Available online at www.sciencedirect.com
SCIENCE DIRECT
Developmental Biology 294 (2006) 406–417
DEVELOPMENTAL BIOLOGY
www.elsevier.com/locate/ydbio

Deadlock, a novel protein of *Drosophila*, is required for germline maintenance, fusome morphogenesis and axial patterning in oogenesis and associates with centrosomes in the early embryo

Kristina Wehr, Andrew Swan, Trudi Schüpbach *

Howard Hughes Medical Institute, Department of Molecular Biology, Princeton University, Princeton, NJ 08544, USA

Received for publication 5 August 2005, revised 26 January 2006, accepted 3 March 2006
Available online 17 April 2006

Abstract

The *deadlock* gene is required for a number of key developmental events in *Drosophila* oogenesis. Females homozygous for mutations in the *deadlock* gene lay few eggs and those exhibit severe patterning defects along both the anterior–posterior and dorsal–ventral axis. In this study, we analyzed eggs and ovaries from *deadlock* mutants and determined that *deadlock* is required for germline maintenance, stability of mitotic spindles, localization of patterning determinants, oocyte growth and fusome biogenesis in males and females. *Deadlock* encodes a novel protein which colocalizes with the oocyte nucleus at midstages of oogenesis and with the centrosomes of early embryos. Our genetic and immunohistological experiments point to a role for Deadlock in microtubule function during oogenesis.

Keywords: *Drosophila*; Oogenesis; Fusome; Germline stem cell; Microtubules; Spermatogenesis

Introduction

Analyses of genes required for female fertility in *Drosophila* have provided insights into the diverse cellular processes required for stem cell maintenance and egg development. While some of these genes encode proteins with very specific functions, many others appear to function at several stages during oogenesis giving rise to complex phenotypes (Huynh and St Johnston, 2004). We report here that the *deadlock* gene is required for a wide range of processes including germline maintenance, mitosis, patterning and growth. All of these processes require an organized and dynamic microtubule network and *deadlock* appears to be required for microtubule functions.

Oogenesis begins in a specialized region called the germarium found at the anterior tip of the ovariole. Between 2 and 3 germline stem cells (GSCs) reside there in close contact with somatic cells whose role it is to regulate their division and to maintain their fate as stem cells (Song et al., 2002; Spradling et al., 2001; Szakmary et al., 2005; Wang and Lin, 2004). GSCs undergo an asymmetric cell division to yield a new stem cell and a daughter cystoblast. These cystoblasts subsequently undergo 4 rounds of mitosis with incomplete cytokinesis to give rise to a cyst of 16 cells. Cyst cells stay connected and share cytoplasm via intercellular bridges termed ring canals. One of the cells becomes the oocyte while the other 15 differentiate into nurse cells.

Each GSC harbors a spectrin-rich membranous structure called a spectrosome positioned at the anterior cortex of the cell (de Cuevas and Spradling, 1998; Deng and Lin, 1997; Lin et al., 1994). It has been suggested that the spectrosome is required to anchor stem cells in their niche in conjunction with adherens junctions (Deng and Lin, 1997; Song et al., 2002). Departure from the niche allows germline cells to differentiate into cystoblasts as they move further away from stem cell maintenance signals (Cox et al., 2000; King and Lin, 1999; Szakmary et al., 2005; Xie and Spradling, 1998, 2000). Proper orientation of GSCs divisions is therefore important for maintenance of GSC fate and cystoblast production. This spatially directed division involves the association of the spectrosome with a single pole of the mitotic spindle. In the

* Corresponding author. Fax: +1 609 258 1547.
E-mail address: gschupbach@molbio.princeton.edu (T. Schüpbach).

