Developmental Biology 375 (2013) 33–44

Contents lists available at SciVerse ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

The kinase domain of Drosophila Tribbles is required for turnover of fly C/EBP during cell migration

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ARTICLE INFO

Article history:
Received 21 November 2012
Received in revised form
22 December 2012
Accepted 28 December 2012
Available online 7 January 2013

Keywords:
Drosophila oogenesis
Trib protein family

ABSTRACT

Drosophila Tribbles (Trbl) encodes the founding member of the Trib family of kinase-like proteins that regulate cell migration, proliferation, growth and homeostasis. Trbl was identified in a misexpression screen in the ovary as an antagonist of border cell migration and acts in part by directing turnover of the C/EBP protein encoded by the gene *slow border cells* (*slbo*). The ability of mammalian Trib isoforms to promote C/EBP turnover during tissue differentiation indicates that this function is highly conserved. To better understand the role of Trbl in cell migration, we tested specific Trbl antisera, a *trbl* null allele and Trbl transgenes bearing site-directed mutations. Trbl is expressed at high levels in the nuclei of follicle cell epithelia and is downregulated in delaminating epithelia as expression of Slbo (C/EBP) is upregulated. This complementary pattern of expression during subsequent cell migration is achieved by negative feedback whereby *slbo* represses Trbl expression and *trbl* is necessary and sufficient to promote Slbo protein turnover. A series of point mutations that scan the conserved kinase domain of Trbl reveal that the conserved DLK catalytic loop is required for Trbl–Slbo binding and turnover, as well as for interactions between Trbl subunits, suggesting a mechanism of Trbl function.

Introduction

Cell migration is a tightly regulated process, in which loss of polarity and delamination of epithelial cells is followed by changes in cell adhesion and the formation and extension of cellular processes during a migratory phase. During normal development as well as abnormal tumor progression, the levels of key regulatory proteins that trigger, direct and terminate cell migration are strictly controlled. The Drosophila ovary presents two excellent model tissues to study collective cell migration: (1) the border cells (BC), which delaminate as a small cluster and migrate through the nurse cells to reach the posterior oocyte from stages 9-10B and (2) the centripetal follicle cells (centripetal migrating FC or CMFC) which migrate as a cell sheet between the nurse cells and oocyte from stages 10B-13 (Fig. 1A). A key regulator of migration in both these tissues is the C/EBP protein Slow border cells (Slbo). Levels of Slbo are critical for proper migration, and negative feedback regulates Slbo expression both transcriptionally (Levine et al., 2010) and post-transcriptionally. A search for genes that regulate Slbo protein levels during BC migration identified the gene *tribbles* (*trbl*; Rorth et al., 2000), which encodes a member of the Trib protein family, which include mammalian isoforms Trib1, Trib2 and Trib3.

Trib family members share three conserved motifs (reviewed in Angyal and Kiss-Toth, 2012; Dobens and Bouyain, 2012; Kiss-Toth, 2011; Yokoyama and Nakamura, 2011). First, members have a central kinase-like domain that retains a conserved DLK catalytic loop but lacks other conserved motifs critical for kinase activity. Consequently, it remains unclear whether Tribs are pseudokinases or act to phosphorylate substrates via a novel mechanism. The presence of conserved binding sites for Mitogen-activated protein kinase or extracellular signal-regulated kinase kinase (MEK1) and constitutive photomorphogenesis protein 1 (COP1) in the C-terminal region has led to competing ideas that Tribs act either as docking kinases (Remenyi et al., 2006) or as 'molecular scaffolds,' pseudokinases that bind multiple regulatory proteins and balance the activities of several signaling pathways simultaneously (Kiss-Toth et al., 2006). In support of both these ideas, work done in vertebrates has connected Tribs to regulation of the TGFβ/BMP, ras/MAPK and insulin/Akt signaling pathways (Hegedus et al., 2006, 2007; Chan et al., 2007; Hua et al., 2011).

During mammalian and fly development, Tribs regulate cell proliferation during tissue differentiation (reviewed in Dobens

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http://dx.doi.org/10.1016/j.ydbio.2012.12.016

