**Research Article**

**vasa is expressed in somatic cells of the embryonic gonad in a sex-specific manner in *Drosophila melanogaster***

**Andrew D. Renault**

Max Planck Institute for Developmental Biology, Spemannstrasse 35, 72076 Tübingen, Germany

Author for correspondence (andrew.renault@tuebingen.mpg.de)

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**Summary**

Vasa is a DEAD box helicase expressed in the *Drosophila* germline at all stages of development. *vasa* homologs are found widely in animals and *vasa* has become the gene of choice in identifying germ cells. I now show that *Drosophila* *vasa* expression is not restricted to the germline but is also expressed in a somatic lineage, the embryonic somatic gonadal precursor cells. This expression is sexually dimorphic, being maintained specifically in males, and is regulated post-transcriptionally. Although somatic Vasa expression is not required for gonad coalescence, these data support the notion that Vasa is not solely a germline factor.

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Key words: *vasa*, *Drosophila*, Germline, Somatic gonadal precursor, Germ cell

**Introduction**

Vasa is the founder member of the class of DEAD box proteins (Hay et al., 1988a; Lasko and Ashburner, 1988) and was originally discovered as a maternal effect gene required for the formation of germ cells and abdominal segments and in *Drosophila* (Schüpbach and Wieschaus, 1986).

Vasa protein is expressed throughout the life cycle of *Drosophila* germ cells and is frequently cited as being germline specific. Germ cells form early in development at the posterior pole of the embryo where Vasa is maternally provided. At stage 10 the germ cells migrate from the posterior midgut pocket to mesodermally derived clusters of somatic gonadal precursor cells (SGPs) which are specified bilaterally (reviewed by Richardson and Lehmann, 2010). During this migration the germ cells become transcriptionally active and switch on zygotic *vasa* transcription (Van Doren et al., 1998). The germ cells associate with the SGPs and coalesce to form two compact rounded embryonic gonads, one on each side of the embryo. In males, the embryonic gonad is already oriented along its antero-posterior axis, the anterior cells somatic become hub cells (the germline stem cell niche) and a group of male-specific SGPs (msSGPs) occupy the posterior (DeFalco et al., 2003). During larval, pupal and adult stages germ cells continue to express Vasa.

Flies mutant for *vasa* illustrate how Vasa is required at several stages of development. Firstly, Vasa is required during oogenesis. Females homozygous for *vasa* null alleles are viable but sterile due to a number of defects during oogenesis including defects in proper encapsulation of the oocyte by the follicular epithelium, positioning of the oocyte within the egg chamber, and integrity of the oocyte nucleus (Styhler et al., 1998). In contrast, *vasa* null males are viable and fertile (Lasko and Ashburner, 1990).

Secondly, Vasa is required for germ cell formation and embryonic patterning. Germ cell formation is dependent on pole plasm, a specialised yolk-free cytoplasm containing electron rich structures called polar granules. Vasa accumulates at the posterior pole of developing oocytes and is a component of pole plasm (Hay et al., 1988a; Hay et al., 1988b), and is required for polar granule assembly (Schüpbach and Wieschaus, 1986). Females homozygous for weak alleles of *vasa*, which are sufficient to allow progression through oogenesis, lay embryos which fail to form germ cells (Hay et al., 1988a) and lack posterior segments.

Vasa contains a DEAD motif and DEAD-box family members are generally considered RNA helicases and Vasa shows RNA-binding and helicase activity *in vitro* (Liang et al., 1994). During oogenesis Vasa regulates translational initiation of germline mRNAs including *gurken* (Styhler et al., 1998; Tomancak et al., 1998) and *mei-P26* (Liu et al., 2009) via an interaction with eukaryotic initiation factor 5B (eIF5B) (Carrera et al., 2000; Johnstone and Lasko, 2004). In addition, Vasa has a translation-independent function in regulating mitotic chromosome condensation in the female germline stem cells (Pek and Kai, 2011) and is required for Piwi-interacting RNA (piRNA) mediated transposable element silencing (Vagin et al., 2004; Lim and Kai, 2007; Malone et al., 2009).

