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Report

A Role for Vasa in Regulating Mitotic Chromosome Condensation in *Drosophila*

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Summary

Vasa (Vas) is a conserved DEAD-box RNA helicase expressed in germline cells [1] that localizes to a characteristic perinuclear structure called nuage [2, 3]. Previous studies have shown that Vas has diverse functions, with roles in regulating mRNA translation, germline differentiation, pole plasm assembly, and piwi-interacting RNA (piRNA)-mediated transposon silencing [1, 4–10]. Although *vas* has also been implicated in the regulation of germline proliferation in *Drosophila* and mice [1, 11], little is known about whether Vas plays a role during the mitotic cell cycle. Here, we report a translation-independent function of *vas* in regulating mitotic chromosome condensation in the *Drosophila* germline. During mitosis, Vas facilitates robust chromosomal localization of the condensin I components Barren (Barr) and CAP-D2. Vas specifically associates with Barr and CAP-D2, but not with CAP-D3 (a condensin II component). The mitotic function of Vas is mediated by the formation of perichromosomal Vas bodies during mitosis, which requires the piRNA pathway components *aubergine* and *spindle-E*. Our results suggest that Vas functions during mitosis and may link the piRNA pathway to mitotic chromosome condensation in *Drosophila*.

Results

vas Promotes Mitotic Chromosome Condensation

The *Drosophila* germarium (Figure 1A) is a favorable system in which to study mitotically dividing germline cells. At the anterior tip of each germarium, there are two to three germline stem cells (GSCs) that divide asymmetrically to produce a GSC and a cystoblast (CB). The CB undergoes four mitotic divisions, forming a 16-cell cyst, which later differentiates into an oocyte and 15 nurse cells [12]. To address whether *vasa* (*vas*) has any function during mitosis, we first examined mitotic defects in *vas* mutant germline cells. Like other piRNA pathway mutants, *vas* mutants exhibited an increased incidence of DNA double-strand breaks (DSBs) in differentiating germline cells, but not in GSCs and CBs (see Figure S1A available online) [13]. To avoid any indirect contributions from DNA damage checkpoint, we focused only on mitotically dividing GSCs and CBs in this study.

During mitosis, chromosomes condensed during prophase and metaphase in wild-type cells, but progression of chromosome condensation appeared delayed in *vas* mutants (Figure 1B). Wild-type ovaries contained a higher percentage of mitotic GSCs and CBs in metaphase than in prometaphase. However, *vas* mutant ovaries exhibited an increased percentage of GSCs and CBs in prometaphase with a concomitant drop of GSCs and CBs in metaphase (Figure 1C), suggesting a delay in the prometaphase-metaphase transition. In addition, many *vas* mutant GSCs and CBs exhibited lagging chromosomes during anaphase, which were not seen in wild-type cells (Figure 1B; 0% in wild-type cells, n = 36, versus 56.7% in *vas* mutants, n = 30). We further confirmed a requirement for *vas* in mitotic chromosome condensation and segregation by analyzing *bag-of-marbles* (*bam*) mutant ovaries, which have large populations of GSC-like cells (Figures S1B–S1D; see Experimental Procedures). Mitotic chromosomal defects were rescued by a full-length *vas* transgene (Figure 1C; 0%, n = 20), confirming that the phenotype we observed was due to the impaired function of *vas*.

Because Vas has been reported to function as a general regulator of translation via its interaction with the eukaryotic initiation factor 5B (eIF5B) [5, 7], the observed delay in prometaphase and segregation defects could be a consequence of general perturbation of gene expression. This was not the case, because the defects in *vas* mutants were rescued by a $vas^{\Delta 617}$ transgene, which is unable to interact with eIF5B and therefore lacks the ability to regulate translation [5, 7] (Figure 1C; 0%, n = 20). This indicates that *vas* promotes chromosome condensation and segregation independently of its role in regulating translation and that the observed chromosomal defects are unlikely to be indirect consequences of altered gene expression. Consistent with this view, the expression of chromosomal and cell-cycle proteins was unaffected in *vas* mutant ovaries (Figure S1E).