0012-1606/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2006.03.002

first asymmetric division, the daughter cystoblast inherits roughly one third of the spectrosome, which, thereafter is called a fusome. The fusome anchors one mitotic spindle pole in each subsequent division, giving rise to a stereotypical cluster of cells. The fusome remains associated with the progenitor cells of each division until cytokinetic arrest at which point a 'plug' of fusomal material forms in each new ring canal. The plug fuses with the original fusome to form a branched structure that extends into each cell of the cyst (de Cuevas and Spradling, 1998; Deng and Lin, 1997; Lin et al., 1994; McGrail and Hays, 1997).

Two classes of mutants have been described that disrupt fusome morphology. Mutations in genes encoding structural components of the fusome such as *hu-li tai shao* (*hts*) and α-*spectrin* (α*spc*) cause severe disruptions in cystocyte divisions and mutant egg chambers usually degenerate before completing oogenesis (de Cuevas et al., 1996; Lin et al., 1994; Snapp et al., 2004; Yue and Spradling, 1992). The heavy chain of the microtubule motor, Dynein (Dhc64C), and its regulator, *Drosophila* Lissencephany1 (DLis1), are required for fusome biogenesis as are the non-motor microtubule-associated proteins, Abnormal Spindle (Asp) and Orbit/Mast. Mutations in these microtubule-associated proteins disrupt fusome growth and branching suggesting that fusome assembly is a microtubule-dependent process (Liu et al., 1999; Mathe et al., 2003; McGrail and Hays, 1997; Riparbelli et al., 2004; Swan et al., 1999). In addition to defects in fusome biogenesis, these mutants exhibit aberrations in oocyte growth, differentiation and axial patterning as well.

In this study, we analyzed the defects that underlie early arrest and axial patterning phenotypes of *del* mutants. We found that ovarian phenotypes in *del* flies are strikingly similar to aberrations associated with defects in microtubule-associated proteins and microtubule motors. In addition, we found that *del* mutants are very sensitive to normally well-tolerated modifications of microtubule-associated proteins. These results strongly implicate Del in playing a role in the organization of, or transport along, microtubules.

Materials and methods

Drosophila strains and transgenes

Deadlock alleles used in this study, del^{HN} and del^{WK}, were from our lab collection, but had been induced on different chromosomes (Schupbach and Wieschaus, 1991). The CG9252 P-element insertion line, *P[SUPor-P] CG9252*^{KG10262}, the Glu^{1} allele, as well as the *deadlock* Deficiency, *Df(2L) DS6*, were provided by the Bloomington Stock Center. Flies carrying the *UAS-GFP-tub* transgene, were provided by Allan Spradling, and the *NOD-lacZ* transgenic line was provided by Ira Clark (Clark et al., 1997). Mutations in *Dhc64C* were provided by Tom Hays, *UAS-HA-del* was injected into *yw* flies. The transgene was made by fusing 2 copies of a hemagglutinin tag (HA) in frame at the 5′ end of full-length *del* cDNA derived from the SD07269 EST clone (ResGen) and cloned into the pUASp vector (Rorth, 1998). Expression was driven by the nos-Gal4-VP16 (Van Doren et al., 1998), or *mat-alpha4-GAL-VP16* (Hacker and Perrimon, 1998).

Histological staining techniques

For in situ hybridizations, ovaries were dissected in PBS and fixed for 20 min in 4% paraformaldehyde in PBS +0.1% Tween20, 10% dimethyl sulfoxide and 3vol of heptane, as well as without heptane. Subsequent steps were performed as previously described, except that proteinase K treatment was varied in length between 1 min. and 5 min. (Queenan et al., 1997; Tautz and Pfeifle, 1989). Testes were dissected in Testes Buffer (183 mM KCl, 47 mM NaCl, 10 mM Tris–HCl, 1 mM EDTA, 1 mM PMSF), and the procedure of White-Cooper et al. (1998) was followed.

