Vasa Homolog Genes in Mammalian Germ Cell Development

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ABSTRACT. Many vasa homologue genes to Drosophila vasa have been isolated in various animal species. They provide specific molecular probes to analyze the establishment and the differentiation of germ cell lineage. In mammals, the expression of VASA protein becomes detectable in PGCs at the late migrating stage. Interestingly, during spermatogenesis the intracellular localization of VASA protein is closely associated with the chromatoid body.

Key words: primordial germ cell (PGC)/vasa/spermatogenesis/chromatoid body

VASA gene family

Germ cells play an important role as a carrier for the species-specific genomic information during the evolution of multicellular organisms. From this point of view, the germ cell lineage is believed to be potentially immortal and set aside under a special developmental program different from that of the somatic cell lineage. In many animals, germ cells are specified by germplasm deposited in the fertilized egg, which contains germ cell-specific cytoplasmic determinants known as polar granules in Drosophila and P-granule in Caenorhabditis elegans (for review see Eddy, 1975). As special phenomena, it has been known that chromatin diminution in certain nematodes (Ascaris) and chromosome elimination in certain insects (Miaster) are closely associated with germ cell determination (for review see Beams and Kessel, 1974), in which germplasm in germ cell precursors is involved in retaining their full set of genomes. It is therefore conceivable that germ cell determination should be linked not only with a commitment to cell fate leading to gametogenesis but also with a regulatory mechanism to protect the genetic information transmitted to the next generation.

The molecular characteristics of germ cell determinants have been well investigated in the case of Drosophila (Rongo et al., 1995). The polar granules are formed during oogenesis while closely associated with mitochondrial clouds and are composed of RNAs and proteins. Interestingly, recent studies suggested that the polar granule contains polysomes consisting of mitochondrial rRNAs (Kobayashi et al., 1993). In Drosophila, after fertilization only the nuclei that migrate into the pole plasm localized at the egg are destined to form the germline progenitors, pole cells. Among the components of Drosophila germplasm such as Oskar, Vasa, Nanos and Tudor, which have been molecularly identified, vasa is the best characterized and encodes a DEAD-family protein of ATP-dependent RNA helicase (Hay et al., 1988; Lasko and Ashburner, 1988; Liang et al., 1994). Vasa is required for pole cell formation and the zygotic expression is exclusively restricted to the germ cell lineage throughout the development. The molecular function of the VASA protein is estimated to bind target mRNAs involved in germ cell establishment such as Oskar and Nanos, and in oogenesis such as Gruken, and to control the onset of the translation (Gavis et al., 1996; Dahanukar and Wharton, 1996; Tomancak et al., 1998; Styhler et al., 1998).

On the basis of structural conservation, genes homologous to vasa have been cloned in many animal species (for review see Raz, 2000) including mammals such as mouse, rat and human (Fujiiwara et al., 1994; Komiya and Tanigawa, 1995; Castrillon et al., 2000). Up to present vasa expression provides the most reliable molecular marker for the germ cell lineage in most animal species. Alignment of three vasa homolog proteins of those mammal species, in which only one ortholog has been identified in each species to date, shows a high similarity over the entire region of amino acid sequences (Fig. 1A). The eight conserved
motifs, which have been presumed to compose functional domains essential for ATP binding, ATP hydrolysis, RNA binding and RNA unwinding activities (Schmid and Linder, 1991; Pause et al., 1993), are included in the identical core region. Subtle species variation is found in approximately one quarter of the N-terminal region and approximately 5–10 amino acids stretches at the C-terminal end.

VASA expression in primordial germ cells (PGCs)

Lineage studies in the mouse have showed the presence of cells, which give rise to primordial germ cells (PGCs), within the proximal margin of the 6.5 dpc epiblast (Lawson and Hage, 1994). By 7.5 dpc, these cells have migrated to the extraembryonic mesoderm at the posterior of the primitive streak and become distinguishable by their strong alkaline phosphatase (AP) activity. They then migrate along the hindgut to arrive at the genital ridges by 10.5 dpc (Ginsburg et al., 1990). Remarkable expression of mouse vasa homolog (MVH) protein is detected specifically in germ cells just after their colonizing the genital ridges and is not detected in ES cells derived from inner cell mass of blastocysts and embryos by 8.5 dpc (Toyooka et al., 2000).

