Minireview

The function and regulation of *vasa*-like genes in germ-cell development

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Abstract

The *vasa* gene, essential for germ-cell development, was originally identified in *Drosophila*, and has since been found in other invertebrates and vertebrates. Analysis of these *vasa* homologs has revealed a highly conserved role for Vasa protein among different organisms, as well as some important differences in its regulation.

Germ-cell development in vertebrates and invertebrates

In sexually reproducing organisms, primordial germ cells (PGCs) give rise to gametes that are responsible for the development of a new organism in the next generation. These cells must remain totipotent - able to differentiate into each and every cell type of all the different organs. In many organisms, maintenance of totipotency is achieved by the specification of germ cells early in embryogenesis: a small group of cells is set aside to follow a unique pathway of differentiation into gametes (reviewed in [1-3]).

Information on the specification of PGCs has been gained from detailed microscopical analysis, embryological experiments (for example transplantation of cells or cytoplasm) and gene identification through genetic screens for maternal-effect mutations in *Drosophila* and *Caenorhabditis elegans* (reviewed in [1-4]). The main conclusion from these two invertebrate model organisms is that asymmetrical localization of cytoplasmic determinants - the germ plasm - is responsible for the early specification of the germline lineage. The importance of localized cytoplasmic determinants for germ-cell development has been most clearly shown in *Drosophila*. Here, for example, cytoplasmic germ plasm determinants concentrated at the posterior pole of the embryo in 'pole plasm' can direct cells towards a germ-cell fate when transplanted to an ectopic location. In mutants in which the formation of this morphologically characteristic cytoplasm is disrupted, germ-cell formation is impaired (reviewed in [5]). The pole plasm is characterized by the presence of the polar granules, electron-dense structures not delimited by a membrane that contain many RNAs and proteins and that are associated with mitochondria. The distribution of the pole plasm correlates with the site of PGC formation. On the basis of their unique morphology, germ plasm components have also been identified in other organisms such as *C. elegans* (where they are termed P granules), *Xenopus laevis* (germinal granules), chick and zebrafish [1-3,6-9].

The specification of the germline is different in mammals. Several lines of evidence argue that here inherited cytoplasmic factors do not have a role in germ-cell specification, but that germ cells are induced through cellular interactions during gastrulation (reviewed in [1-3]). Morphologically distinct germ plasm has not so far been identified in early mammalian embryos. In addition, mouse eggs from which the animal or vegetal pole was experimentally removed developed into fertile mice, arguing against any polarity in the egg that is functionally important for germ-cell specification [10]. Mouse germ cells arise just before and during early gastrulation, around the proximal part of the epiblast adjacent to the extra-embryonic region. Transplantation experiments

