Tbc1d15-17 Regulates Synaptic Development at the Drosophila Neuromuscular Junction

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Members of the Tre-2/Bub2/Cdc16 (TBC) family of proteins are believed to function as GTPase-activating proteins (GAPs) for Rab GTPases, which play pivotal roles in intracellular membrane trafficking. Although membrane trafficking is fundamental to neuronal morphogenesis and function, the roles of TBC-family Rab GAPs have been poorly characterized in the nervous system. In this paper, we provide genetic evidence that Tbc1d15-17, the Drosophila homolog of mammalian Rab7-GAP TBC1d15, is required for normal presynaptic growth and postsynaptic organization at the neuromuscular junction (NMJ). A loss-of-function mutation in tbc1d15-17 or its presynaptic knockdown leads to an increase in synaptic bouton number and NMJ length. tbc1d15-17 mutants are also defective in the distribution of the postsynaptic scaffold Discs-large (Dlg) and in the level of the postsynaptic glutamate subunit GluRIIA. These postsynaptic phenotypes are recapitulated by postsynaptic knockdown of Tbc1d15-17. We also show that presynaptic overexpression of a constitutively active Rab7 mutant in a wild-type background causes a synaptic overgrowth phenotype resembling that of tbc1d15-17 mutants, while a dominant-negative form of Rab7 has the opposite effect. Together, our findings establish a novel role for Tbc1d15-17 and its potential substrate Rab7 in regulating synaptic development.

INTRODUCTION

Rab GTPases constitute the largest subgroup of the Ras GTPase superfamily and play key roles in controlling membrane trafficking (Stenmark, 2009). Like other Ras-superfamily members, they serve as molecular switches that cycle between an active, GTP-bound state and an inactive, GDP-bound state. Activated, GTP-bound Rabs perform their biological functions by recruiting and activating specific effector molecules that are involved in vesicle budding, motility, tethering, and fusion (Stenmark, 2009). Interconversion between these alternate states is primarily regulated by guanine nucleotide exchange factors (GEFs) and by GTPase-activating proteins (GAPs). GEFs facilitate the release of GDP from Rab GTPases, thereby promoting the exchange of GDP for GTP, while GAPs enhance the intrinsic GTPase activity of Rab proteins, converting GTP-bound Rabs to the GDP-bound state (Barr and Lambright, 2010). Except Rab3GAP, all proteins that have been proven or are expected to be Rab GAPs have a Tre-2, Bub2, and Cdc16 (TBC) domain, which has a catalytic 'arginine finger' analogous to those of Ras and Rho family GAPs (Albert et al., 1999; Barr and Lambright, 2010).

It has recently emerged that Rab GTPases and their regulators play essential roles in the regulation of neuronal development. Protrudin mediates nerve growth factor (NGF)-induced neurite formation via its antagonistic action on Rab11 (Shirane and Nakayama, 2006). The mammalian homolog of Drosophila Lethal giant larva (Lgl1) is shown to control axonal outgrowth by activating Rab10 (Wang et al., 2011a). In addition, Drosophila Rab11 is required for normal synaptic morphogenesis at the neuromuscular junction (NMJ) (Khodosh et al., 2006). Finally, mammalian Rab17 is shown to play an essential role in the regulation of dendritic morphogenesis and postsynaptic development (Mori et al., 2012). Although these studies suggest the importance of Rab-dependent membrane trafficking in multiple aspects of neuronal morphogenesis, little is known about the roles of Rab-specific GEFs and GAPs in the developing nervous system.

