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Bazooka regulates microtubule organization and spatial restriction of germ plasm assembly in the *Drosophila* oocyte

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ABSTRACT

Localization of the germ plasm to the posterior of the *Drosophila* oocyte is required for anteroposterior patterning and germ cell development during embryogenesis. While mechanisms governing the localization of individual germ plasm components have been elucidated, the process by which germ plasm assembly is restricted to the posterior pole is poorly understood. In this study, we identify a novel allele of *bazooka (baz)*, the *Drosophila* homolog of Par-3, which has allowed the analysis of *baz* function throughout oogenesis. We demonstrate that *baz* is required for spatial restriction of the germ plasm and axis patterning, and we uncover multiple requirements for *baz* in regulating the organization of the oocyte microtubule cytoskeleton. Our results suggest that distinct cortical domains established by Par proteins polarize the oocyte through differential effects on microtubule organization. We further show that microtubule plus-end enrichment is sufficient to drive germ plasm assembly even at a distance from the oocyte cortex, suggesting that control of microtubule organization is critical not only for the localization of germ plasm components to the posterior of the oocyte but also for the restriction of germ plasm assembly to the posterior pole.

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Introduction

Axial patterning during embryonic development often relies on molecular asymmetries that are established during oogenesis and propagated in the early embryo. Anteroposterior (A–P) patterning of the *Drosophila* embryo requires the asymmetric localization of *bicoid* (*bcd*) and *nanos* (*nos*) mRNAs during oogenesis, with *bcd* targeted to the anterior and *nos* to the posterior (reviewed in Berleth et al., 1988; Wang et al., 1994). After fertilization, opposing protein gradients produced by translation of these localized maternal mRNAs specify cell fates along the A–P axis (Driever and Nusslein-Volhard, 1988; Gavis and Lehmann, 1992). Consequently, mutations that disrupt *bcd* function or mRNA localization affect development of head and thoracic segments whereas mutations that disrupt *nos* function or mRNA localization produce embryos lacking abdominal segments (Driever and Nusslein-Volhard, 1988; Frohnhofer et al., 1986; Lehmann and Nusslein-Volhard, 1991; Wang et al., 1994).

nos is localized to the germ plasm, a specialized cytoplasm at the posterior of the oocyte. In addition to containing *nos* mRNA, and, consequently, directing abdominal segmentation, this assemblage of localized RNAs and proteins is necessary and sufficient for the formation of the germ cells at the posterior of the embryo (reviewed in Mahowald, 2001). Germ plasm assembly occurs by a hierarchical pathway that begins with the transport of *oskar* (*osk*) mRNA to the posterior of the oocyte (Ephrussi et al., 1991; Kim-Ha et al., 1991). In turn, *osk* localization relies on the polarization of the A–P axis of the oocyte, a process initiated earlier in oogenesis with local signaling by Gurken (Grk), a TGFα-like ligand (Gonzalez-Reyes et al., 1995). *Drosophila* oogenesis proceeds through 14 morphologically distinct stages (reviewed in Spradling, 1993) during which the oocyte is supplied with maternal mRNAs, proteins and organelles by 15 accessory nurse cells. Early in oogenesis, microtubules nucleated by a microtubule-organizing center (MTOC) at the posterior of the oocyte mediate transport of maternal mRNAs from the nurse cells into the oocyte (Theurkauf et al., 1992). Using this network, *grk* mRNA becomes localized to the posterior of the oocyte where the resulting Grk protein signals to the overlying somatic follicle cells, triggering the disassembly of the posterior MTOC (reviewed in Steinhauer and Kalderon, 2006). The subsequent nucleation of microtubules at the anterior and lateral oocyte cortex leads to a reorganization of the oocyte microtubule cytoskeleton and a bias of microtubule plus ends oriented toward the posterior pole (Cha et al., 2001; Theurkauf et al., 1992; Zimyanin et al., 2008).

