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asunder is required for dynein localization and dorsal fate determination during *Drosophila* oogenesis

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ABSTRACT

We previously showed that *asunder* (*asun*) is a critical regulator of dynein localization during *Drosophila* spermatogenesis. Because the expression of *asun* is much higher in *Drosophila* ovaries and early embryos than in testes, we herein sought to determine whether ASUN plays roles in oogenesis and/or embryogenesis. We characterized the female germline phenotypes of flies homozygous for a null allele of *asun* (*asun*^{d93}). We find that *asun*^{d93} females lay very few eggs and contain smaller ovaries with a highly disorganized arrangement of ovarioles in comparison to wild-type females. *asun*^{d93} ovaries also contain a significant number of egg chambers with structural defects. A majority of the eggs laid by *asun*^{d93} females are ventralized to varying degrees, from mild to severe; this ventralization phenotype may be secondary to defective localization of *gurken* transcripts, a dynein-regulated step, within *asun*^{d93} oocytes. We find that dynein localization is aberrant in *asun*^{d93} oocytes, indicating that ASUN is required for this process in both male and female germ cells. In addition to the loss of *gurken* mRNA localization, *asun*^{d93} ovaries exhibit defects in other dynein-mediated processes such as migration of nurse cell centrosomes into the oocyte during the early mitotic divisions, maintenance of the oocyte nucleus in the anterior-dorsal region of the oocyte in late-stage egg chambers, and coupling between the oocyte nucleus and centrosomes. Taken together, our data indicate that *asun* is a critical regulator of dynein localization and dynein-mediated processes during *Drosophila* oogenesis.

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Introduction

Drosophila oogenesis is a powerful model system for studying various aspects of cell and developmental biology such as control of the cell cycle, axis formation, epithelial morphogenesis, cellular polarity, and cell fate determination. A wild-type *Drosophila* female has a pair of ovaries, each made up of 16–18 independent "egg assembly lines" known as ovarioles (Bastock and St Johnston, 2008; Spradling, 1993). Each ovariole consists of a specialized anterior region (the germarium) where the progeny of germline and somatic stem cells are organized into distinct egg chambers. Each egg chamber consists of a cyst of 16 germ cells (15 nurse cells and 1 oocyte) interconnected by cytoplasmic bridges called ring canals and surrounded by a single layer of somatic follicle cells. The development of the egg chambers into mature eggs has been divided into 14 stages based on egg chamber morphology (Spradling, 1993). The polarity of the mature egg, formed at the end of oogenesis, is characterized by certain prominent structures: an anteriorly positioned, cone-shaped micropyle that facilitates sperm entry prior to fertilization and, located above the micropyle, a pair of dorsal appendages that facilitate embryonic respiration.

Determination of eggshell polarity depends on key patterning events that occur during *Drosophila* oogenesis. Within the germarium, centrosomes migrate from the nurse cells into the future oocyte in a manner dependent on a branched cytoplasmic organelle called the fusome, which extends into all the germline cells within a cyst (Bolivar et al., 2001; Lin et al., 1994). A microtubule-organizing center (MTOC) forms in the oocyte posterior; microtubules originating from this MTOC pass through cytoplasmic bridges into adjacent nurse cells and are required for transport of maternal mRNAs and proteins from the nurse cells into the oocyte (Pokrywka and Stephenson, 1991; Theurkauf et al., 1992). Transport and asymmetric localization within the oocyte of *oskar* (*osk*), *nanos* (*nos*), *bicoid* (*bcd*), and *gurken* (*grk*) transcripts are critical for proper establishment of the embryonic body axes (Becalska and Gavis, 2009).

grk mRNA is localized to the posterior of the *Drosophila* oocyte prior to its translation to generate Gurken (Grk) protein, a TGFα-like ligand, which signals posterior follicle cells to adopt a posterior fate (Gonzalez-Reyes et al., 1995; Neuman-Silberberg and Schupbach, 1993). The posterior follicle cells in turn trigger reorganization of the microtubule cytoskeleton of the oocyte that