Recent studies in mammals suggest that Tbc1d15 has a GAP activity selective for Rab7 (Peralta et al., 2010), which is a key regulator of endosomal maturation and endo-lysosomal trafficking (Wang et al., 2011b). Here, we describe a synaptic role of Tbc1d15-17, the single Drosophila ortholog of mammalian TBC domain-containing proteins Tbc1d15 and Tbc1d17. Our genetic data demonstrate that Tbc1d15-17 is required presynaptically and postsynaptically for normal presynaptic growth and postsynaptic organization, respectively, at the neuromuscular junction (NMJ). Consistent with expectation for Rab7 GAP, Tbc1d15-17 is targeted to Rab7-positive intracellular structures in cultured cells. Moreover, neuronal expression of a constitutively-active form of Rab7 produces an NMJ overgrowth phenotype resembling that of tbc1d15-17 mutants. Together, our data establish a novel role for Tbc1d15-17 and Rab7 in regulating synapse development.
MATERIALS AND METHODS

Drosophila stocks

Flies were maintained on standard fly food at 25°C. w1118 was used as the wild-type strain in this study. A transposable P-element insertion line of tbc1d15-17 (tbc1d15-17d05023) and transgenic lines carrying UAS-tbc1d15-17, UAS-YFP-rab7Q67L, or UAS-YFP-rab7T22N were obtained from the Bloomington Stock Center (USA). UAS transgenes were driven by C155-GAL4 (Lin and Goodman, 1994), BG57-GAL4 (Budnik et al., 1996), and da-GAL4 (Wodarz et al., 1995).

Molecular biology

A full-length cDNA clone encoding Tbc1d15-17 was obtained from the Drosophila Genomics Resource Center (Clone ID: LD27216; USA). For expression in S2 cells, the entire tbc1d15-17 open reading frame was PCR amplified and subcloned into pUAST-HA, a derivative of the GAL4-based expression vector pUAST (Brand and Perrimon, 1993), to produce pUAST-HA-tbc1d15-17.

Cell transfection

Drosophila Schneider S2R+ cells were cultured in Schneider’s medium (Invitrogen, USA) containing 10% heat-inactivated fetal bovine serum (FBS) at 25°C and were transiently transfected with pUAST-HA-tbc1d15-17 and an actin5C promoter-GAL4 construct along with pAc-rab5-GFP or pAc-rab7-GFP, in six-well plates using Cellfectin (Invitrogen, USA), according to the manufacturer’s instructions.

Reverse transcription (RT)-PCR

Levels of tbc1d15-17, CG11617, and rp49 mRNAs in tbc1d15-17d05023 or larvae expressing UAS-tbc1d15-17d05023 driven by da-GAL4 were determined by RT-PCR. Briefly, total RNA was extracted from third-instar larvae using TRizol reagent (Invitrogen, USA) and reverse-transcribed into cDNA with an oligo-dT primer and the SuperScript II RT kit (Invitrogen, USA), as previously described (Nahm et al., 2010). The resulting cDNA was amplified by PCR using the following primers: tbc1d15-17, 5′-TGTTCCTGCAAGGAGTCTCG-3′ and 5′-AAATGCGCAAGCAGATCGGACA-3′; CG11617, 5′-TATTCGCGAGATAGTTCTCCACTCAAAGGAC-3′; and rp49, 5′-AACAGGATGATGATGTGTTTG-3′.

Immunohistochemistry and morphological quantification of NMJs

Wandering third-instar larvae were dissected in Ca2+-free HL3 saline (70 mM NaCl, 5 mM KCl, 20 mM MgCl2, 10 mM NaHCO3, 5 mM trehalose, 115 mM sucrose, 5 mM HEPES pH 7.2) (Stewart et al., 1994). Dissected larval fillets were fixed in either 4% formaldehyde in PBS for 20 min or Bouin’s fixative for 10 min at room temperature, washed with PBS containing 0.1% Triton X-100 (PBT) for 10 min three times, and incubated with primary antibodies for 2 h at room temperature or overnight at 4°C. The samples were then incubated with fluorescence-conjugated secondary antibodies for 1 h at room temperature. The following antibodies were used in this study: anti-Dlg (4F3, DSHB; 1:500), anti-GluRIIA (8B4D2, DSHB; 1:10), anti-Csp (1G12, DSHB; 1:100), anti-Bruchpilot (NC82, DSHB; 1:10) and anti-CaMKII (1B4, DSHB; 1:200), anti-Ttn (1D8, DSHB; 1:100), anti-Parkin (1B6, DSHB; 1:500), anti-MAP2 (1B10, DSHB; 1:500).