One consequence of the reorganization of oocyte microtubules is the relocation of the oocyte nucleus and *grk* mRNA from the posterior to the dorsal anterior corner of the oocyte (Gonzalez-Reyes et al., 1995). Here, Grk is again synthesized and signals to the overlying follicle cells to specify the dorsoventral (D–V) axis of the embryo (Neuman-Silberberg and Schupbach, 1993). Another consequence is the kinesin-dependent transport of *osk* mRNA to the posterior of the oocyte, which initiates the assembly of the germ plasm (Brendza et al., 2000). Upon localization, *osk* is translated and the resulting protein

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recruits other germ plasm components to the posterior including the RNA helicase Vasa (Vas) (Breitwieser et al., 1996; Hay et al., 1988; Schupbach and Wieschaus, 1986), which, together with Osk, is required for the localization of *nos* mRNA later in oogenesis (Wang et al., 1994). *osk* localization is sufficient to dictate the site of germ plasm assembly, as mistargeting of *osk* mRNA to the anterior of the oocyte results in ectopic germ plasm assembly, germ cell formation, and abdominal development at the anterior of the embryo (Ephrussi and Lehmann, 1992). Interestingly, while translational repression of *osk* mRNA during its localization to the posterior of the oocyte is required to prevent ectopic Osk function (Kim-Ha et al., 1995), the mechanism by which this repression is alleviated at the posterior pole remains unclear. In addition, the mechanisms that govern the polarity of the oocyte microtubule cytoskeleton and the restriction of the germ plasm to the posterior pole are not fully understood.

The oocyte cortex itself is polarized by the Par proteins. First characterized in *Caenorhabditis elegans*, Par proteins are key regulators of cell polarity in many cell types in diverse organisms (reviewed in Goldstein and Macara, 2007). The Par proteins function at the cell cortex where they form two mutually exclusive domains containing either Par-1 or Par-3. In the *C. elegans* embryo, as well as in mammalian epithelial cells, these complementary cortical domains define an axis of cell polarity and are regulated by antagonistic interactions between the Par proteins (reviewed in Suzuki and Ohno, 2006). In the *Drosophila* oocyte, Par-1 is localized to the posterior pole where it accumulates by a microfilament based mechanism, before *osk* mRNA localization, and is required for correct polarization of the microtubule cytoskeleton, posterior localization of *osk*, and, consequently, the assembly of the germ plasm (Doerflinger et al., 2006; Shulman et al., 2000). In contrast, Bazooka (Baz), the *Drosophila* homolog of Par-3, is enriched on the anterior and lateral cortex (Benton and St Johnston, 2003b). The function of Baz in the oocyte is poorly understood because all previously characterized *baz* alleles display defects in oocyte specification (Cox et al., 2001; Huynh et al., 2001). While forcible localization of Baz to the posterior of the oocyte, by expression of an unregulated form of the protein, leads to A–P patterning defects (Benton and St Johnston, 2003b), a bona fide role for Baz in axis determination has yet to be demonstrated.

Here, we describe the identification and characterization of a novel allele of *baz*, $baz^{X\text{-}82}$, which supports oocyte development. We find that $baz^{X\text{-}82}$ causes defects in microtubule organization and germ plasm localization in the oocyte that lead to ectopic foci of germ plasm in the embryo. In addition, we show that mutation of *grk*, which disrupts microtubule polarity by eliminating the necessary oocyte-to-follicle cell signaling events, leads to ectopic *osk* translation and germ plasm assembly. Together, these results reveal a novel role for Baz in regulating oocyte microtubule polarity and axis patterning. Furthermore, our results suggest that a focus of microtubule plus ends is sufficient to trigger *osk* translation and germ plasm assembly even at a location distant from the oocyte cortex.

Materials and methods

Fly stocks and genetics

Oregon-R or *y* w^{67c23} were used as wild-type controls. The following mutants and transgenic lines were used: *X-82* (Luschnig et al., 2004), baz^{EH171} (Eberl and Hilliker, 1988), baz^{4} (Muller and Wieschaus, 1996), *osk-gfp* (Snee et al., 2007), *vas-gfp* (gift of R. Lehmann), *khc-lacZ* (Clark et al., 1994), *nod-lacZ* (Clark et al., 1997), *pnt-lacZ* (Gonzalez-Reyes and St Johnston, 1998), *UASp-baz-gfp* (Benton and St Johnston, 2003a), grk^{2B}, and grk^{HF} (Neuman-Silberberg and Schupbach, 1993).