Table 1

Expression and function of *vasa* homologs*

Species	Gene	RNA expression	RNA localization	Protein expression	Protein localization	Function	References
Fruit fly (*Drosophila melanogaster*)	*vasa*	Early embryo: uniform, followed by specific expression in germ cells. Ovary: uniform in nurse cells and oocyte. Testis: early stage spermatocytes and germline stem cells.	No	Germ cells: as soon as they form and throughout embryonic development. Ovary: germline stem cells, nurse cells, oocytes. Testis: early stages of spermatogenesis.	Oocyte and cleavage-stage embryos: in polar granules, nuclear bodies and dense cytoplasmic masses.	Early embryogenesis: abdomen development, *nanos* mRNA translation, formation of pole plasm and germ cells. Oogenesis: germline cyst development, oocyte differentiation, *gurken* mRNA translation, oocyte polarity, translation of *oskar* mRNA.	[13,14,16, 17,40,41]
Mouse *(Mus musculus)*	*Mvh*	Testis: spermatocytes and early spermatids. Ovary: ND	No	Germ cells: as they arrive at the genital ridge. Ovary: early stages of oogenesis. Decreases during maturation. Not detected in mature oocytes. Testis: in spermatogonium to round spermatid stages.	Granules near nucleus in pachytene-diplotene spermatocytes. After meiosis: a large perinuclear granule up to spermatid nucleus elongation. Sperm: corresponds to that of the 'chromatoid body'.	Testis: required in PGCs during premeiotic stages of spermatogenesis for sperm differentiation before pachytene spermatocyte stage. Male mutants: PGC proliferation defects. Female mutants: fertile and show no defects of oogenesis.	[19, 20,38]
Zebrafish *(Danio rerio)*	*vasa*	Maternally supplied to embryo. From the 32-cell stage, detected in four blastomeres. Gastrulation: expressed in four PGC clusters as they migrate towards the gonad. Testis: spermatogenesis, excluding mature spermatozoa. Ovary: all stages of oogenesis.	One-cell embryo: ring between yolk and cytoplasm. Two- to four-cell embryo: near cleavage furrow, inherited by four cells. Then asymmetrically localized and distributed between dividing blastomeres until late blastula, when it fills PGCs' cytoplasm. Late oogenesis: oocyte cortex.	Maternal protein uniformly distributed during blastula stages. At late blastula, distinct expression in PGCs as expression in somatic cells decreases.	Associated with germinal vesicle in early oogenesis. From late blastula on, associated with nuclear envelope in a pattern distinct from that of the RNA	ND	[6,9,23,31]
Planarian (*Dugesia japonica*)	*DjvlgA* *DjvlgB*	Testis: *DjvlgA* in spermatogonia, spermatocytes, spermatids. *DjvlgB* only in spermatocytes. Ovary: both expressed in oocytes. Outside gonad *DjvlgA* expressed in totipotent neoblasts.	No	ND	ND	ND	[26]
Frog *(Xenopus laevis)*	*XVLG1*	Gonad in both sexes. Ovary: all stages of oogenesis. Gastrulation: PGC-specific expression.	No	Oocytes and unfertilized eggs. Blastula and gastrula: all cells, intensity of staining decreasing with time. After gastrulation: level increases in PGCs.	Embryos: appears to be perinuclear.	Survival or differentiation of PGCs.	[22,42-44]
Chick *(Gallus gallus)*	*Cvh*	Testis: spermatogonia to mature spermatocytes. Ovary: ND	ND	Embryos: maternal protein from the one-cell stage. Before gastrulation: *Cvh*-positive PGCs in center of area pellucida. Migrating and post-migratory PGCs. Testis: spermatogonia to round spermatids. Ovary: immature oocytes.	Cleaving embryos: basal part of cleavage furrows.Testis: granular staining in spermatocytes. Ovary: underneath plasma membrane, near germ-plasm-associated organelles and molecules such as mitochondria and spectrin.	ND	[8]

Table 1 (continued)

Species	Gene	RNA expression	RNA localization	Protein expression	Protein localization	Function	References
Ascidian (*Ciona intestinalis*)	*Ci-DEAD1*	Embryo: mesenchymal cells and posterior cells presumed to be germ cells. Ovary: early oogonic cells. Testis: early spermatogenic cells.	Early embryos: posterior during two- to four-cell stage, inherited by presumed germ cells.	ND	ND	ND	[27]
Nematode (*Caenorhabditis elegans*)	*glh-1* *glh-2* *glh-3* *glh-4*	*glh-1*, *glh-2*, *glh-3* and *glh-4* expressed in all cells of early cleavage stages followed by a decrease to background levels. All expressed in germline cells in male and hermaphrodite.	No	All expressed throughout life, first in germline blastomeres and later in germ cells in gonad	All localized to P-granules that segregate to germline blastomeres. Granules are cytoplasmic in oocyte and early embryo and perinuclear at later stages.	*Glh-1 glh-2* and *glh-4* products are required for germ-cell proliferation and gametogenesis. Oogenesis and spermatogenesis defective in *glh-1/4*(RNAi), with many cells not proceeding beyond pachytene.	[28,45]
Human (*Homo sapiens*)	*VASA*	In the fetus and adult, gonad-specific expression in both sexes.	ND	Germ cells: expressed in PGCs as they populate the gonadal ridge as well as in PGCs that have not yet reached this target. Testis: spermatogonia, spermatocysts and spermatids. Ovary: oocytes.	Testis: granular staining in spermatocytes. Ovary: in fetal oocytes: within a compact perinuclear body. In adult oocytes no subcellular localization.	ND: hereditary infertility syndromes that map to the chromosomal region of *VASA* have not yet been found.	[30]

*Not all the known *vasa* homologs are listed in the table. Homologs for which little functional information is available have been omitted unless they shed light on the function of the gene in a certain class of organism. ND, not determined.

showed that, at these stages, these cells are not yet committed to the germline, and when grafted into distal positions they can develop into somatic tissues [1,2]. Consistent with the notion of germ-cell induction through cell-cell interactions, distal cells that would develop as somatic cells can develop into germ cells when grafted into the region where germ cells normally form [1,2]. Indeed, formation of the founding population of PGCs in the mouse was shown to depend on the function of at least one extracellular factor - bone morphogentic protein 4 (Bmp4) [3].

Some of the methodologies used in the invertebrate models cannot be applied to vertebrates. In particular, the maternal-effect screens that were instrumental in analyzing the specification of PGCs in invertebrates are not practicable in the frog, chick or mouse. Fortunately, such screens can be carried out in the zebrafish [11], although in this system too, it would be very difficult to achieve saturation for all maternal-effect mutations involved in PGC specification using classical 'forward' genetic analysis.

