscular and neuronal function. J Cell Biol. 2000;149:915-30. doi:10.1083/jcb.149.4.915. PMID:10811831
26. Pielage J, Fetter RD, Davis GW. A postsynaptic spectrin scaffold defines active zone size, spacing, and efficacy at the Drosophila neuromuscular junction. J Cell Biol. 2006;175:491-503. doi:10.1083/jcb.200607036. PMID:17088429
27. Fricke R, Gohl C, Dharmalingam E, Grevelhorster A, Zahedi B, Harden N, Kessels M, Qualmann B, Bogdan S. Drosophila Cip4/Toca-1 integrates membrane trafficking and actin dynamics through WASP and SCAR/WAVE. Curr Biol. 2009;19:1429-37. doi:10.1016/j.cub.2009.07.058. PMID:19716703