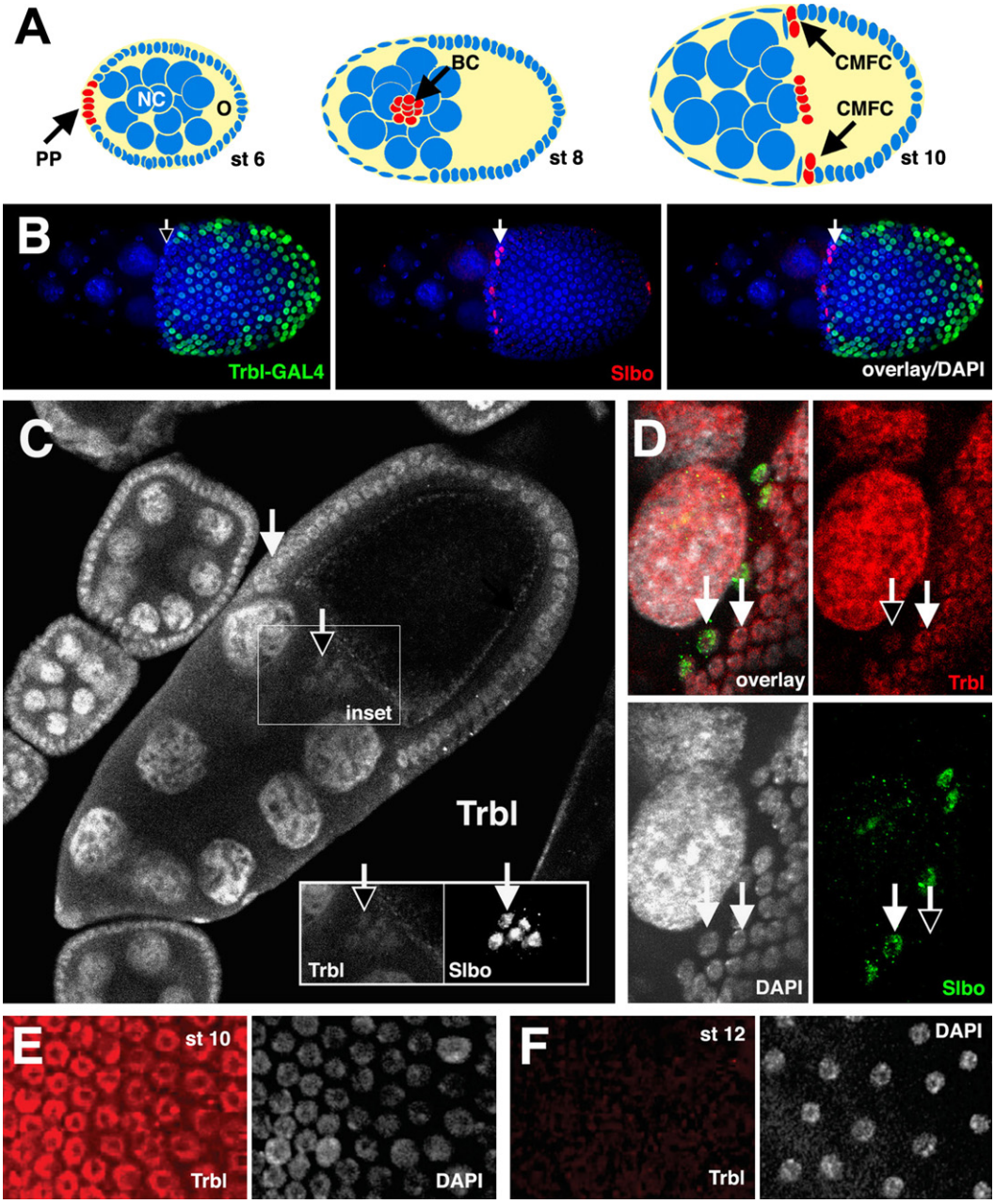

Fig. 1. Trbl accumulates in the nuclei of non-migratory FC groups (A). Outline of migratory cell types during oogenesis. At stage 9, posterior pole FC (PP) delaminate to form the border cell cluster (BC) which migrate through the nurse cells (NC) to reach the oocyte (O) at stage 10, when the centripetal FC (CMFC) migrate between the NC/O boundary. (B). Trbl-GAL4 enhancer trap ($trbl^{P\{GawB\}NP4027}$) expresses UAS-GFP (green) in the main body FC with low levels in the centripetal FC (empty arrow) that express Slbo (red). (C). At stage 10B, Trbl accumulates at high levels in the nuclei of the main body FC. Lower levels of Trbl occur in the border cells (black arrow) at stage 10B. Inset contrasts high Slbo protein levels in the border cells (white arrow, right) with low levels of Trbl (left, empty arrow). (D). Low levels of Trbl (red) accumulate in the centripetal FC that express Slbo (green). DAPI reveals the location of all nuclei in the cell sheet. (E) At stage 10, Trbl levels are high and nuclear. (F) At stage 12, Trbl levels are low and non-nuclear.

and Bouyain, 2012). In flies, Trbl blocks (1) embryonic cell division during mesodermal migration (Grosshans and Wieschaus, 2000; Seher and Leptin, 2000), (2) the step-wise cell division connected to patterning of the bristle primordia in the peripheral neurons (Abdelilah-Seyfried et al., 2000; Norga et al., 2003; Fichelson and Gho, 2004) and (3) stem cell proliferation during germ line differentiation (Mata et al., 2000; Schulz et al., 2004). Mammalian Trib isoforms regulate differentiation linked to cell division during hematopoesis (Lin et al., 2007; Eder et al., 2008; Sathyanarayana et al., 2008), myogenesis (Kato and Du, 2007; Sung et al., 2007), lymphogenesis (Selim et al., 2007) and adipogenesis (Naiki et al., 2007). The connections observed between Tribs and diverse cell signaling pathways regulating cell growth, proliferation and differentiation likely underlie the involvement of Tribs in cancer and disease (Kiss-Toth, 2011; Yokoyama and Nakamura, 2011; Angyal and Kiss-Toth, 2012; Prudente et al., 2012).

One common mechanistic feature shared by all Tribs is the ability to bind key regulatory proteins and either block their activity or direct their turnover by the proteosome. In the mesoderm and germ line of the fly, Trbl degrades String/cdc25 phosphatase to regulate the entry