Because the piRNA pathway is required for heterochromatin formation in *Drosophila* germline cells [14], we wondered whether mitotic chromosomal defects in *vas* mutants were due to defective interphase heterochromatin formation because of an impairment in the piRNA pathway. In *vas* mutants, despite the perturbation of the piRNA pathway [6, 8, 10], we did not observe any global effects on interphase heterochromatin as assessed by the expression and localization of the heterochromatin markers HP1a (heterochromatin protein 1a), trimethylation of H3K9 (lysine 9 of histone 3), and CID (centromere identifier, homolog of the centromere-specific H3-like protein CENP-A) (Figures S1F–S1H). Furthermore, heterochromatin formation was unaffected during mitosis in *vas* mutants (Figure S1I), suggesting that the chromosomal defects in *vas* mutants were unlikely to be caused by the failure of heterochromatin formation. Taken together, our data suggest a role for *vas* in regulating chromosome condensation and segregation during mitosis.

vas Facilitates Robust Chromosomal Localization of Barr

Chromosomal defects in *vas* mutants are similar to those observed in the absence of Barren (Barr, or CAP-H), a conserved component of the condensin I complex [15, 16]. Barr localizes to chromosomes during mitosis and is required for proper chromosome condensation and segregation [15–17]. Although the expression of Barr was unaffected in *vas* mutant ovaries (Figures S1E and S2A), the localization of Barr to mitotic chromosomes was diminished in over 80% of *vas* mutant GSCs and CBs in prometaphase and metaphase

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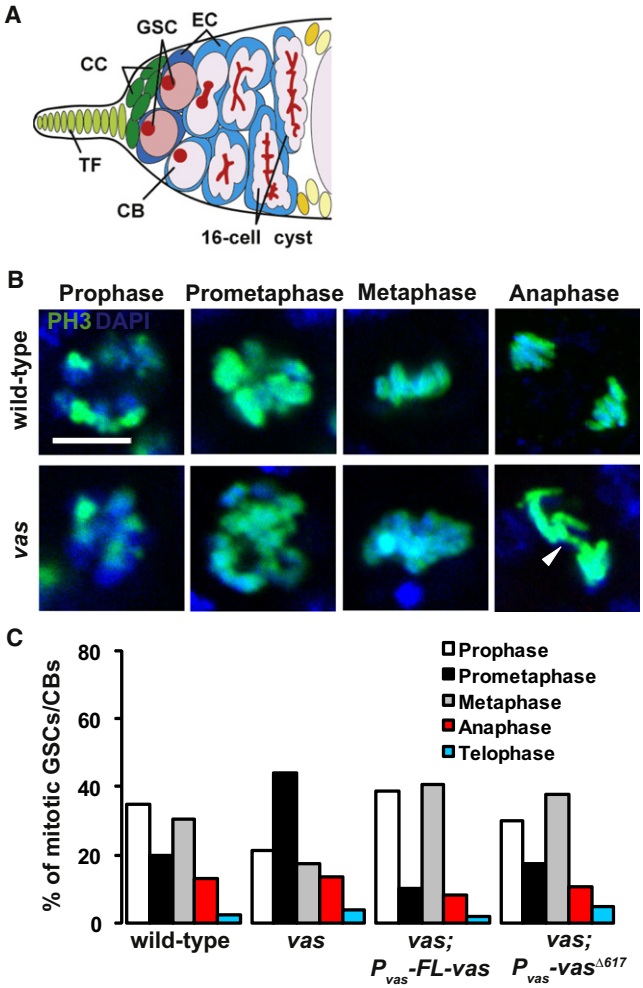


Figure 1. *vas* Promotes Mitotic Chromosome Condensation

(A) Schematic diagram of a *Drosophila* ovary germarium. The following abbreviations are used: CC, cap cells; GSC, germline stem cells; EC, escort cells; TF, terminal filament; CB, cystoblast.

(B) Wild-type and *vas* mutant ovarian GSCs and CBs at various stages of mitosis stained for PH3 (green) and DAPI (blue). Mitotic chromosomes in *vas* mutants appear less condensed and frequently exhibit lagging chromosomes (arrowhead). Scale bar represents 10 μm.

(C) Chart showing percentage of GSCs and CBs from the indicated genotypes at each stage of mitosis (n > 35 for each genotype). Mitosis is delayed in prometaphase in *vas* mutants, and this can be rescued by either a *FL-vas* or a $vas^{\Delta 617}$ transgene.