Homologs of *vasa* in invertebrates and vertebrates

The *vasa* gene was originally identified in *Drosophila* as a maternal-effect gene required for the formation of the abdominal segments and for germ-cell specification [12]. The Vasa protein can be detected in the germline cells of *Drosophila* throughout their development and in early embryos it is specifically localized to polar granules, which are located where the germ cells are specified. *Drosophila* embryos that inherit mutant maternal *vasa* RNA and protein fail to form germ cells, and females carrying null mutations in *vasa* display a range of defects in oogenesis. The *vasa* gene encodes an ATP-dependent RNA helicase of the DEAD-box family and is required for promoting translation of at least two known mRNAs, *nanos* and *gurken* [13-19].

Following the isolation of the *Drosophila vasa* gene, *vasa*-like DEAD-box RNA helicase genes that are expressed in germ cells were identified in many species, including mouse, rat, frog, zebrafish, medaka (*Oryzias latipes*), trout, planarian, chick, ascidian, nematode, silkworm, human and the flour beetle (Table 1; [8,20-30], and R. Schröder and D. Tautz, personal communication). The distribution of *vasa* RNA or protein was determined during different stages of development, thus providing information on the possible function of *vasa* during germ-cell development in these species. The *vasa* loss-of-function phenotype in the fly, the mouse and the nematode provided direct evidence for the role of *vasa* in the development of germ cells in these organisms. And in cases where the origin and precise route of germ-cell migration towards the gonad were unknown (for example in fish, chick and ascidians), it proved possible, using *vasa* as a molecular marker, to trace back the migration path and establish the position in which these cells originate [8,23,27,31].

Regulation of expression and subcellular localization of *vasa*-like gene products

As can be seen from Table 1, *vasa* RNA is expressed in the germ cells of many organisms. But translational control, post-translational control and subcellular localization of the Vasa protein, as well as interaction with other proteins, appear to play a major part in controlling Vasa function. For example, *vasa* RNA is uniformly distributed in early *Drosophila* embryos but, consistent with its function, the protein is found localized to the posterior pole, where it is associated with the polar granules in the germ plasm. Similar discrepancies between RNA and protein expression, in which the Vasa protein is found in a restricted number of cells relative to the RNA and is localized to specific subcellular structures, have also been described in *Xenopus* and the nematode.

A recent thorough analysis of the distribution of the *vasa* gene products in zebrafish revealed a unique and surprising difference between the localization of RNA and that of the protein [9]. The RNA is localized through a microtubule-dependent process in a novel pattern to the first and second cleavage planes of the early zebrafish embryo [23,32]. Knaut *et al*. [9] also showed that the *vasa* RNA aggregates reside within an electron-dense matrix similar to structures associated with germ plasm in other organisms. The localization of *vasa* RNA to the germ plasm allowed them to follow precisely the distribution of the germ plasm to the cells of the early embryo [9]. Until late blastula stages, the four cells that contain germ plasm divide asymmetrically, so that only one of the blastomeres resulting from each division inherits the germ plasm labeled by *vasa* RNA; these will become the future PGCs. Towards the end of the blastula stage, the RNA fills the cytoplasm of the *vasa*-positive cells and is inherited by both daughter cells after cell division. The distribution of the Vasa protein at these early stages of development differed from that of the RNA in a striking and unexpected manner. When the *vasa* RNA is asymmetrically segregating to the future PGCs, the Vasa protein is uniformly distributed in the cytoplasm of all blastomeres. This initial pattern of protein expression changes at late blastula stages, when stronger protein expression is detected in the germ cells, which now divide symmetrically. At this time, the distribution of the protein within the cells becomes perinuclear.

Given that Vasa protein is implicated in establishing functional germ plasm and in the specification of germ cells in other organisms, the findings in zebrafish seem paradoxical. In *Drosophila*, for example, loss-of-function mutations in *vasa* that affect the formation of the pole plasm were shown to affect either the biochemical activity of the protein or its localization [19]. In zebrafish, on the other hand, the *vasa* RNA, rather than the protein, is initially localized to the germ plasm. There are several possible explanations for this paradox. One is that in zebrafish it is the *vasa* RNA and not the protein that is important for the early determination of germ plasm. It is important to note, however, that in zebrafish, *vasa* RNA expressed ectopically during embryogenesis is unable by itself to alter the number or position of the PGCs [31]. Furthermore, cloning of the medaka *vasa* homolog and analysis of its expression pattern showed that in this fish, which like the zebrafish is also a teleost, the RNA is uniformly expressed until gastrulation, when germ-cell-specific expression is observed [24].