*vasa* homologs are present throughout the animal kingdom and are considered excellent makers to study germ cell formation and germline development (reviewed by Raz, 2000). However, a number of recent studies have observed *vasa* message or protein in somatic cell lineages of other organisms, including the sea urchin *Strongylocentrotus purpuratus* (Yajima and Wessel, 2011), the polychaete *Platynereis dumerilii* (Rebscher et al., 2007), the
annelid *Tubifex tubifex* (Oyama and Shimizu, 2007), the planarian *Dugesia japonica* (Shibata et al., 1999), the flatworm *Macrostomum lignano* (Pfister et al., 2008) the cnidophore *Pleurobrachia pileus* (Aliè et al., 2011), the cephalochordate *Branchiostoma floridae* (Wu et al., 2011), and the cnidarian *Hydractinia echinata* (Rebscher et al., 2008) leading to the broader concept of Vasa being required for both germline and somatic multipotent or stem cell function (Gustafson and Wessel, 2010).

During our lab’s work on germ cell migration in *Drosophila* I noticed that some non-germline embryonic cells were positive for Vasa expression so I investigated whether *vasa* is truly germline specific in *Drosophila*.

**Results**

*vasa* is expressed outside of the *Drosophila* germline

Firstly I examined the expression of *vasa* RNA in wild-type *Drosophila* embryos. As previously reported (Hay et al., 1988a; Lasko and Ashburner, 1988), there is strong uniform maternal expression in the syncytial embryo (Fig. 1A) which is degraded upon cellularization in both the somatic cells and germ cells (Fig. 1B). Zygotic expression is detectable in the germ cells by stage 11 (Fig. 1C) and RNA is strongly expressed in the region of the embryonic gonads from stage 13 onwards (Fig. 1D,F).

The embryonic gonad is composed of two cell types, the germ cells and the SGP s, which are closely intermingled making it difficult to assess in which cells *vasa* is expressed. Therefore I made use of an *osk* mutant allelic combination (*osk^{301/C24}* in which females lay embryos with normal embryonic patterning but no germ cells are formed (Lehmann and Nüsslein-Volhard, 1986). Surprisingly, in such embryos strong *vasa* RNA expression was still observed in the region of the embryonic gonads at stage 14 (Fig. 1H) which became restricted to a smaller number of cells by stage 16 (Fig. 1J). Thus *vasa* is expressed by somatic cells. At the protein level, *Vasa* positive cells are present in the region of the embryonic gonads in embryos from *osk* mutant mothers (Fig. 1I), although the staining intensity is lower than in wild-type embryos which appear darker due to the presence of germ cells (Fig. 1E).

To confirm that the somatic *vasa* expression that occurs in embryos that lack germ cells is not specific to *osk* mutants, I examined *vasa* expression in embryos that have very few germ cells due to germ cell death. *wunen2* (*wun2*) encodes a lipid phosphate phosphatase that when over-expressed in somatic cells results in germ cell death, with only a few germ cells remaining by stage 13 (Starz-Gaiano et al., 2001). In such embryos, *vasa* expression was clearly evident in the region of the embryonic gonads (Fig. 1L), the extent of which was too large to be accounted for by a few remaining germ cells. These embryos frequently showed gonads with a large germ cell with strong Vasa staining surrounded by smaller somatic cells with weaker Vasa staining (Fig. 1K, insert). I conclude that somatic cells normally express *vasa* RNA and protein and that this occurs independently of the presence of germ cells.

To verify whether germ cells normally express *vasa* at late embryonic stages I examined *wun wun2* M–Z– mutant embryos in which the germ cells fail to migrate and remain inside of the gut (Renault et al., 2010) (Fig. 1M). At stage 16 in such embryos *vasa* RNA positive clusters of cells were observed inside the midgut (Fig. 1N) confirming that germ cells switch on *vasa* expression and this occurs independently of initiating their migration.

Overall I conclude that *vasa* RNA is highly expressed in both the germ and somatic cells of the embryonic gonads and that *Vasa* protein is highly expressed in the germ cells and is weakly expressed in surrounding somatic cells. The close association of the germ and somatic cells and the difference in protein levels explains why Vasa expression was not previously reported in somatic cells.

*vasa* is expressed in the somatic gonadal precursor cells

To verify that the somatic Vasa expression observed in Fig. 1 is not a consequence of the detection method or antibody used, I examined embryos stained fluorescently with an independent anti-Vasa antibody and co-stained with an antibody against Abdominal B (AbdB), a homeotic gene that is expressed in msSGPs (DeFalco et al., 2008). In male embryos, Vasa is present...
in both the germ cells and also in smaller AbdB positive cells (Fig. 2B) at the posterior of the gonad (Fig. 2A) although with weaker intensity.

