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Trio gene is required for mouse learning ability

Wen Zong[a], Shuoyang Liu[a], Xiaotong Wang[b], Jian Zhang[a], Tingting Zhang[a], Ziyi Liu[a], Dongdong Wang[b], Aizhen Zhang[a], Minsheng Zhu[c,*], Jiangang Gao[a,**]

[a]Key Laboratory of the Ministry of Education for Experimental Teratology and School of Life Science, Shandong University, Jinan 250100, China
[b]Department of Neurobiology, Shandong Provincial Key Laboratory of Mental Disorders, School of Medicine, Shandong University, Jinan, Shandong 250012, China
[c]Model Animal Research Center, Key Laboratory of Model Animal for Disease Study of Ministry of Education, Nanjing University, Nanjing210061, China

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ABSTRACT

Trio is a guanine nucleotide exchange factor with multiple guanine nucleotide exchange factor domains. Trio regulates cytoskeleton dynamics and actin remodeling and is involved in cell migration and axonal guidance in neuronal development. The null allele of the Trio gene led to embryonic lethality, and Trio null embryos displayed aberrant organization in several regions of the brain at E18.5, including hippocampus. Nestin-Trio−/− mice, in which the Trio gene was deleted specifically in the neuronal system by the Nestin-Cre system, displayed severe phenotypes, including low survival rate, ataxia and multiple developmental defects of the cerebellum. All Nestin-Trio−/− mice died before reaching adulthood, which hinders research on Trio gene function in adult mice. Thus, we generated EMX1-Trio−/− mice by crossing Trio-floxed mice with EMX1-Cre mice in which Cre is expressed in the brain cortex and hippocampus. EMX1-Trio−/− mice can survive to adulthood. Trio gene deletion results in smaller brains, an abnormal hippocampus and disordered granule cells in the dentate gyrus (DG) and cornu ammonis (CA). Behavior tests showed that Trio deletion interfered with the hippocampal-dependent spatial learning in the mice, suggesting that Trio plays critical roles in the learning ability of adult mice. We conclude that the Trio gene regulates the neuronal development of the hippocampus and that it affects the intelligence of adult mice.

*Corresponding author.
**Corresponding author.
E-mail addresses: zhums@nju.edu.cn (M. Zhu), jggao@sdu.edu.cn (J. Gao).

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1. Introduction

GTPases act as molecular switches in many cellular processes, and these proteins function in cell growth, differentiation, vesicle-mediated transport, and cytoskeletal organization (Bourne et al., 1991). Rho-family small GTPases, which are activated by guanine nucleotide exchange factors (GEFs), are key regulators of cytoskeleton dynamics (Etienne-Manneville and Hall, 2002). In mammals, the GEF family consists of several subfamilies with different amino acid sequences (Liu et al., 1998). Dbl-homology guanine nucleotide exchange factors (DH-GEFs) can activate Rho GTPases, and this activation regulates cell activities. The Trio gene belongs to a subfamily of DH-GEFs, and it was originally identified in humans in 1996 (Debant et al., 1996; Alam et al., 1997). Since then, Trio orthologs have been identified in the invertebrates *C. elegans* and *Drosophila*, which are called UNC-73 and DTrio, respectively (Steven et al., 1998; Lin and Greenberg, 2000). In mammals, there is a Trio orthlog called kalirin that was originally identified in rats. The structural organization of Trio is evolutionarily very well conserved (O'Brien et al., 2000; Peng et al., 2010).

Trio is a complex protein with multiple domains. Trio consists of 3 domains, including 2 GEF domains and a protein serine/threonine kinase (PSK) domain (Seipel et al., 1999). Each GEF domain has an adjacent pleckstrin homology (PH) domain and a SRC homology 3 (SH3) domain, and the PSK domain has an adjacent immunoglobulin (Ig)-like domain and multiple spectrin-like domains (Seipel et al., 1999). In two GEF domains, the Trio amino-terminal GEF domain (GEF-D1) can catalyze nucleotide exchange for Rac1, activate Jun kinase and produce membrane ruffles (Medley et al., 2000). In addition, Trio GEF-D1 has also been shown to activate RhoG (Blangy et al., 2006). The Trio carboxyl-terminal GEF domain (GEF-D2) acts as an exchange factor for RhoA and induces the synthesis of stress fibers (Seipel et al., 1999). Trio GEF-D2 can promote RhoA binding to the Ig-like domain of Trio, and this process activates RhoA (Medley et al., 2000). In cell cytoskeleton regulation, Trio GEF-D1 activates Rac1 and Rho G, while Trio GEF-D2 modulates Rho A (Bellanger et al., 2000).