See also Figure S1.

(Figures 2A and 2B), suggesting that chromosomal defects in *vas* mutants may be due to impaired Barr localization. Further analysis showed that localization of CAP-D2 (another condensin I component), but not the chromosomal protein topoisomerase II (Topo II), was aberrant in *vas* mutant GSCs and CBs (Figures S2B–S2D), indicating a specific defect in the loading of condensin I components in *vas* mutants. The condensin II components CAP-D3 and CAP-H2 neither accumulated in the nucleus nor localized to chromosomes in the germarium [18] (data not shown), ruling out the possibility that Vas regulates condensin II.

Because a small percentage of *vas* mutant GSCs and CBs had normal localization of Barr (Figure 2B), *vas* may function to facilitate robust chromosomal localization of Barr. We thus investigated whether overexpression of Barr could compensate for the requirement of *vas* in promoting chromosome condensation and segregation in *vas* mutants (Figure S2E). In *vas* mutant ovaries that ectopically expressed Barr-GFP, localization of Barr and CAP-D2 to the mitotic chromosomes was increased (Figures 2A and 2B; Figures S2B and S2C), and the delay in prometaphase and chromosome segregation defects was rescued (Figure 2C; 3.2% segregation defects in *vas/Df;UASp-barr/nosGal4VP16*, n = 31, versus 56.7% in *vas/Df*, n = 30). This genetic interaction suggests that *vas* promotes mitotic chromosome condensation at least in part by facilitating robust chromosomal localization of Barr.

Vas Localizes as Foci near Mitotic Chromosomes and Interacts with Barr

To determine whether Vas plays a direct role in regulating chromosome condensation, we examined the localization of Vas during mitosis. Immunostaining with a specific Vas antibody revealed a cell-cycle-dependent localization (Figures S1B and S3A). During interphase and prophase, Vas localized as perinuclear foci, as reported previously (Figures S1B and S3B) [4]. During prometaphase/metaphase, Vas appeared as foci in close proximity to the chromosomes (Figure 3A; Figure S1B). During anaphase, these foci were displaced between separating sister chromatids, with a few Vas foci remaining near the chromosomes (Figures S1B and S3B). Consistently, transgenic Vas-GFP also exhibited this dynamic localization during mitosis (Figure S3C).

Whereas *vas* is required for Barr chromosomal localization (Figures 2A and 2B), the converse is not true. In *cap-g* (a condensin I component) mutants, which have defective chromosomal localization of Barr and impaired prometaphase progression, formation of perichromosomal Vas foci was unaffected (Figures S3D and S3E; n > 20). These data support the idea that Vas first localizes as foci near mitotic chromosomes to facilitate robust chromosomal localization of Barr during mitosis. We further tested this idea by examining the interaction between Vas and Barr in vivo. In *bam* mutant ovaries, which have enhanced numbers of mitotically dividing GSC-like cells, Barr coimmunoprecipitated with Vas, but not with an IgG control or with Vas from *vas* mutant extracts (Figure 3B). Furthermore, CAP-D2, but not CAP-D3 (a condensin II component), was also pulled down by Vas (Figure 3B), suggesting a specific interaction between Vas and the condensin I components Barr and CAP-D2. In reciprocal coimmunoprecipitations (CoIPs), we also detected an interaction between Barr-GFP and Vas that was absent in IgG and nontransgenic controls (Figure 3B). Barr-GFP also interacted with CAP-D2, but not with CAP-D3 (Figure 3B), again confirming a specific interaction between condensin I components and Vas. Interaction between Vas and Barr was confirmed in an S2 cell line transfected with epitope-tagged Vas and Barr (Figure S3F), indicating that Vas and Barr can interact in the absence of germline factors. The interaction between Vas and Barr was not abrogated by RNase treatment (Figure S3G), further suggesting a direct interaction. Taken together, our data support a direct role for Vas in regulating Barr chromosomal localization.

