Glutathionylation promotes Gbb degradation by proteasome-mediated pathway

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Synapse, the specialized contacting site between neurons and their target cells, is crucial for brain function. One main interest in the laboratory is to understand how synapse development is regulated. To identify a novel gene that regulates Neuromuscular Junction (NMJ) growth, we have carried out an RNAi knockdown screen for NMJ overgrowth phenotype. We categorized the candidate genes into calcium signaling, transcription factors, redox and miscellaneous subgroup.

From the unbiased genetic screen, we found postsynaptic knockdown of glutathione S- transferase omega 1 (GstO1) showed significantly more synaptic boutons at the NMJs. To understand the function of GstO1 in synapse development, we generated multiple mutations of GstO1, the *Drosophila* ortholog of GSTO1 by CRIPR/Cas9 technology.

Phenotypic analysis of tissue-specific alterations of GstO1 showed that GstO1 restrains NMJ growth post-synaptically. Additionally, GstO1 mutants showed impaired larval locomotion including larval roll-over and crawling. Several signaling pathways that regulate synapse development. To determine if GstO1 loss-of-function does indeed lead to NMJ overgrowth through a BMP signaling pathway, we quantified the level of phosphorylated MAD (pMad), an indicator of BMP signaling strength, in NMJ synapses and found pMad staining intensity was upregulated in GstO1 mutants. To examine possible involvement of BMP signaling in mediating the overgrowth of synaptic boutons in GstO1 mutants, we examined genetic interactions between GstO1 and the BMP pathway components. Removal of one copy of gbb (heterozygous gbb4 mutation) did not effect on NMJ growth but suppressed the excess bouton and satellite bouton phenotype of GstO1 mutants. As GstO1 acts in the postsynaptic muscles and Gbb is secreted from muscles to retrogradely activate BMP signaling. Intracellular Gbb intensity around the synaptic terminals and the number of Gbb positive puncta were increased in GstO1 mutants. Along with increased intracellular Gbb, extracellular Gbb at NMJ synapses was also upregulated in GstO1 mutants.

To verify the effect of GstO1 on secreted Gbb, we knocked down GstO1 in *Drosophila* S2 cells and found an increased level of unprocessed cytoplasmic full-length Gbb in the cell lysates and the processed mature Gbb level in the culture medium.

Glutathione (GSH) is a universal antioxidant in aerobic cells. The GSH/GSSG ratio is a redox state indicator. We found that in GstO1 mutants, the ratio of GSH/GSSG was increased, indicating disrupted redox homeostasis. Grx1 acts as the main deglutathionylating enzyme and plays a critical role in redox homeostasis as well as redox signaling. Overexpression of glutaredoxin 1 (Grx1) produced similar phenotype as GstO1 mutants. As Gbb

level was upregulated in GstO1 mutant and GstO1 genetically interact with Gbb, we speculated that GstO1 might interact physically with Gbb. By co-immunoprecipitation experiment, we found that GstO1 physically interacted with Gbb and was perfectly co-localized with Gbb. To find out the localization of GstO1, we found that GstO1 localized in ER, early endosomes and recycling endosomes but not in late endosomes.

GstO1 specifically glutathionylates Gbb using anti-GSH antibody under non-reducing conditions. Mass spectrometry revealed two potential glutathionylated residues, cysteines 420 and 354, in the C-terminal of Gbb. We set out to dissect the mechanisms mediating negative regulation of Gbb by GstO1 through examining the stability of Gbb protein in S2 cells at various time points after treatment with the protein synthesis inhibitor cycloheximide (ChX). GstO1 knockdown increases Gbb stability. We observed a relatively stable level of Gbb after co-treated with a proteasome inhibitor (MG132) and cycloheximide (ChX). Further biochemical results demonstrate that GstO1 promotes ubiquitin-mediated degradation of Gbb. Knockdown of ctrip mimics the increased satellite bouton phenotype of GstO1 mutants. The intensity of extracellular Gbb at NMJ synapses was increased in knowndown of ctrip. These results indicate that Ctrip may be a major E3 ligase to degrade Gbb in postsynaptic muscles.

Biography

Md. Shafayat Hossain is a talented and resourceful academician and researcher, offering 10 years' research and teaching experience bearing field of Neurobiology, Molecular Biology, Pharmacology, Physiology, Protein Biochemistry, Biotechnology, and Genetics. Successfully awarded a doctorate from world-leading research organization, Chinese Academy of Sciences (CAS) with most prestigious CAS- TWAS (Italy) President fellowship of China. Excellent collaborative capabilities with top-ranking research laboratories/organizations (University of Cambridge and University of York) for solving unanswered biological questions. Dedicated biological researchers for more than 7 years of research experiences in the state key laboratory of molecular developmental biology. Self-motivated and goal-oriented individual with multiple oversee projects handing capabilities. Having proven leadership, excellent communication skills, and strong student management skills to achieve high productivity and improve the institute.

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