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Multiple novel isoforms of Trio are expressed in the developing rat brain

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Abstract

Mammalian Trio is a multifunctional, multidomain Rho guanine nucleotide exchange factor (GEF) closely related to Kalirin. Trio is important for proper axon guidance in *Drosophila*, and mice lacking Trio exhibit both skeletal muscle and neuronal disorders. Full length mammalian Trio and Kalirin both consist of a Sec14P-like domain, several spectrin-like domains, two Rho GEF domains each containing a Dbl-homology (DH) and a pleckstrin-homology (PH) domain, two src homology 3 domains (SH3), Ig/fibronectin-like domains (Ig/FN), and a kinase domain. We have previously described multiple isoforms of Kalirin derived through alternative splicing and multiple transcription start sites, but multiple isoforms of Trio containing different functional domains have not been described. Using a new antibody directed against the spectrin-like region of rat Trio coupled with reverse transcription PCR and cDNA sequencing, we have identified 4 novel isoforms of Trio expressed in rat cortex and cerebellum. Two isoforms, Trio 9S and Trio 9L, are derived through alternative splicing of Trio exon 48 and are abundantly expressed in rat brain. Trio 8 is expressed in postnatal day 30 and adult cerebellum, but not in cortex or skeletal muscle. Trio/duet is expressed in adult cortex and cerebellum. In the rat brain, each of these Trio isoforms is expressed at a higher level than full length Trio.

Keywords: Kalirin; Duet; Alternative splicing; Rho GEF; Dbl

1. Introduction

Both mammalian Trio and Kalirin contain a Sec14p-like domain, several spectrin-like repeats, 2 guanine nucleotide exchange factor (GEF) domains, two src homology 3 (SH3) domains, an Ig/fibronectin (FN) domain, and a kinase domain (Fig. 1A). *Drosophila* Trio, a paralog of both mammalian Trio and Kalirin, lacks the second SH3, the Ig/FN, and the kinase domains. The *C. elegans* paralog of Kalirin and Trio, Unc-73, closely resembles *Drosophila* Trio except that it contains Ig-like and FN-like domains. *Drosophila* expresses a single isoform of Trio, while alternative splicing and transcription start site usage yields 5 isoforms of *C. elegans* Unc-73. Thus, Unc-73 resembles Kalirin with its multiple 5′ and 3′ ends, and *Drosophila* Trio resembles the published data for mammalian Trio.

In *Drosophila*, Trio is involved in photoreceptor axon guidance and CNS and motor axon development (Newsome et al., 2000; Liebl et al., 2000; Bateman et al., 2000; Awasaki et al., 2000). In *C. elegans*, Unc-73 is expressed in both neurons and non-neuronal tissue and plays a role in growth cone guidance (Steven et al., 1988). Kalirin is widely expressed during brain development and plays a role in neurite outgrowth and maintenance (Alam et al., 1997; Ma et al., 2001, 2003; May et al., 2002). Mice lacking Trio exhibit defects in secondary myogenesis as well as defects in the organization of several brain regions (O'Brien et al., 2000). Analysis of Trio mutants in *Drosophila* identifies its

Abbreviations: GEF, guanine nucleotide exchange factor; SH3, src homology 3; DH, Dbl homology; PH, Plekstrin homology; Ig, immunoglobulin; FN, fibronectin; GST, glutathione *S*-transferase; BLAT, BLAST-like alignment tool; EST, expressed sequence tag; kDa, kilo Dalton; PCR, polymerase chain reaction; RTPCR, reverse transcription PCR; RACE, rapid amplification of cDNA ends; UTR, untranslated region; SMART, simple modular architecture research tool.

