

brain tumor regulates neuromuscular synapse growth and endocytosis in Drosophila by suppressing Mad expression

Short title: Brat regulates synaptic growth by suppressing Mad

Wenwen Shi^{1#}, Yan Chen^{1#}, Guangming Gan², Dan Wang¹, Jinqi Ren¹, Qifu Wang¹, Zhiheng Xu¹, Wei Xie², Yong Q. Zhang^{1*}

1, Key Laboratory of Molecular and Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China.

2, The Key Laboratory for Developmental Genes and Human Disease, Ministry of Education, Institute of Life Sciences, Southeast University, Nanjing 210096, China

equal contributors

*Correspondence: Yong Q. Zhang, PhD

Institute of Genetics and Developmental Biology

Chinese Academy of Sciences

Datun Road, Chao Yang District

Beijing 100101, China

Tel: 86 10 6480 7611; fax: 86 10 6480 7611; Email: yqzhang@genetics.ac.cn

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Abstract

The precise regulation of synaptic growth is critical for the proper formation and plasticity of functional neural circuits. Identification and characterization of factors that regulate synaptic growth and function have been under intensive investigation. Here we report that *brain tumor (brat)*, which was identified as a translational repressor in multiple biological processes, plays a crucial role at *Drosophila* neuromuscular junction (NMJ) synapses. Immunohistochemical analysis demonstrated that *brat* mutants exhibited synaptic overgrowth characterized by excess satellite boutons at NMJ terminals, while electron microscopy revealed increased synaptic vesicle size but reduced density at active zones compared to wild types. Spontaneous miniature excitatory junctional potential amplitudes were larger and evoked quantal content was lower at *brat* mutant NMJs. In agreement with the morphological and physiological phenotypes, loss of Brat resulted in reduced FM1-43 uptake and decreased immunostaining of the endocytic proteins AP180 and dynamin at the NMJ terminals, indicating that *brat* regulates synaptic endocytosis. Genetic analysis revealed that the actions of Brat at synapses are mediated through Mothers against decapentaplegic (Mad), the signal transduction effector of the bone morphogenetic protein (BMP) signaling pathway. Furthermore, biochemical analyses showed upregulated levels of Mad protein but normal mRNA levels in the larval brains of *brat* mutants, suggesting that Brat suppresses Mad translation. Consistently, knockdown of *brat* by RNA interference in *Drosophila* S2 cells also increased Mad protein level. These results together reveal an important and previously unidentified role for Brat in synaptic development and endocytosis mediated by suppression of BMP signaling.

Introduction

The synapse is a specialized intercellular junction devoted to communication between neurons and their targets. Proper growth and regulation of synapses are critical to the normal neuronal function. *Drosophila* neuromuscular junction (NMJ) is

an effective model system to dissect molecular mechanisms of synaptic development. Multifarious factors and molecular signaling pathways such as actin regulators, endocytic proteins, ubiquitin-mediated protein degradation, bone morphogenetic protein (BMP), and Wingless (Wnt) pathways play important roles at *Drosophila* NMJ synapses (Collins and DiAntonio, 2007; O'Connor-Giles, et al., 2008; Giagtzoglou et al., 2009; Ball et al., 2010; Bayat et al., 2011).

BMP signaling is a major retrograde growth-promoting pathway at *Drosophila* NMJ synapses (Collins and DiAntonio, 2007; O'Connor-Giles, et al., 2008; Ball et al., 2010; Bayat et al., 2011). The retrograde BMP signaling cascade is initiated by release of the ligand Glass bottom boat (Gbb) from the postsynaptic muscle and subsequent binding to the presynaptic type II BMP receptor Wishful Thinking (Wit). Upon ligand binding, Wit forms a complex with the type I receptors Thickveins (Tkv) and Saxophone (Sax), resulting in their phosphorylation. In turn, phosphorylated type I receptors phosphorylate the Smad family transcriptional factor Mothers against decapentaplegic (Mad). Mad is a signal transduction effector that, when phosphorylated, translocates to the nucleus of motoneurons to regulate transcription of target genes that control NMJ growth (Collins and DiAntonio, 2007; Ball et al., 2010; Bayat et al., 2011).

Brain tumor (Brat) contains multiple protein-protein interaction domains and is conserved throughout evolution from *Caenorhabditis elegans* to humans (Arama et al., 2000). Brat acts as a translational repressor in multiple developmental contexts through distinct mechanisms. During early embryogenesis, Brat forms a complex with the RNA-binding proteins Pumilio (Pum) and Nanos (Nos) and the RNA 5' cap-binding protein d4EHP (the *Drosophila* homolog of eIF4E) to suppress the translation of the morphogen Hunchback in the posterior (Sonoda and Wharton, 2001; Edwards et al., 2003; Cho et al., 2006). In the female germline, Brat acts together with Pum to repress the expression of Mad and the growth regulator dMyc to promote germline differentiation (Harris et al., 2011). During larval neurogenesis, Brat controls neuroblast self-renewal and neuronal differentiation (Bello et al., 2006; Betschinger et al., 2006; Lee et al., 2006). In the postmitotic neurons, Brat interacts

with Pum and Nos to translationally repress the voltage-gated sodium channel subunit *paralytic (para)* and thereby modulate the excitability of motor neurons (Muraro et al., 2008). Pum and Nos regulate NMJ synapse development (Menon et al., 2004, 2009), but a possible role for Brat at synapses has not been demonstrated.

We report here that the NMJ terminals of *brat* mutants exhibit more numerous satellite boutons than do wild types and that these mutant NMJs have reduced neurotransmission efficiency and defective endocytosis. Furthermore, our data indicate that Brat regulates synapse development and endocytosis by suppressing translation of the BMP signaling component Mad. Thus, our study unravels a novel role for *brat* at the NMJ and offers new insight into the regulation of BMP signaling for NMJ growth.