To verify this somatic expression results from the vasa locus I examined if this expression was present in male embryos zygotically null for vasa (vas$^{BC69}$/Df(2L)osp29). Although the germ cells remain positive for Vasa due to pedurance of the maternal provision the somatic expression was never observed in male vasa zygotic null embryos (Fig. 2D).

Based on their location and co-expression of AbdB the somatic vasa positive cells are SGPs and in male embryos are the msSGPs. To ascertain if Vasa is required in SGPs, their behaviour in vasa zygotic null embryos was examined using antibodies against AbdB and against the general SGP marker Eyes absent (Eya) also known as Clift (Boyle et al., 1997). SGPs are present and coalesce with germ cells in vasa zygotic null embryos (Fig. 2B,E,H,K) indicating that SGP coalescence behaviour is independent of Vasa. In addition the expression of these makers was not altered in the mutant embryos indicating that vasa null SGPs maintain appropriate marker gene expression.

To test whether the differences in Vasa expression levels between germ cells and somatic cells reflects differences in transcription I made use of an enhancer trap in the vasa locus, vas$^{BC69}$. In this line, a nuclear lacZ containing P-element is inserted into the first exon of vasa and anti-LacZ staining recapitulates endogenous vasa expression (Sano et al., 2001). To avoid perdurance of LacZ from maternal expression from the vasa locus I stained embryos from wild-type females mated to vasa$^{BC69}$ males. As expected LacZ positive germ cells were observed, but LacZ positive cells were also seen intermingled around the germ cells (Fig. 2M–O). Thus zygotic transcription from the vasa promoter and subsequent translation occurs both in germ cells and SGPs during embryogenesis. The LacZ staining intensity in germ cells and SGPs was comparable whilst the staining intensity of Vasa was much lower in the SGPs (Fig. 2A,N, quantified in Fig. 2P). The intensity of vasa RNA staining between germ cells and somatic cells was also similar (Fig. 1N). These data indicate that the difference in Vasa protein levels between these cell types is not due to differences in vasa transcription but likely reflect differences in translation or protein stability.

**vasa expression shows sex-specific differences**

Not all embryos from osk mutant mothers expressed vasa RNA at late embryonic stages (data not shown). To test if this heterogeneity in detection resulted from sex-specific differences in vasa expression I mated osk mutant females to males carrying a LacZ expressing transgene on the X chromosome to differentiate between female (XX) and male (XY) embryos, which would be positive and negative for LacZ expression respectively.

At stage 13 in both female and male embryos vasa expression was equivalent (Fig. 3A,B). However, whilst this expression decreased and eventually became undetectable in female embryos (Fig. 3C,E), in male embryos this expression was maintained and became restricted to the posterior of the gonad (Fig. 3D,F) the location of the msSGPs.

**vasa is required only in the female germ line**

Given the expression of Vasa in embryonic msSGPs I tested whether the male gonad was affected by the loss of Vasa in larvae and adults. Male 3rd instar larval gonads appeared normal in size in vasa null animals (Fig. 4). The msSGPs give rise to the terminal epithelium, which in larval stages appears as a cluster of Eya positive cells at one end of the gonad (Nanda et al., 2009). In wild-type, Vasa is no longer detectable in the terminal epithelium (Fig. 4E–H) indicating that Vasa is not essential for survival of these cells.
Fig. 3. Sex specific somatic expression of vasa RNA. Lateral views of embryos laid by osk^{EX}/osk^{M1} females (which therefore lack germ cells) mated to males carrying a Df(2L)osp29 transgene on the X chromosome stained for vasa RNA (blue) and LacZ protein (brown). The XX female embryos will therefore be positive for LacZ (A,C,E) whereas the XY male embryos will be LacZ negative (B,D,F). At stage 13 vasa RNA is present in somatic cells in both males and female embryos (A,B), and becomes enriched at the posterior of the gonad in stage 15 (D) and stage 16 (F) males but decreases in stage 15 (C) and is not detected in stage 16 (E) females.

I examined the testis of vasa null adults. I found that such testis were phenotypically normal (data not shown) in agreement with these flies being fertile. I conclude, that as previously reported, vasa is not essential in the adult testis (Lasko and Ashburner, 1990).