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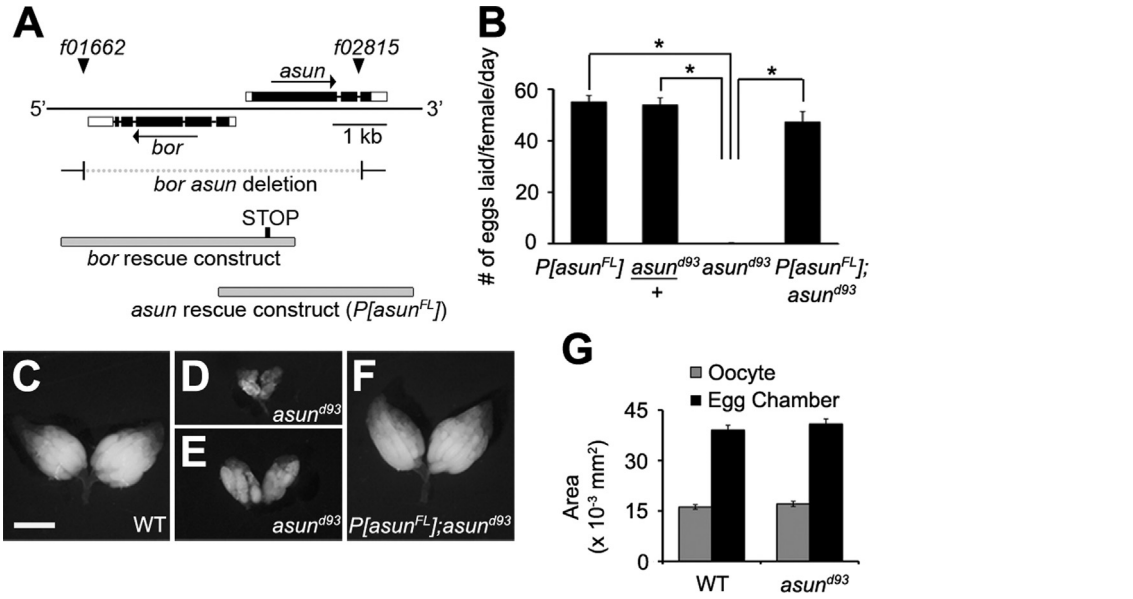

Fig. 1. Reduced egg-laying rates and ovary size of *asun*^{d93} females. (A) Schematic diagram of the *asun* gene region. Coding regions and UTRs are represented as filled and unfilled boxes, respectively, introns as thin lines, and *piggyBac* transposons *f01662* and *f02815* as triangles. Breakpoints of a *bor asun* two-gene deletion (generated through FLP-mediated recombination of FRT sites within the transposons) and design of a *bor* transgene are shown; as previously described, *asun*^{d93} flies are homozygous for the *bor asun* two-gene deletion and *bor* transgene (Sitaram et al., 2012). Design of the full-length *asun* transgene (*P*[*asun*^{FL}]) generated for this study is also shown. (B) Quantification of egg-laying rates for females of the indicated genotypes. Asterisks, $p < 0.0001$. (C–F) Whole ovaries dissected from 2-day old fattened females of the indicated genotypes. Ovaries from *asun*^{d93} females (D and E) are highly reduced in size compared to those from wild-type (C) or *P*[*asun*^{FL}];*asun*^{d93} rescue (F) females. Scale bar, 1 mm. (G) Quantification of the average area of stage 10B egg chambers and oocytes isolated from females of the indicated genotypes.

promotes localization of *bcd* transcript to the anterior pole and *osk* and *nos* transcripts to the posterior pole, thus establishing the anterior–posterior axis of the embryo. This microtubule reorganization also results in migration of the oocyte nucleus to the anterior-dorsal region of the oocyte (Zhao et al., 2012). *grk* mRNA, which associates with the oocyte nucleus, begins to accumulate in this region (Neuman-Silberberg and Schupbach, 1993). The resulting localized secretion of Grk protein, which signals to overlying dorsal-anterior follicle cells, initiates a signaling cascade that ultimately establishes the dorsal–ventral axis of the embryo (Peri and Roth, 2000; Sen et al., 1998; Van Buskirk and Schupbach, 1999; Wasserman and Freeman, 1998).