The piRNA Pathway Components *aub* and *spn-E* Mediate Vas Mitotic Localization and Chromosome Condensation

Vas has been reported to interact with Aubergine (Aub), a piwi-subfamily protein that binds germline piRNAs [19–22]. Consistently, Aub coimmunoprecipitated with Vas in

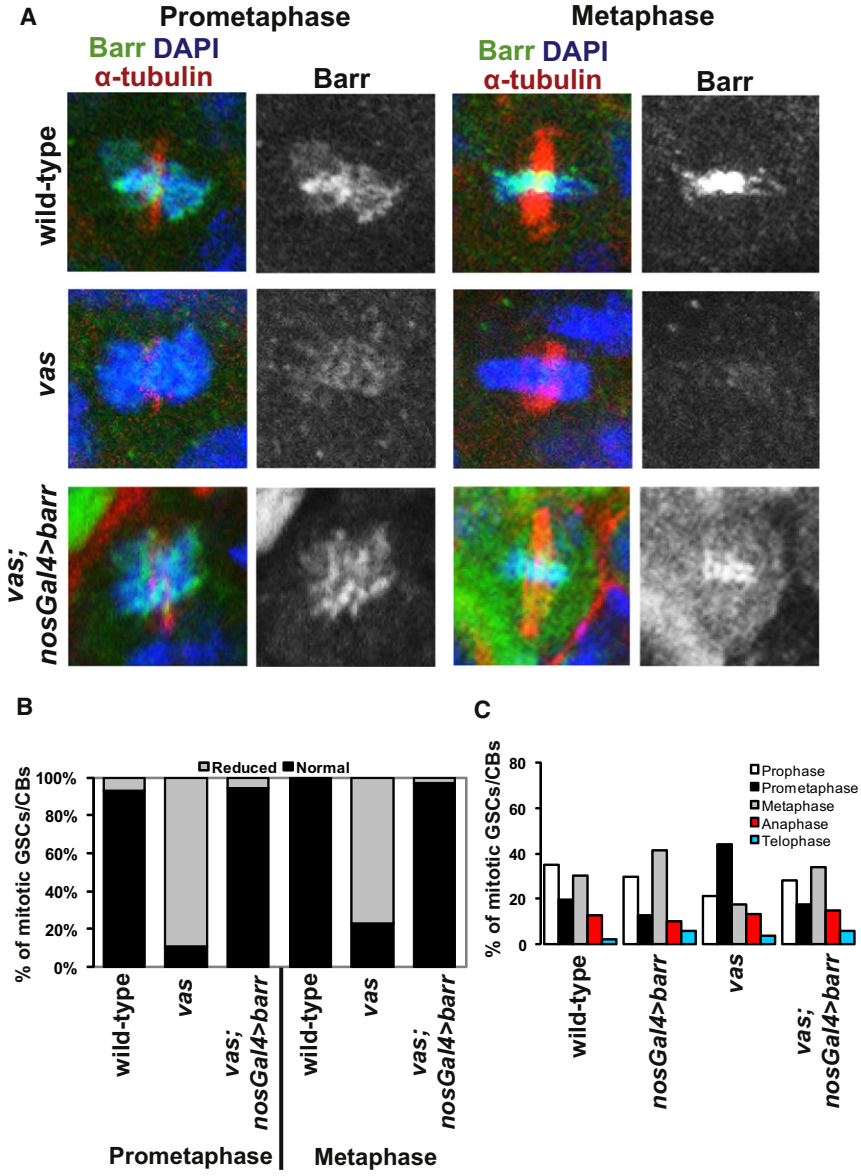


Figure 2. *vas* Facilitates Robust Chromosomal Localization of Barr

(A) Prometaphase and metaphase GSCs and CBs from the indicated genotypes stained for Barr (green), α-tubulin (red), and DAPI (blue). Chromosomal localization of Barr is reduced in *vas* mutants; proper localization can be compensated by overexpression of Barr.

(B) The percentage of GSCs and CBs with normal or reduced chromosomal localization of Barr as depicted in (A) (n > 25 for each genotype).

(C) The percentage of GSCs and CBs from the indicated genotypes at various stages of mitosis (n > 45 for each genotype). Overexpression of Barr can rescue the delay in prometaphase in *vas* mutants.

See also Figure S2.