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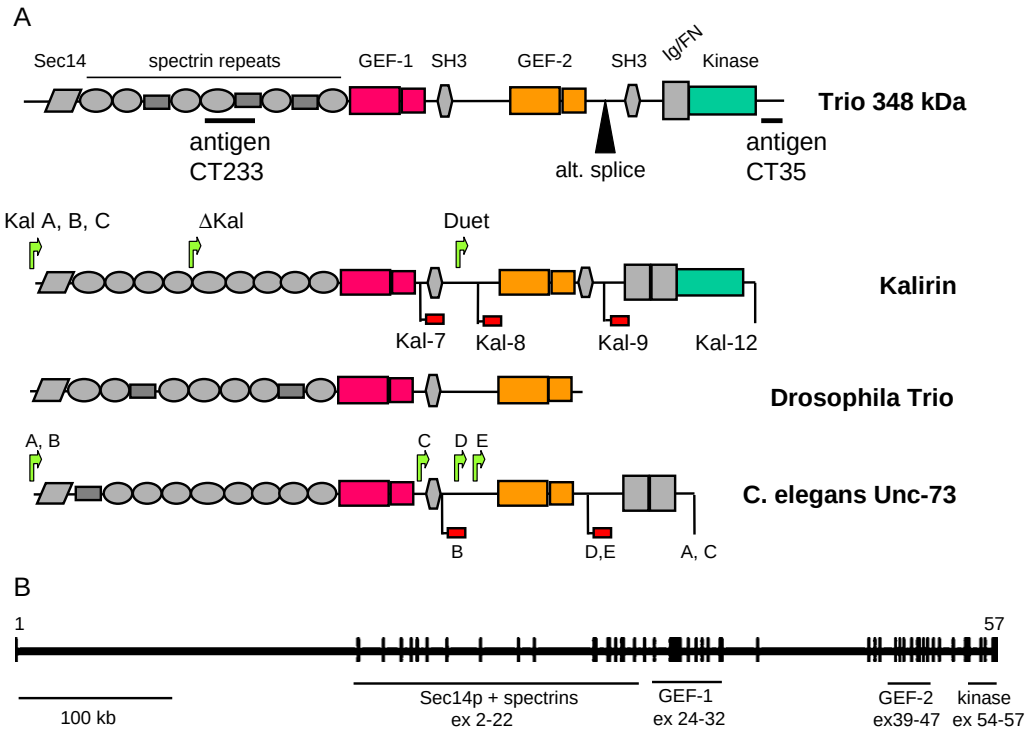

Fig. 1. Domain and genomic organization of Trio and Kalirin. (A) The functional domains of Trio, Kalirin, and their paralogs are indicated. Rectangles in the Spectrin repeat region indicate sequences not recognized as Spectrin-like domains by SMART. Green arrows above diagrams indicate internal transcription start sites; red boxes below diagrams indicate alternate 3′ ends. All possible combinations of alternate 5′ and 3′ ends have been detected for Kalirin (McPherson et al., 2002, 2004). For Unc-73, only the indicated 5′ and 3′ end combinations have been reported (www.wormbase.org). (B) Genomic organization of human Trio is indicated with vertical bars representing exons. Because of the scale, some vertical lines represent more than one exon. Exons encoding domains of Trio are indicated. (For the interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Rho GEF activity as essential (Newsome et al., 2000; Liebl et al., 2000; Bateman et al., 2000; Awasaki et al., 2000).

Several observations suggested that a search for alternate forms of Trio would be informative. First, Northern blot analysis of human RNA using a probe from the Trio 3′ end reveals at least two prominent Trio mRNAs in several tissues (Debant et al., 1996). Second, using a Trio-spectrin specific probe, we detected multiple Trio mRNAs in rat cortex (unpublished). Third, the effects of exogenous Trio expression have been investigated, but the endogenous protein has gone largely undetected (Estrach et al., 2002; Bellanger et al., 2003). Finally, although Trio mRNA is abundantly expressed in rat and mouse brains, Trio-specific antibodies directed against the C-terminal third of the protein detect only low levels of full length Trio protein expression (Debant et al., 1996; O'Brien et al., 2000; McPherson et al., 2002).

2. Experimental procedures

2.1. Generation of Trio spectrin antibody and Western blot analysis

The portion of the rat Trio gene encoding spectrins 5 and 6, amino acids 674 to 900, was cloned in the pGEX-6P vector (Amersham Biosciences) and expressed in *E. coli* as a glutathione *S*-transferase (GST) fusion protein. After purification of the fusion protein using glutathione-sepharose, the recombinant Trio portion was cleaved from GST using PreScission Protease (Amersham Biosciences). The resulting rat Trio spectrin 5/6 protein was used to immunize rabbits (Covance Research Products). Protein extractions and Western blot analyses were performed as described (Johnson et al., 2000; Penzes et al., 2000; McPherson et al., 2002). Protein extracts were separated on 4% acrylamide gels (Invitrogen) and transferred to PVDF membranes at 150 mA for 4 h on ice. Experiments to determine the specificity and sensitivity of Trio antibodies were performed four times, and the results were averaged. Western blots of rat protein probed with Trio antibody CT233 were performed four times.

2.2. RNA isolation and RTPCR

Isolation of rat total RNA and analysis by RTPCR was performed as described (McPherson et al., 2002). 3′ RACE was carried out using the GeneRacer Kit (Invitrogen) according to the manufacturer's protocol. The TOPO TA Cloning Kit (Invitrogen) was used to clone RTPCR products for sequencing. The oligonucleotides used were: exon 48 top strand: CATCGCCAATCGAGTACCAGAGGAA; exon 50