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RESEARCH ARTICLE

Microtubule-severing protein Katanin regulates neuromuscular junction development and dendritic elaboration in *Drosophila*

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ABSTRACT

Microtubules (MTs) are crucial for diverse biological processes including cell division, cell growth and motility, intracellular transport and the maintenance of cell shape. MT abnormalities are associated with neurodevelopmental and neurodegenerative diseases such as hereditary spastic paraplegia. Among many MT regulators, katanin was the first identified MT-severing protein, but its neuronal functions have not yet been examined in a multicellular organism. Katanin consists of two subunits; the catalytic subunit katanin 60 contains an AAA (ATPases associated with a variety of cellular activities) domain and breaks MT fibers while hydrolyzing ATP, whereas katanin 80 is a targeting and regulatory subunit. To dissect the *in vivo* functions of Katanin, we generated mutations in *Drosophila Katanin 60* and manipulated its expression in a tissue-specific manner. Null mutants of *Katanin 60* are pupal lethal, demonstrating that it is essential for viability. Loss-of-function mutants of *Katanin 60* showed excess satellite boutons, reduced neurotransmission efficacy, and more enlarged cisternae at neuromuscular junctions. In peripheral sensory neurons, loss of *Katanin 60* led to increased elaboration of dendrites, whereas overexpression of *Katanin 60* resulted in the opposite. Genetic interaction analyses indicated that increased levels of MT acetylation increase its susceptibility to Katanin-mediated severing in neuronal and non-neuronal systems. Taken together, our results demonstrate for the first time that Katanin 60 is required for the normal development of neuromuscular synapses and dendrites.

KEY WORDS: *Drosophila melanogaster*, Katanin, Spastin, HDAC6, Neuromuscular junction

INTRODUCTION

Microtubules (MTs) play a crucial role in a diverse array of fundamental cellular processes including cell division, cell growth and the maintenance of cell shape. MT dynamics are tightly regulated by a host of proteins that stabilize or destabilize MTs. Three MT-severing proteins, katanin, spastin and fidgetin, are members of the diverse ATPases associated with a variety of cellular activities (AAA) protein superfamily (Roll-Mecak and McNally, 2010; Sharp and Ross, 2012). Katanin, the founding member of the MT-severing protein family, was initially identified from *Xenopus* egg extracts exhibiting MT-severing activity (Vale, 1991). It consists of two subunits, katanin 60 and katanin 80 (McNally and Vale, 1993). Katanin 60 is the catalytic subunit that breaks MT fibers while hydrolyzing ATP, whereas katanin 80 is a targeting and regulatory subunit (Hartman and Vale, 1999).

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The *in vivo* functions of katanin 60 have been characterized in a range of organisms. Genetic analyses of katanin 60 in *Tetrahymena* showed that it plays a crucial role in the formation of cilia and is essential for locomotion (Sharma et al., 2007). *Caenorhabditis elegans* mutant for the *katanin 60* homolog *mei-1* show meiotic spindle abnormalities (Srayko et al., 2006). In *Drosophila* S2 cells, overexpression of Katanin 60 results in MT severing and depolymerization (Díaz-Valencia et al., 2011; Zhang et al., 2007; Zhang et al., 2011). The susceptibility of MTs to katanin-mediated severing is controlled by many factors; for example, acetylation of MTs sensitizes their severing by katanin (Sudo and Baas, 2010) whereas tau binding to MTs protects them from katanin severing (Qiang et al., 2006; Qiang et al., 2010; Sudo and Baas, 2010; Yu et al., 2008).

Katanin is distributed in all neuronal compartments including axons, dendrites and cell bodies, and is particularly abundant in axons, whereas spastin is mostly concentrated at axonal branching points (Ahmad et al., 1999; Karabay et al., 2004; Sharp and Ross, 2012; Yu et al., 2008). In the rodent brain, katanin levels are high during rapid phases of axonal growth but diminish as axons reach their targets (Karabay et al., 2004). In cultured rat sympathetic neurons, expression of a dominant-negative form of katanin 60 inhibits MT severing and axonal growth, whereas overexpression of wild-type katanin 60 results in excess MT severing, but can also be deleterious to axonal growth in a subset of neurons (Karabay et al., 2004). Thus, katanin is a crucial regulator of axonal growth.

Dendrites play an essential role in information processing in the nervous system as they are involved in synapse formation and signal integration (Jan and Jan, 2010). MTs are crucial for dendrite elaboration. However, how MT regulators including MT-severing proteins affect dendritic development is poorly understood.

Previous studies have uncovered an important role for spastin in neuromuscular junction (NMJ) growth and dendritic elaboration (Jinushi-Nakao et al., 2007; Ozdowski et al., 2011; Trotta et al., 2004; Sherwood et al., 2004; Yao et al., 2011; Ye et al., 2011). However, how katanin affects neuronal development has not been characterized at an organism level. To dissect the neuronal function of katanin we generated mutants of *Drosophila melanogaster Katanin 60* and transgenic lines that could be used to overexpress Katanin 60 in a tissue-specific manner. We then examined the effects of altered Katanin 60 expressions on NMJ synapses, neuronal morphogenesis and the MT cytoskeleton. We report for the first time that Katanin 60 is required for the normal development of NMJs and dendrites in *Drosophila*.

RESULTS

The *Drosophila* genome encodes an ortholog of human katanin 60

Sequence comparisons revealed that the *Drosophila* genome contains a gene that encodes an ortholog of human katanin 60. *Drosophila* Katanin 60 protein is overall 50% identical and 63%