GSC/CB-enriched *bam* mutant ovaries and colocalized with Vas to mitotic chromosomes (Figures S4A and S4B). We also found that another major piRNA pathway component, Spindle-E (Spn-E), coimmunoprecipitated with both Vas and Barr-GFP in GSC/CB-enriched ovaries and colocalized with Vas to mitotic chromosomes (Figures S4A, S4C, and S4D). Thus, Vas may function together with Aub and Spn-E during mitosis.

We next addressed whether Vas localizes to piRNA loci on chromosomes by chromatin immunoprecipitation using ovaries enriched with mitotic GSCs and CBs. Vas was enriched at pericentromeric germline piRNA clusters, but not at somatic piRNA, endogenous siRNA (endo-siRNA), or control clusters (Figure 4A) [21, 23], suggesting that Vas chromosomal localization may be mediated by the piRNA pathway. To address this, we examined whether Vas chromosomal localization depended on two major piRNA pathway components, *aub* and *spn-E*. In *aub* and *spn-E* mutants, perinuclear localization of Vas during interphase was unaffected (Figure 4B; Figure S4E) [8, 24]. However, Vas foci near mitotic chromosomes were undetectable in these mutants (Figure 4B; Figure S4E), indicating that, unlike perinuclear Vas foci, formation of Vas chromosomal foci has a unique genetic requirement for *aub* and *spn-E*.

In addition to the disruption of mitotic Vas chromosomal foci, both *aub* and *spn-E* mutants exhibited delays in prometaphase and defects in chromosome segregation and chromosomal localization of Barr (Figures 4C–4F; Figures S4F–S4I). These defects were not observed in GSCs and CBs of the endo-siRNA pathway mutants *ago2* and *dcr-2* (Figures S4F, S4G, and S4J) [23], suggesting that the germline piRNA pathway components *aub* and *spn-E* are specifically required for chromosomal localization of Vas to regulate chromosome condensation during mitosis.

Discussion

In *Drosophila*, the association of Barr with mitotic chromosomes is highly dynamic, and Barr is loaded primarily at the centromeric regions before spreading distally [17]. How such a dynamic event is regulated remains unclear. We suggest that during mitosis, Vas forms bodies that are in close proximity to pericentromeric piRNA-generating loci, where they function to facilitate chromosomal recruitment of Barr to participate in the robust condensation of chromosomes (Figure 4G). Alternatively, despite the localization to pericentromeres, Vas may promote the long-range stable association of Barr with entire mitotic chromosomes by a yet unknown mechanism. Barr appears to be a principal effector of Vas, because ectopic expression and enhanced localization of Barr to mitotic chromosomes suppresses, to a large extent, chromosomal defects in *vas* mutants. Furthermore, mitotic localization of Vas correlates with the timing of active chromosomal loading of Barr during prometaphase, and Vas interacts specifically with Barr and CAP-D2 (condensin I components). Interestingly, we also observed relocalization of Vas bodies to regions between segregating chromosomes during