Another possible explanation of the paradox is that although most of the Vasa protein is uniformly distributed in all cells, it is active only in the germ cells, as a result of cell-specific post-translational modifications. Vasa activity can indeed be affected by post-translational modification, as described during oogenesis in *Drosophila* [33]. Finally, there could be a higher concentration of Vasa protein in germ cells as a result of translation of the maternally localized RNA. In this scenario, localization of *vasa* RNA, and presumably other RNAs, important for germ-cell determination, generates high levels of these proteins in some cells, thereby inducing the zygotic PGC differentiation path. An interesting recent discovery was recently made by Schröder and Tautz, who cloned a *vasa*-like gene from the 'short germ band' beetle, whose embryogenesis represents a more ancestral form of embryogenesis in insects. *Tribolium castaneum* (R. Schröder and D. Tautz, personal communication). They found that the distribution of *vasa* RNA in this insect is more reminiscent of that in zebrafish rather than the 'long germ band' *Drosophila*. After early uniform distribution of the RNA, the *Tribolium vasa* RNA appears to be located exclusively at the posterior of the early embryo, where the germ cells presumably form.

The function of *vasa* in germ-cell development

The function of the *vasa* gene can be inferred from its expression pattern in different organisms and from phenotypic analysis of animals lacking a functional gene. With the exception of the mouse (and probably other mammals), the *vasa* gene product is expressed in or localized to the PGCs very early in development, consistent with the idea that its activity is required for specification of this cell lineage. Interestingly, in planarians, where a *vasa* homolog is also expressed in the soma, the somatic cells that express the gene were identified as neoblasts - a totipotent cell type that functions in regeneration. The function of *vasa* could therefore be described as important for preserving totipotency. One mechanism for preserving totipotency is to inhibit expression of genes that would lead to somatic differentiation [34]. An indirect role for *vasa* in transcriptional inhibition is suggested by the finding that one of the few known targets of Vasa, *nanos,* can repress gene expression in the *Drosophila* germline [15,35,36].

In the mouse, where germ cells are induced through cellular interactions rather than by inheritance of maternal cytoplasmic determinants, the expression of *vasa* is initiated relatively late [20,37]. Vasa is expressed in the PGCs as they

arrive at the gonad, and expression is induced by interaction between the germ cells and the somatic cells of the developing gonad [37]. The expression of *vasa* at this stage in the mouse, as well as in all the other organisms described above, is likely to reflect a requirement for the gene product for differentiation of the germ cells into gametes. Indeed, loss of *vasa* function in the mouse affects differentiation of the male germ cells, resulting in male sterility (no other phenotype is observed in the knockout mice) [38]. Similarly, a late function of *vasa* during gametogenesis has been described in the nematode and the fly [16,17,28,33]. The first mechanistic evidence coupling progression in gamete differentiation and *vasa* function is the demonstration that a meiotic checkpoint during oogenesis in *Drosophila* appears to control the activity of the Vasa protein [33].

The search for new factors involved in germ-cell development

To gain a more comprehensive understanding of germline development in different species, one would obviously seek to identify most or all of the components relevant to the process and determine the functional relationships between them. Genome sequencing and the availability of expressed sequence tag (EST) libraries now allow us to identify homologs through database screens of different organisms. Isolation of new genes essential for germ-cell development is more demanding. Classical genetic analyses in invertebrates and in zebrafish are likely to identify new genes and proteins. This approach will fail, however, where there is functional redundancy among genes or when a zygotic requirement for the gene complicates the analysis of its function as a maternal factor. By using DNA microarray chips and tissue-specific probes, on the other hand, one could identify genes that are specifically expressed in the germ cells. The yeast two-hybrid system can be used to reveal new proteins that physically interact with previously identified gene products (see [19]). In addition, screening cDNA libraries by *in situ* hybridization can identify new genes even if they are expressed in other cell types as well as in germ cells. This approach has led to identification of many homologs of known genes in the zebrafish, as well as of new genes and proteins that are expressed in the germline (C. Thisse, B. Thisse and E.R., unpublished observations). Functional analysis of zebrafish genes isolated by such 'reverse' genetics approaches can be carried out using morpholino antisense oligonucleotides [39], which inhibit translation of their specific mRNAs in this organism (S. Ekker, personal communication).

The approaches described above are most useful in model organisms for which investment in genomic resources has provided the necessary tools. Nevertheless, other species such as planarian, flour beetle, silkworm and medaka display interesting parallels as well as important differences in the way the *vasa* gene is expressed and regulated. Continuing and expanding the work in different model systems is likely to contribute to our understanding of the molecular mechanisms of specification and differentiation of the germ cells across the animal kingdom.

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