The microtubule motors, dynein and kinesin, are critical for the transport of various mRNAs to their specific sites during *Drosophila* oogenesis (Becalska and Gavis, 2009; Duncan and Warrior, 2002; Januschke et al., 2002). Localization of *grk* mRNA, which is required for the formation of both major axes, is dependent on the minus-end-directed motor, dynein (MacDougall et al., 2003; Rom et al., 2007; Swan et al., 1999). Dynein is a large complex composed of four types of subunits: heavy, intermediate, light intermediate, and light chains (Hook and Vallee, 2006; Susalka and Pfister, 2000). Dynein regulates multiple cellular processes such as organelle transport, chromosome movements, nucleus–centrosome coupling, nuclear positioning, and spindle assembly (Anderson et al., 2009; Gusnowski and Srayko, 2011; Hebbar et al., 2008; Huang et al., 2011; Jodoin et al., 2012; Salina et al., 2002; Sitaram et al., 2012; Splinter et al., 2010; Stuchell-Brereton et al., 2011; Wainman et al., 2009). During *Drosophila* oogenesis, dynein is required within the germ cells for maintenance of fusome integrity, centrosome migration, oocyte determination, migration of the oocyte nucleus, transport into the oocyte of various mRNAs and proteins that play critical roles in axis determination of the embryo, and localization of these mRNAs and proteins within the oocyte (Bolivar et al., 2001; Januschke et al., 2002; Lei and Warrior, 2000; McGrail and Hays, 1997; Schnorrer et al., 2000; Swan et al., 1999). Dynein is also important within the somatic follicle cells for maintenance of their apical-basal polarity as well as for the migration of the border cells from the anterior of the egg chamber to the anterior of the oocyte (Horne-Badovinac and Bilder, 2008; Van de Bor et al., 2011; Yang et al., 2012).

We previously identified *asunder* (*asun*) as a critical regulator of dynein localization during *Drosophila* spermatogenesis (Anderson et al., 2009). Dynein enrichment on the nuclear surface of G2 spermatocytes and round spermatids is lost in *asun* testes; as a result, *asun* male germ cells exhibit defects in nucleus–centrosome and nucleus–basal body coupling. Northern blot analysis of *Drosophila* tissues revealed that *asun* transcripts, while detected in the testes, were present at much higher levels in ovaries and early embryos, suggesting that *asun* may play roles in oogenesis and/or embryogenesis (Stebbings et al., 1998). In this study, we investigate the role of *asun* during *Drosophila* oogenesis by characterizing the phenotypes of females homozygous for a null allele of *asun* (*asun*^{d93}). We provide evidence to show that, similar to its role in spermatogenesis, *asun* is required during *Drosophila* oogenesis for regulating dynein localization and dynein-mediated processes such as nuclear positioning, centrosome migration, and dorsal–ventral patterning.

Materials and methods

Drosophila stocks

y w was used as "wild-type" stock. The *asun*^{d93} allele was previously described (Sitaram et al., 2012). *png*^{50} and *png*^{1058} were gifts from T. Orr-Weaver (Whitehead Institute, Cambridge, MA). *TrolGFP*^{ZCL1700}, which was used for observing the localization of *Drosophila* Perlecan, was a gift from A. Page-McCaw (Vanderbilt University School of Medicine, Nashville, TN).

Transgenesis

A 3.6-kb genomic fragment containing *asun* and its flanking regions (Fig. 1A) was PCR-amplified from BAC clone BAC37I18

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