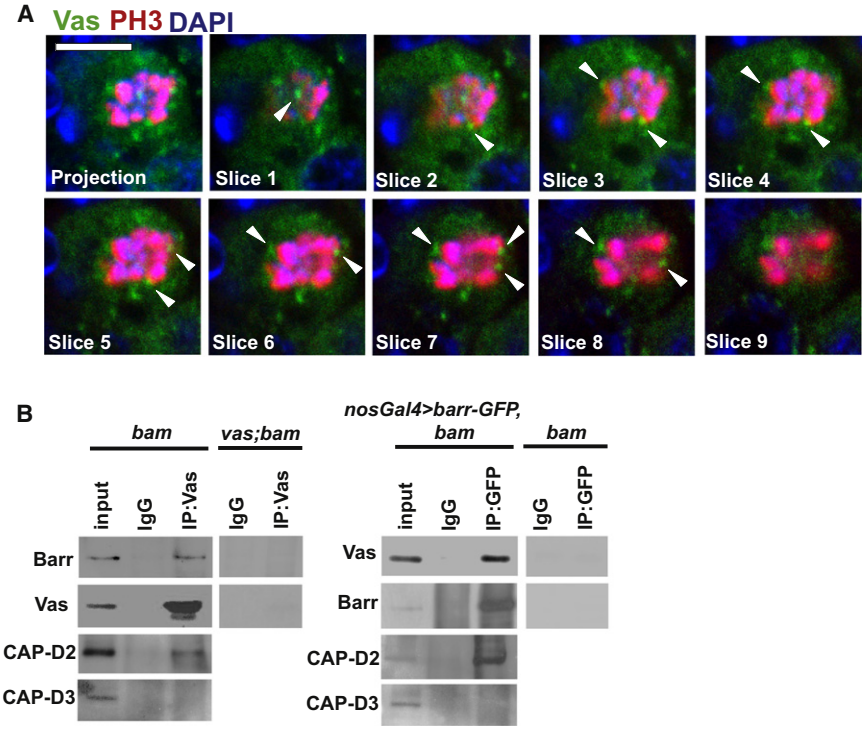


Figure 3. Vas Localizes as Foci near Mitotic Chromosomes and Interacts with Barr

(A) Wild-type GSC at prometaphase stained for Vas (green), PH3 (red), and DAPI (blue). A series of nine confocal sections of 0.5 μm intervals and their projection are shown. During prometaphase, Vas localizes as foci (arrowheads) in close proximity to the chromosomes. Scale bar represents 10 μm.

(B) Western blots showing coimmunoprecipitation of Vas and Barr in GSC/CB-enriched *bam* mutant ovaries. Barr coimmunoprecipitated with Vas, but not with the IgG control or the *vas* mutant extract. Reciprocally, Vas coimmunoprecipitated with Barr-GFP expressed in germline cells, but not with IgG or nontransgenic controls. In both experiments, CAP-D2, but not CAP-D3, also coimmunoprecipitated with Vas or Barr-GFP, indicating specificity.

See also Figure S3.

anaphase. In a small fraction of *vas* mutant germline cells (3.2%), segregation defects are not suppressed by ectopic expression of Barr, suggesting a condensin I-independent role for Vas during anaphase. It would be interesting to examine whether Vas also functions in a tether-based mechanism to facilitate the chromosome segregation seen in neuroblasts [25].

Studies have shown that loss of condensin function triggers the spindle assembly checkpoint (SAC) [26–28]. Consistent with our observation that *vas* promotes robust chromosomal localization of condensin I components, we observed that the delay in mitotic progression in *vas* mutants is partially rescued by reducing a copy of the SAC genes *bubR1* or *zw10* (Figure S2F), suggesting that the SAC may be activated in *vas* mutants. However, we cannot completely exclude the possibility that Vas may also regulate other factors that trigger or suppress the SAC.

Because Vas has a wide range of functions, *vas* mutants exhibit a pleiotropic phenotype, including showing a mild defect in stem cell maintenance and germline differentiation [1]. In *aub* and *spn-E* mutants, in which the mitotic function of Vas is perturbed, we observed less robust Barr localization and a delay in chromosome condensation during prometaphase, but germline differentiation was unaffected. Similarly, *cap-g* (condensin I) mutants are female sterile and have impaired Barr localization and delayed chromosome condensation during prometaphase (Figures S3D and S3E). In these mutants, although the dynamics of the synaptonemal complex during meiosis are perturbed, progression of oogenesis is unaffected [29]. Moreover, introduction of the $vas^{\Delta 617}$ or *barr-GFP* transgene into *vas* mutants restores mitotic defects of prometaphase delay and lagging chromosomes, but not the later-stage germline differentiation defects (Figure 1; Figure 2; data not shown). Taken together, these data suggest that the function of *vas* in chromosome condensation is not required for progression of germline differentiation but is required to ensure the fidelity of chromosome segregation, which may contribute in part to the age-dependent atrophy, oocyte differentiation defect, and sterility in *vas* mutants. Analysis of a Vas variant that specifically abrogates the interaction of Vas with Barr would give us insight into the molecular function of Vas in regulating Barr.

Our study identifies a possible link between the piRNA pathway and chromosome condensation and segregation during mitosis. This is particularly intriguing because recent studies in *C. elegans* have shown that 22G-RNAs and several P granule components function to organize chromosomes with holocentric centromeres during mitosis [30, 31]. Together with our study in *Drosophila*, these studies raise an interesting possibility that components of the nuage and small RNA pathways may play a unique role in organizing chromosomes during the cell cycle in eukaryotes. It will be interesting to dissect the molecular involvement of piRNAs, siRNAs, and other small RNA pathway components in regulating mitotic chromosome condensation and segregation in germline cells.