All ovaries for immunostaining, with the exception of BamC staining, were dissected in phosphate-buffered saline (PBS) and fixed in 4% paraformaldehyde in PBST (PBS + 0.3% Triton X-100) plus three volumes of heptane at room temperature for 20 min. Ovaries were next blocked and permeabilized in 3% BSA in PBS plus Triton X-100 (1% Triton X-100 for Grk and Orb staining, 0.3% Triton X-100 for all other antibody staining except BamC staining) for 1 h at room temperature. Ovaries were incubated in primary antibody overnight at 4°C and in secondary antibody for 1 h at room temperature. BamC staining was performed as described above using Tween 20 in place of Triton X-100. Monoclonal Grk antibody ID12 was used at 1:10 dilution (Queenan et al., 1999), rabbit anti-Osk (gift from Paul MacDonald) was diluted 1:500 and monoclonal Orb antibodies 4H8 and 6H4 (gift from Paul Schedl) were each diluted 1:60 and mixed. Rabbit anti-Vas (gift from Paul Lasko) was diluted 1:2000, rabbit anti-Caspase-3 was diluted 1:100 (Cell Signaling Technology) and rat anti-BamC (gift from Dennis McKearin) was used at 1:1000. Monoclonal anti-αSpc 3A9 was diluted 1:100 (Developmental Studies Hybridoma Bank), rat anti-αTub YL1/2 (Cappel) was used at 1:100 and rabbit anti-Asp (gift from David Glover) was used at 1:100. We also used a rabbit anti-Prod antibody (provided by Tibor Torok) at 1:500, and a rat anti-HA antibody (Roche) at 1:500. All secondary antibodies, 568 goat α-rat, 568 goat α-mouse (Molecular Probes), Cy3 donkey α-rat (Jackson Immunoresearch), 488 goat α-rabbit (Molecular Probes), were diluted 1:1000 in PBST (0.3% Triton X-100). Oregon green and Alexa Fluor 546 phalloidin were used at 1:1000 and Hoechst was used at 1 μg/ml (Molecular Probes).

A polyclonal antibody against Del was generated by injecting rabbits with a peptide corresponding to amino acids 262–280 (NH2-CLKKKTEKVHN-KIMDKPKN-COOH) of the CH9252 gene (Bio Synthesis Inc.). The third bleed from rabbit #65 was used at a 1:200 dilution to immunostain ovaries and embryos.

Testes were dissected in Testes Buffer (183 mM KCl, 47 mM NaCl, 10 mM Tris–HCl, 1 mM EDTA, 1 mM PMSF). Fixing and staining of testes was performed in the same manner as described for ovaries. Immunostaining of embryos for Tubulin and DNA was performed as described in Swan et al. (2005). For Del immunostaining, embryos were fixed 10 min in 3.7% formaldehyde in PBST and blocked in PBST, 0.3% Triton X-100, 1% BSA. Mouse anti Histones (Chemicon) and mouse anti γ-Tubulin GTU-88 (Sigma) antibodies were both used at 1/500.

Assay for mitotic divisions

Ovaries were dissected in Grace's medium at room temperature and incubated in Grace's medium containing 10 μM BrdU for 1 h and 45 min. After 2 washes in Grace's medium, ovaries were fixed for 20 min in 3.7% formaldehyde in PBST (PBS plus 0.1% Triton). Ovaries were washed 3 times for 5 min and acid-treated for 30 min in 2N HCl followed by neutralization in 100 mM borax solution for 2 min. Ovaries were next washed 3 times for 10 min and blocked in 3% BSA for 1 h. BrdU incorporation was detected by immunocytochemistry using a rat anti-BrdU antibody diluted 1:40 in PBST overnight at 4°C followed by a 1-h incubation in Cy3 anti-rat at room temperature (Jackson Immunoresearch).

Results

Deadlock mutations cause mitotic defects in germline divisions

Deadlock was identified in an EMS screen for genes required for female fertility in *Drosophila* (Schupbach and Wieschaus, 1991). This screen yielded four alleles of *deadlock (del)*, del^{HN}, del^{WK}, del^{PS} and del^{WH}, all of which lay eggs with axial patterning defects. Females homozygous for the two strongest