Vasa null females lay only very few eggs (Fig. 5A) which all display a ventralised phenotype as measured by the absence of (Fig. 5C, middle egg) or defects in the number or length of the dorsal appendages (Fig. 5C, upper and lower eggs, respectively). To examine whether vasa expression in the soma is required during oogenesis I tested whether germline specific expression of vasa using the nanos-Gal4VP16 driver was sufficient to rescue the number and patterning defects of eggs from vasa null females. I found that germline vasa expression was able to increase the egg laying rates of vasa null females but not to wild-type levels (Fig. 5A). The eggs laid by the rescued females were no longer ventralised but appeared wild-type (Fig. 5D) and indeed many hatched (data not shown). The lack of eggs laid by vasa null females is due to a large amount of degenerating ovarioles compared to wild-type (Fig. 5E,F). The ovaries of the rescued females on the other hand appeared mostly wild-type with only the occasional degenerating ovariole (Fig. 5G). I conclude that vasa is only essential in the female germline.

Fig. 4. Larval gonads of vasa null males appear wild-type. 3rd instar larval gonads from a sibling control (A–D) or vasa null males (E–H) showing DAPI stained nuclei (A,E), Vasa (B,F) and Eya (C,G) and merged image (D,H). Arrows indicate terminal epithelium precursors. Scale bar = 25 μm.

Discussion

The vasa gene is often cited as the most conserved germline marker in animals. The germline restricted vasa expression pattern, first demonstrated in Drosophila, has been instrumental for identifying germ cells in many animal species. In zebrafish (Braat et al., 2000; Knaut et al., 2000), the crustacean Parhyale hawaiensis (Özhan-Kizil et al., 2009), and mouse (Fujiwara et al., 1994) vasa expression is indeed germline specific but increasingly it is being recognized that, for other species, vasa is also expressed in somatic cell types.

In this paper I report that vasa expression in Drosophila melanogaster, both at the RNA and protein level, is not restricted to the germline but is also expressed in a somatic lineage, the embryonic SGPs. This embryonic expression at the RNA level occurs transiently in female embryos but is maintained in the posterior somatic gonadal precursor cells in male embryos. These posterior cells are the msSGPs which give rise to the terminal epithelium.

The situation in late Drosophila embryogenesis in which vasa positive germ cells are surrounded by vasa positive somatic cells is highly reminiscent of the situation in a number of other species. In amphioxus, presumptive germ cells are specified by
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vasa domains are proliferative zones and the urges caution when using vasa animal it was first discovered in, strengthens this argument and Drosophila tempting to speculate that Vasa may also support proliferation of during embryogenesis is not essential for establishment or genes including Drosophila DEAD-box RNA helicases of which there are several in the X chromosome (used to sex embryos) and made according to Renault et al. (Renault et al., 2010). A courtesy of Ruth Lehmann, New York, USA; wun wun2 (1:40, Developmental Studies Hybridoma Bank, Iowa City, USA), mouse anti-lacZ (courtesy of Renault et al., 2012). In both cases the vasa positive somatic domains are proliferative zones and the vasa positive cells are likely somatic stem cells.

What is the function of somatic vasa expression in Drosophila? In the embryo, gonad coalescence is wild-type in vasa null animals (Fig. 2A–L). Although it cannot be ruled out that there are subtle changes in vasa null SGPs, in expression profile for example, these do not affect embryonic SGP behaviour. The terminal epithelium is present in the gonads of vasa null male larvae (Fig. 4) indicating that vasa expression during embryogenesis is not essential for establishment or maintenance of this tissue. Given that Vasa is expressed by proliferating lineages in a number of different species, it is tempting to speculate that Vasa may also support proliferation of Drosophila SGPs. If this is the case, the lack of a discernible phenotype would mean that Vasa acts redundantly with other DEAD-box RNA helicases of which there are several in Drosophila.

Germline establishment and maintenance has traditionally been thought to require a conserved set of germline specific genes including vasa, nanos and pumilio. Recent studies indicating a broader expression of these genes in many organisms, in cells with stem cell properties, have argued their role maybe more multipotent than purely germline (Gustafson and Wessel, 2010). The finding that another member of this conserved set of genes is not germline specific, even in the animal it was first discovered in, strengthens this argument and urges caution when using vasa expression alone to identify germ cells in non-model organisms.