GTPase family proteins play important roles in synaptic communication and plasticity in the brain (Ishikawa et al., 2002). As important members of GTPase family, Rac1, Rho G and Rho A can modulate the neuronal cell cytoskeleton. RhoA regulates focal adhesion assembly and actin stress fiber formation; Rac1 induces membrane ruffling and lamellipodia at the plasma membrane; RhoG is involved in nerve growth factor-induced neurite outgrowth and participates in the regulation of neuritogenesis (Ishikawa et al., 2002). In the developing brain, RhoA and Rac1 have been suggested to regulate dendritic growth and remodeling (O'Kane et al., 2003). The Trio protein can affect neuronal cell migration and axon guidance by modulating RhoG, Rac1 and RhoA through its GEF domains (Debant et al., 1996).

Trio can also function in conjunction with other proteins (Astigarraga et al., 2010).Trio interacts with leukocyte common antigen-related protein (LAR), and the Trio-LAR complex coordinates the cell-matrix interactions and cytoskeletal rearrangements (Astigarraga et al., 2010). LAR is a broadly expressed transmembrane protein tyrosine phosphatase (PTPase) with a cell adhesion-like extracellular region that regulates cell-matrix interactions (Serra-Pages et al., 1995). Trio binds to the cytoplasmic region of the LAR, which localizes to the ends of focal adhesions (Ridley and Hall, 1994; Debant et al., 1996). Focal adhesions are large, dynamic protein complexes through which the cytoskeleton of the cell connects to the extracellular matrix (ECM). Focal adhesion dynamics affect the migration of cells by orchestrating cell-matrix and cytoskeletal rearrangements. Through involvement in the regulation of focal adhesion dynamics, the Trio-LAR complex protein can regulate the migration of polarized cells (Bateman and Van Vactor, 2001; Wozniak et al., 2004).

Research on different animals shows the irreplaceable role of the Trio protein in the neuronal development process. UNC-73 and Dtrio, the Trio protein orthologs in *C. elegans* and *Drosophila*, respectively, were reported to be important regulators of axon guidance during nervous system development (Steven et al., 2005). In mammals, Trio is expressed ubiquitously in various tissues, including the central nervous system (Estrach et al., 2002). To determine the role of Trio during mammalian development, a null allele of Trio was generated in mice, and loss of Trio caused embryonic lethality. Sixty percent of Trio null embryos died between E15.5 and E18.5, and the remainder died perinatally. Trio null E18.5 embryos displayed aberrant organization in several regions within the brain, including the hippocampus and olfactory bulb (O'Brien et al., 2000). To address the role of Trio in postnatal brain development, Nestin-Trio−/− mice were generated by crossing Trio-floxed mice with Nestin-Cre mice, in which Cre is mainly expressed in the central and peripheral nervous systems (Dubois et al., 2006). Approximately 90% of Nestin-Trio−/− mice died within 1 day after birth, and the remainder survived for a further 5–22 days. These surviving Nestin-Trio−/− mice showed reduced body weight and smaller brain size. Nestin-Trio−/− mice displayed severe ataxia and defects in the cerebrum and cerebella (Peng et al., 2010). In the cerebellum of Nestin-Trio−/− mice, cerebellar granule neurons were disordered, showing an abnormal pattern of migration. In an in vitro assay, Trio-deficient migrating granule cells showed reduced spreading distance and moved in random directions (Peng et al., 2010). Research using knockout mice models suggests that the Trio gene regulates neural organization and is essential for neural development. However, all Nestin-Trio−/− mice died in infancy, and no adult mice survived (Peng et al., 2010).This phenomenon limited the behavioral studies and Trio functional research in adult mice.

In this study, EMX1-Trio−/− mice were generated by crossing Trio-floxed mice with EMX1-Cre mice in which Cre expression is restricted to the cerebral cortex and hippocampus (Gorski et al., 2002). EMX1-Trio−/− mice can survive to adulthood. The adult mice were useful for Trio gene research and allowed behavioral tests and adult brain analysis of these mice.

2. Results

2.1. *Trio gene is deleted in the hippocampus of EMX1-Trio−/− mice*

Trio-floxed mice, with exons 22–25 flanked by LoxP sites, were crossed with EMX1-Cre mice to generate EMX1-Trio−/− mice.