An unlinked lethal mutation present on the original *X-82* chromosome was removed by recombination of $baz^{X\text{-}82}$ onto the *FRT19A* chromosome (gift from M. Metzstein). Germline clones were induced by the dominant female sterile technique (Chou et al., 1993). Follicle cell clones were generated using the FRT/UAS-Flp/GAL4 system (Duffy et al., 1998). Clones were induced in females of the following genotype: $baz^{X\text{-}82}$ *FRT19A/ubi-GFP FRT19A;e22c-GAL4 UAS-FLP/+* or *FRT19A/ubi-GFP FRT19A;e22c-GAL4 UAS-FLP/+* as a control.

UASp-baz-gfp and *UASp-*$baz^{X\text{-}82}$*-gfp* were expressed to equivalent levels using the *matα-tubulin-GAL-VP16*^{V2H} driver at 18 °C and the *matα-tubulin-GAL-VP16*^{V37} driver at 29 °C, respectively (Bloomington).

Positional cloning

X-82 was mapped proximal to *sd* by meiotic recombination between the *X-82* chromosome and the *t v m wy sd os* and *y sn lz ras v m* chromosomes (Bloomington). Recombinants carrying the *X-82* mutation were followed by examining embryos under oil for segmentation defects and confirmed in cuticle preparations. *X-82* was further mapped using in situ hybridization to *nos* mRNA in early embryos from females heterozygous for *X-82* and each of the following deficiencies: *Df(1)ED7355*, *Df(1)4b18*, *Df(1)ED7374*, *Df(1)B25*, *Df(1)BK10*, *Df(1)RR79*, *Df(1)N19*, *Df(1)Exel6291*, *Df(1)ED7441*, and *Df(1)JA27* (Bloomington). In this analysis, *Df(1)B25* failed to complement the ectopic *nos* mRNA phenotype of *X-82*. Of the 14 genes specific to *Df(1)B25*, we selected *baz* as a likely candidate gene. Genomic DNA was isolated from individual homozygous *X-82* adult males (Mansfield et al., 2002) and exonic regions of the *baz* gene were amplified by PCR and sequenced. A nonsense mutation that creates a TAG stop codon was identified in exon 7 of *baz*, and we designate this allele as $baz^{X\text{-}82}$.

Immunoblotting

Immunoblotting was performed as previously described (Kalifa et al., 2006) except that the ovaries were dissected in PBS. Membranes were probed with rabbit anti-Baz (1:2000) (Wodarz et al., 1999), and proteins were detected using Lumi-Light Western blot substrate (Roche).

Embryonic cuticle preparation, in situ hybridization, and immunostaining

Cuticle preparations and in situ hybridization to embryos for individual mRNAs were performed as previously described (Gavis and Lehmann, 1992). Double fluorescence in situ hybridization (FISH) using tyramide signal amplification was performed as described (Kosman et al., 2004). Immunofluorescence was performed as previously described (Duchow et al., 2005) using embryos heat-fixed in 68 mM NaCl/0.03% Triton X-100, with rabbit anti-Vas (1:10,000; gift from R. Lehmann) and Alexa Fluor 568 goat anti-rabbit (1:1000; Molecular Probes). Embryos were mounted in 90% glycerol/100 mM Tris pH 8.0 and imaged with a Zeiss LSM 510 confocal microscope.

Ovary immunostaining, in situ hybridization, and GFP imaging

To visualize microtubules, ovaries from well-fed females were dissected in 10% EM grade formaldehyde (Polysciences, Inc.) in PBST (PBS/2% Tween-20). Fixation was stopped 7 minutes after dissection started. Ovaries were washed 3 × 10 minutes in PBST, blocked in PBST/10% BSA for 1 hour and incubated with FITC conjugated anti-α-tubulin (1:250; Sigma) in PBST/10% BSA overnight at 4 °C. Subsequently, ovaries were washed 4 × 15 minutes in PBS/0.1% Tween-20, 4 × 5 minutes in methanol and mounted in 40 µl 2:1 benzyl benzoate/benzyl alcohol.

Anti-Grk immunostaining was performed as described (Pane et al., 2007) except that EM grade formaldehyde was used as a fixative. All other immunostaining was performed as described (Shcherbata et al., 2004) except that 4% EM grade formaldehyde was used as a fixative and PBST contained 0.1% Triton X-100. Primary antibodies were used as follows: mouse anti-Grk 1D12 (1:10) (Queenan et al., 1999), rabbit

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