We propose that Vas has a role in regulating chromosome condensation during mitosis of germline cells at least in part by facilitating robust chromosomal localization of Barr. This process is mediated by the formation of Vas perichromosomal bodies during mitosis and depends on *aub* and *spn-E*.

Experimental Procedures

Fly Strains

The fly strains and allelic combinations *y w*, vas^{PH165} [1], *Df(2L)BCS299* (Bloomington Stock Center), $aub^{N11/HN2}$ [32, 33], $spn\text{-}E^{616/hls}$ [34, 35], $ago2^{414}$ [36], $dcr\text{-}2^{L811fsX}$ [37], $bam^{\Delta 86}$ [38], P_{vas}-*FL-vas* [5], P_{vas}-$vas^{\Delta 617}$ [5], *vas-GFP* [5], *UASp-barr-EGFP* [16], $cap\text{-}g^{Z1/K4}$ [29], $mit(1)15^{5}$ (Bloomington Stock Center), and $bubR1^{k03113}$ (Bloomington Stock Center) were used in this study. Further information on symbols and genes can be found at the FlyBase website (http://flybase.org/).

GSC/CB populations can be enriched by a mutation in *bam* [38, 39]. GSCs and CBs in *y w* and vas^{PH165}/*CyO* and GSC-like cells in $bam^{\Delta 68}$ mutant ovaries had indistinguishable phenotypes (Figures S1B–S1D). Either *y w* or vas^{PH165}/*CyO* was used as the wild-type strain unless otherwise stated.

