Drosophila cyfip regulates synaptic development and endocytosis by suppressing filamentous actin assembly

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Abstract

The formation of synapses and the proper construction of neural circuits depend on signaling pathways that regulate cytoskeletal structure and dynamics. After the mutual recognition of a growing axon and its target, multiple signaling pathways are activated that regulate cytoskeletal dynamics to determine the morphology and strength of the connection. By analyzing *Drosophila* mutations in the cytoplasmic FMRP interacting protein Cyfip, we demonstrate that this component of the WAVE complex inhibits the assembly of filamentous actin (F-actin), and thereby regulates key aspects of synaptogenesis. Cyfip regulates the distribution of F-actin filaments in the presynaptic neuromuscular junction (NMJ) terminals. At the *cyfip* mutant NMJ, F-actin assembly was accelerated, resulting in shorter NMJs, more numerous satellite boutons, and reduced quantal content. Increased synaptic vesicle size and failure to maintain excitatory junctional potential amplitudes under high-frequency stimulation in *cyfip* mutants indicated an endocytic defect. Consistently, *cyfip* mutants exhibited upregulated bone morphogenetic protein (BMP) signaling, a major growth promoting pathway known to be attenuated by endocytosis at the *Drosophila* NMJ. We propose that Cyfip regulates synapse development and endocytosis by inhibiting actin assembly.

Key words: Cyfip, actin, endocytosis, synapse, *Drosophila*

Author Summary

Synapses are specialized junctions at which neurons communicate with target cells. To establish properly wired neuronal circuits, synapses must grow in size and strength with a high degree of accuracy. The actin cytoskeleton plays a crucial role in the formation and function of synapses, but the underlying mechanisms remain poorly understood. The *Drosophila* neuromuscular junction (NMJ) is an excellent model for studying synaptic development and function. By analyzing *Drosophila* mutants of the cytoplasmic FMRP interacting protein Cyfip, we establish that this protein inhibits the assembly of filamentous actin (F-actin). At *cyfip* mutant NMJ synapses, F-actin assembly was accelerated and NMJ terminals were shorter and grew supernumerary buds. Furthermore, neurotransmission was not sustained under high-frequency stimulation. These changes could be caused by defects in synaptic endocytosis, which would compromise the endocytic attenuation of signaling pathways such as the NMJ growth-promoting bone morphogenetic protein (BMP) pathway. Indeed, BMP signaling was upregulated in *cyfip* mutants. We propose that Cyfip regulates synaptic development and function by inhibiting F-actin assembly, which in turn downregulates BMP signaling via endocytosis. This study establishes a novel role for Cyfip-mediated regulation of the actin cytoskeleton at the *Drosophila* NMJ.

Introduction

To establish functional neural circuits, synapses must form at specific locations and grow to an appropriate size and strength. A multitude of signaling pathways are required to achieve and maintain these precise patterns of synaptic connectivity [1-3]. Many of these signals regulate local actin cytoskeletal networks, which are crucial for both synapse formation and plasticity [4-6]. Precisely how the actin cytoskeleton integrates the various signaling pathways to regulate synaptic formation and function remains to be elucidated.

At *Drosophila* neuromuscular junctions (NMJs), dysregulation of actin dynamics results in morphological defects, including the formation of excess satellite boutons. For example, mutants of the actin regulator nervous wreck (Nwk), an N-WASP (neuronal Wiskott–Aldrich syndrome protein) interacting protein, show excess satellite boutons at NMJs [7]. Nervous wreck activates WASP-Arp2/3-mediated actin polymerization and coordinates with Cdc42 to regulate actin assembly [8]. Additional actin regulatory proteins implicated in synapse formation include WASP, spectrin, and adducin [6,9,10]. Moreover, these proteins and their interactors are conserved across species, indicating a seminal role for the actin cytoskeleton in synaptic development.

In addition to regulating synaptic development, multiple lines of evidence show that actin and its regulators function in synaptic endocytosis. First, filamentous actin (F-actin) is observed around synaptic vesicle clusters where it facilitates vesicle endocytosis or mobility [11,12]. Second, many actin regulators bind endocytic proteins directly or indirectly. For example, Cdc42, WASP, and Nwk all interact directly with the endocytic protein intersectin-1/Dap160, an important binding partner of dynamin [7,8,13]. Third, disruption of the actin cytoskeleton impairs vesicle recycling at both vertebrate and invertebrate synapses [12,14]. Fourth, actin regulator mutants such as *twinfilin*, *dap160/intersectin*, and *nwk* show defects in synaptic endocytosis [7,15-17]. In addition to endocytosis of synaptic vesicle membrane, receptors must be retrieved from the presynaptic membrane to downregulate specific signaling pathways. At the *Drosophila* NMJ for example, actin-mediated endocytosis

downregulates the bone morphogenetic protein (BMP) signaling pathway that normally promotes synaptic growth [1,7,8], suggesting that actin cytoskeleton may contribute to synaptic development by regulating endocytosis.

The heteropentameric WAVE complex, composed of WAVE (WASP/verprolin homologous protein), Cyfip/Sra-1/Pir121, Kette/Nap1, Abi, and HSPC300 [18-21], relays signals from the Rac GTPase to the actin nucleator Arp2/3 complex to control de novo F-actin assembly. The organization of the WAVE complex is well established in vitro. Specifically, Abi and Nap1 form the core sub-complex and Cyfip binds Nap1, while both WAVE and HSPC300 bind the N-terminus of Abi [20,21]. In the resting state, the verprolin-homology, cofilin-homology, and acidic (VCA) domain of the WAVE protein is sequestered by binding to Cyfip and/or Nap1 [18,21]. Upon Rac1 binding to the N-terminus of Cyfip, together with other coincident signals, the VCA domain is released from the WAVE complex to activate the actin nucleator Arp2/3 [18,21-23]. However, this transduction mechanism has only been demonstrated in vitro, while the exact role of each component in regulating the activity of the WAVE complex in vivo is poorly understood.

We provide evidence that loss of Cyfip leads to enhanced F-actin assembly in *Drosophila*, resulting in altered NMJ morphology. We also report that Cyfip loss disrupts synaptic endocytosis, likely by regulating presynaptic F-actin networks. Consistently, the bone morphogenetic protein (BMP) signaling attenuated by endocytosis is upregulated in *cyfip* mutants. Reducing the level of SCAR, the *Drosophila* homolog of WAVE, partially rescues the morphological anomalies, endocytic defects, and enhanced actin dynamics at *cyfip* mutant NMJs. Thus, our findings demonstrate that Cyfip regulates synapse formation and endocytosis by inhibiting actin dynamics.