Materials and Methods

Fly stocks

The following Drosophila alleles were used: vasa\textsuperscript{Cre\textsuperscript{186}} is a vasa null allele with a 7.3 kb deletion removing the entire vasa coding region (Styhler et al., 1998); Df(2L)osp29 is a deficiency uncovering vasa (courtesy of the Bloomington Stock Center); vasa\textsuperscript{Cre\textsuperscript{186}} contains a P-element enhancer-trap containing a nuclear lacZ transgene inserted the first exon of vasa (Sano et al., 2001) and was courtesy of Stephanie Ronsseray, Institut Jacques Monod, Paris, France; UASp vasa was courtesy of Akira Nakamura, RIKEN Center for Developmental Biology, Kobe, Japan (Sengoku et al., 2006); osk\textsuperscript{Cre} is was courtesy of Anne Ephrussi, EMBL, Heidelberg, Germany; osk\textsuperscript{Cre} is a point mutation, courtesy of the Tibingen stock collection, Tübingen, Germany; nanos-Gal4VP16 and UASwun2myc were courtesy of Ruth Lehmann, New York, USA; wun wun2 M–Z– embryos were made according to Renault et al. (Renault et al., 2010). A df(1)2-lacZ insertion on the X chromosome (used to sex embryos) and fat facets\textsuperscript{2} were preference antibodies which provides LacZ maternally and localized to the posterior pole where germ cells are formed (used to label germ cells) (Fischer-Vize et al., 1992) were courtesy of the Bloomington Stock Center.

Antibody stainings and in situ hybridisation

Embryos were laid at room temperature (except from osk\textsuperscript{Cre\textsuperscript{186}} females which were kept at 18°C) and fixed in 4% formaldehyde, dehydrinulated in methanol. Larvae were dissected in Ringers solution and fixed in 4% formaldehyde. For stainings the following antibodies were used: rabbit anti-Vasa (used for all anti-Vasa stainings except Fig. 2A–F, 1:10,000, a gift from Anne Williamson and Helene Zinszner, Lehmann Lab, New York, USA), rat anti-Vasa (1:40, Developmental Studies Hybridoma Bank, Iowa City, USA), mouse anti-lacZ (1:1000, Promega, Mannheim, Germany), rabbit anti-lacZ (1:10,000, Cappel, MP Biomedicals, Solon, USA), mouse anti-Eya (1:12, 10H6, Developmental Studies Hybridoma Bank), mouse anti-AbdB (1:10, 1AE2, Developmental Studies Hybridoma Bank), anti-rabbit biotin, Cy3 and Cy5 (1:500, Jackson Immunoresearch, Newmarket, Suffolk, UK) and Alexa-488 (1:500, Invitrogen, Life Technologies GmbH, Darmstadt, Germany) coupled secondaries. A VECTASTAIN\textsuperscript{ABC} kit (Vector Labs, Burlingame, USA) followed by diamino-benzidine was used to detect biotinylated secondary antibody. Embryos were either mounted in Epon resin and viewed on a Zeiss Axiosmager or mounted in Aqua-poly-mont (Polysciences) and viewed on an Olympus FV-1000.

Relative levels of Vasa and LacZ in the gonads of vasa\textsuperscript{Cre\textsuperscript{186}} embryos were determined using ImageJ software by measuring the mean fluorescence intensity of Vasa in the cytoplasm (using the rabbit anti-Vasa antibody) and LacZ (using the rabbit anti-LacZ antibody) and DAPI in the nucleus of several germ cells and SGPs in a single gonad. The result for one representative gonad out of a total of 4 quantified is shown in Fig. 2P.

A DIG-labeled vasa RNA antisense probe was synthesized with T7 RNA polymerase using the DIG labeling system (Roche) from a 2.3 kb full length sequence verified vasa cDNA clone (corresponding to transcript vasa-R in Flybase) in pNB40 (courtesy of Ruth Lehmann, New York, USA) linearized with Ava. The probe was chopped by carbododehydrolysis, the embryos were fixed as above and in situ hybridisation carried out according to Lehmann and Tautz (Lehmann and Tautz, 1994). For a double antibody and in situ hybridisation staining, the antibody staining was performed first followed by the in situ hybridisation in a protocol adapted from Manoukian and Krause (Manoukian and Krause, 1992).

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Competing Interests

The author has no competing interests to declare.

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