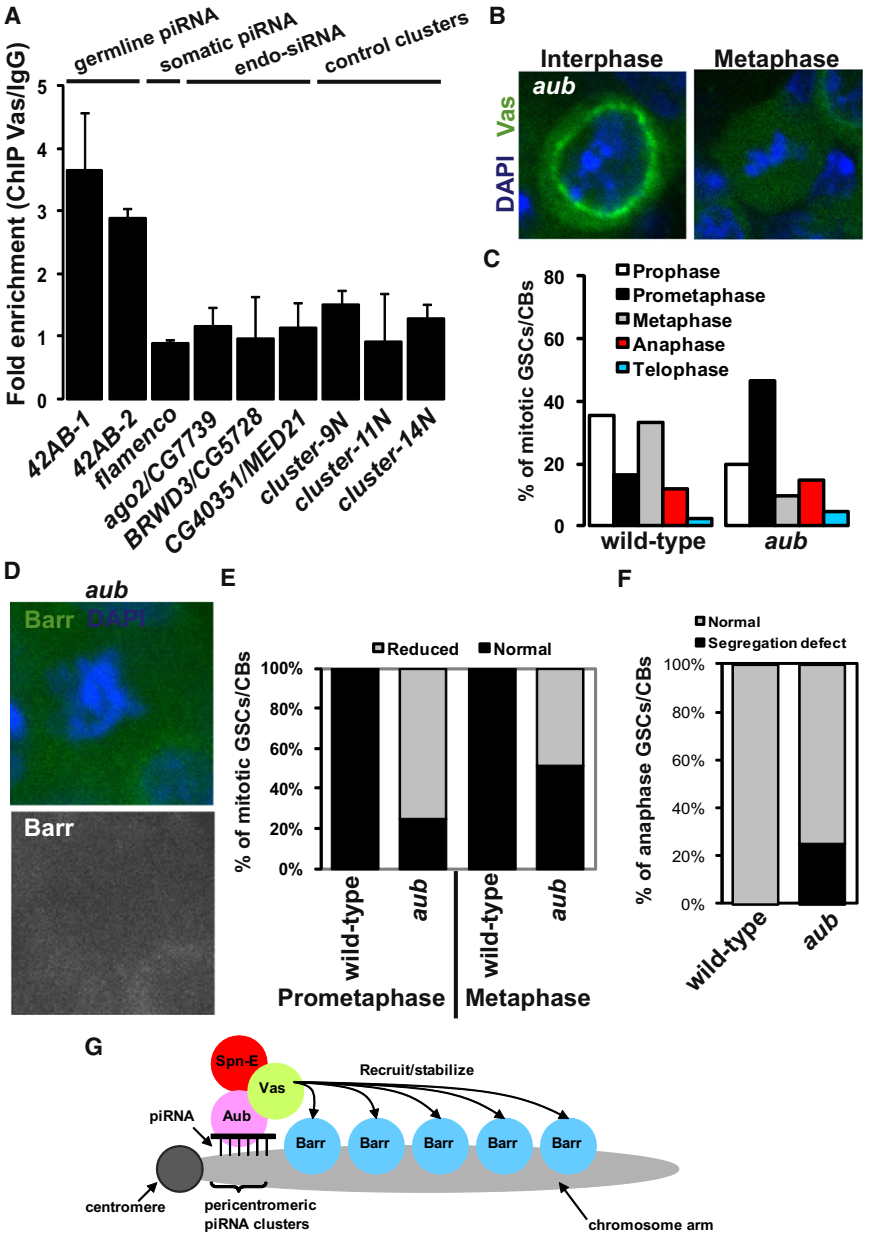


Figure 4. The piRNA Pathway Component *aub* Mediates Vas Localization to Mitotic Chromosomes and Chromosome Condensation

(A) Chromatin immunoprecipitation (ChIP) assay in GSC/CB-enriched *bam* mutant ovaries showing enrichment of germline piRNA loci in Vas immunoprecipitates normalized to IgG controls (n = 3 independent ChIP experiments). Error bars represent standard deviation.

(B) *aub* mutant GSCs and CBs stained for Vas (green) and DAPI (blue). Vas localization is disrupted during metaphase, but not interphase.

(C) The percentage of GSCs and CBs from the indicated genotypes at various stages of mitosis (n > 20 for each genotype). Mutation in *aub* results in a delay in prometaphase.

(D) *aub* mutant GSCs and CBs in delayed prometaphase stained for Barr (green) and DAPI (blue). Chromosomal localization of Barr is disrupted in *aub* mutants.

(E) The percentage of GSCs and CBs with normal or reduced chromosomal localization of Barr as depicted in (D) (n > 32 for each genotype).

(F) The percentage of anaphase GSCs and CBs with normal or defective segregation (n = 20 for each genotype).

(G) Hypothetical model of how Vas, Aub, and Spn-E can function during mitosis to facilitate robust Barr chromosomal localization. Vas may promote local recruitment of Barr near pericentromeric regions. Alternatively, Vas could also promote long-range association of Barr with chromosomes.

See also Figure S4.

NaCl, NaF, 0.05% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate), with each buffer supplemented with protease inhibitor cocktail (Roche). The antibodies used were guinea pig anti-Vasa [41], rabbit anti-GFP (Abcam), mouse anti-GFP (Invitrogen), and mouse anti-FLAG (Sigma). Input corresponded to 0.1%–1.0% of lysates. For RNase treatment, supernatants were incubated with antibody-conjugated beads overnight at 4°C in the presence or absence of RNase A (50 μg/ml).

Supplemental Information

Supplemental Information includes four figures and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.cub.2010.11.051.

Immunostaining

Immunostaining of ovaries was performed as described previously [40]. Ovaries were fixed in fixation solution (one part 16% paraformaldehyde and two parts Grace's medium) for 5 min, rinsed and washed with PBST (phosphate-buffered saline containing 0.2% Triton X-100) twice for 10 min each, and preabsorbed for 30 min in PBST with 5% normal goat serum. Ovaries were incubated with primary antibodies overnight at room temperature and then washed three times for 20 min each with PBST before incubation with secondary antibodies for 4 hr at room temperature. Ovaries were again washed three times for 20 min each with PBST. Images were taken with a Carl Zeiss LSM 5 Exciter Upright microscope and processed using Adobe Photoshop. Quantification of signal intensity was performed using ImageJ software.

CoIP

CoIP was performed as described previously [41] with minor modifications. Lysates were preabsorbed with protein A/G beads (Calbiochem) before incubation with antibody-conjugated beads overnight. Immunoprecipitates were washed three times, and proteins were eluted from the beads at 95°C in SDS buffer. CoIP was performed with IP buffer for ovaries (150 mM NaCl, 50 mM Tris [pH 8.0], 0.1% NP40) and cultured cells (50 mM Tris [pH 8.0], 100 mM

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