PERSPECTIVE

Role of the STRIPAK complex and the Hippo pathway in synaptic terminal formation

To transmit neural information from pre to postsynaptic neurons, the number, morphology, and function of the synapse have to be strictly regulated. Failures in synaptic formations are linked to neural disorders. For example, it is suggested that localization of N-methyl-D-aspartate (NMDA) receptors plays important roles in NMDA receptor-induced excitotoxicity, which is in turn thought to contribute to cell death associated with certain neurodegenerative diseases (Parsons and Raymond, 2014). Furthermore, synaptic overgrowth is observed in Drosophila dfmr1 (homolog of mammalian FMR1) gene mutant, which causes the Fragile X syndrome (Zhang et al., 2001). In this perspective, we introduce the involvement of the striatin-interacting phosphatase and kinase (STRIPAK) complex in synapse formation.

Recent proteomic studies have identified the evolutionarily conserved STRIPAK complex that regulates various cellular processes including cell-cycle control and cell polarity (Figure 1A; Hwang and Pallas, 2014). The main component of STRIPAK complex is striatin, which belongs to the subfamily of regulatory B subunits of the protein phosphatase 2A (PP2A) complex. The A and C subunits of PP2A complex, CCM3, Mob3, Mst3, Mst4, Ysk1, Ccm3, Strip1, and Strip2 form the core mammalian STRIPAK complex together with striatin. This multi-component core complex is capable of assembling in a mutually exclusive manner with other accessory proteins depending on the function that it mediates (Hwang and Pallas, 2014). Although the role of STRIPAK complexes in cellular processes of multiple organisms has been extensively studied in recent years, little is known about the role of STRIPAK complex in synaptic formation. Thus, while it was reported that the Drosophila mutant of Mob4 (homolog of mammalian Mob3) shows abnormal synaptic terminal development (Hwang and Pallas, 2014), no information is available regarding the underlying mechanism.

We have previously identified that Drosophila Strip (homolog of mammalian Strip1 and 2) is expressed at the synaptic sites and regulates axon elongation and dendrite branching in olfactory projection neurons (Sakuma et al., 2014). Since Drosophila larval neuromuscular junction (NMJ) is the established model to study synapse formation, we examined the localization of Strip at larval NMJ and found that Strip showed a punctate distribution at the presynaptic sites (Sakuma et al., 2016). Drosophila NMJs are composed of chains of oval structures called boutons that contain multiple active zones (which serve as neurotransmitter release sites) (Figure 1B) (Menon et al., 2013). When Strip was knocked down specifically in the presynaptic motor neurons, the number of satellite boutons (small boutons that emanate from the normal boutons and are thought to arise from defects in synaptic growth), was increased (Figure 1C). Hence, we concluded that presynaptic Strip regulates bouton formation. Furthermore, we found that Strip not only regulates synapse formation but also affects synaptic function. This was indicated in our observation that the frequency of miniature excitatory junction potential was increased when Strip was knocked down in the presynaptic motor neurons.

To clarify the role of Strip in synapse formation, we focused on the Hippo pathway, which is known to be a major regulator of cell proliferation and cell death (Staley and Irvine, 2012). The Hippo pathway was investigated because a previous report suggested that the Drosophila STRIPAK complex serves as a negative regulator of Hippo (Ribeiro et al., 2010). Using Dro-
sophila S2 cells, we confirmed that Strip could form a complex with Hippo and inactivate it. We also found that overexpression of Hippo in the motor neurons led to the same phenotype in cells as seen with the knockdown of Strip. To control cell proliferation, Hippo forms the core Hippo kinase cassette with its downstream kinase, Warts (Staley and Irvine, 2012). We also found that strip genetically interacts with Hippo and Warts to regulate synapse formation.

The major downstream target of the core Hippo kinase cassette is the transcriptional co-activator, Yorkie, which regulates the transcriptional activity of several genes controlling cell proliferation and apoptosis (Staley and Irvine, 2012). Interestingly, the localization of Yorkie was not affected when Hippo was manipulated in motor neurons, suggesting that Yorkie is not the downstream target of the Strip/Hippo pathway in motor neurons. In an attempt to search for other potential targets of the core Hippo kinase cassette, we focused on a report that identified Enabled, an actin assembly/elongation factor working downstream of the core Hippo kinase cassette in Drosophila border cell migration. Enabled has a conserved Warts consensus phosphorylation site (Luo et al., 2014). The satellite bouton phenotype of Strip knockdown was suppressed by expressing a phospho-mutant form of Enabled that cannot be phosphorylated by Warts, suggesting the involvement of Enabled in satellite bouton formation. Furthermore, F-actin organization was altered when Strip was knocked down in motor neurons, revealing that the Strip/Hippo pathway may be involved in regulating synapse formation by modulating actin organization.

Based on our findings, we hypothesize that the localization of Strip could be used as a marker for new bouton outgrowth (Figure 1D). In presence of Strip, the core Hippo kinase cassette is inactivated, which locally increases the expression of the active (un-phosphorylated) form of Enabled, which in turn prevents satellite bouton formation. On the other hand, in absence of Strip, the core Hippo kinase cassette is activated, which results in satellite bouton formation.

Neurons are usually regarded as post mitotic cells that do not proliferate further. It is surprising that Hippo, a well-known regulator of cell proliferation, is involved in synapse formation. The Hippo pathway may be important for sensing the local environment around NMJ, since the size of muscles and axon termini of motor neurons dynamically change during synaptic terminal development. Many studies have shown that mutants for molecules implicated in endocytosis (Dynamin, Dap-160, Synaptotagmin, etc.) exhibit satellite boutons (Menon et al., 2013). We previously identified that Strip regulates the endocytic pathway and microtubule stability in the axon elongation and dendrite branching of Drosophila olfactory projection neurons (Sakuma et al., 2014). It is therefore possible that Strip and Hippo are involved in the endocytic pathway and microtubule stability in the synapse formation as well. Furthermore, several genes whose mutants exhibit the satellite bouton phenotype also regulate microtubule stability and contribute to neurodegenerative diseases (Carrillo et al., 2013). Thus, further investigations of the function of Strip and Hippo in synapse formation might provide new insights into the development of strategies for the prevention and treatment of neurodegenerative diseases. Furthermore, mechanistic insights we found here might be applicable to mammals, since both vertebrate central nervous system and Drosophila NMJ are glutamatergic, and STRIPAK complex and Hippo pathway molecules are conserved among species.

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