However, our recent analyses showed that MVH expression became detectable in cells which were localized in

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Fig. 1. A) Comparison of human, mouse and rat VASA proteins. The eight conserved motifs (I–VIII) are indicated as shaded boxes. B) Comparison of VASA expression during germ cell development between Drosophila and mouse. The maternal and zygotic expressions are represented as shaded and blacked boxes, respectively.
the gut mesentery of 9.5–10.5 dpc embryos (Fig. 2; a–c), although the level was relatively very low, compared with gonadal stage PGCs. This is also the case in the human, in which the expression of VASA protein became detectable in migratory PGCs in the region of the genital ridges (Castrillon et al., 2000). The timing of the initiation of mammalian vas a gene expression is almost equivalent with that of zygotic transcription in Drosophila (Fig. 1B; Van Doren et al., 1998). This may suggest that the zygotic gene expression of vasa gene reflects a critical step of the germ cell differentiation, which is evolutionally conserved.

In the mouse it is noteworthy that all of the PGCs, which are positively recognized by 4C9 staining (Yoshinaga et al., 1991), do not evenly express MVH protein but only a portion of PGCs expresses MVH protein in the cytoplasm (Fig. 2; d–f). One possible interpretation of the heterogeneity is that the difference may be due to the individual variations in the developmental proceeding, because the difference is not related with the distance to genital ridges and is not found in the PGCs localized in the gonads of 12.5 dpc embryos (Fig. 2; g, h). Similar heterogeneity in the population of migrating PGCs was supposed in the Igf2r genomic imprint characteristics. It was shown that 8.5 dpc-derived EG cell lines were a mixture of imprinted and non-imprinted (erased) cells, suggesting that some of the PGCs may erase their imprint before they reach the genital ridges. The difference in the imprinting state did not correlate with the ability of the germline transmission (Labosky et al., 1994). In this connection, we found that male mice embryos with a targeted mutation of Mvh exhibited a significant delay in the proliferation of PGCs after their colonizing in the gonads (Tanaka et al, 2000). Together with the unequal MVH expression, it

![Fig. 2. MVH expression in embryonic germ cells. Sections of 9.5 dpc mouse embryos (a, b), 10.5 dpc embryos (c–f) and 12.5 dpc embryonic gonads (g, h) were double-stained with rat monoclonal antibody 4C9 (a, c, d, g) and rabbit anti-MVH antibody (e, h) in conjunction with FITC-conjugated goat anti-rat IgG and Alexa594 conjugated goat anti-rabbit IgG, respectively. d), e), f) are high magnification views of the boxed area in c), where a urogenital ridge resides. f) is a merged image of d) and e). Arrows in a), b) indicate PGCs stained with both antibodies. Arrows and arrowheads in d), e), f) indicate a PGC stained with 4C9 but not stained with anti-MVH, and a PGC stained with both antibodies, respectively. No MVH-positive cells were observed in 8.5 dpc embryos using the same staining condition (data not shown). hg; hindgut; dm, dorsal mesentery.]
is therefore conceivable that migrating PGCs are inhomogeneous in the nature of developmental potential and/or proliferative activity.

**VASA expression in gametogenesis**

Mammalian VASA protein is expressed in the cytoplasm of germ cells undergoing gametogenic processes until the post-meiotic stage in both males and females (Toyooka et al., 2000). Mvh knock out mice showed that homozygous mutant males are sterile but female ones are normally fertile. Spermatogenesis in the Mvh homozygous male is blocked at a stage ranging from leptotene to zygotene, indicating the essential role for meiotic progression of spermatogenesis (Tanaka et al., 2000). It is noteworthy that the timing of the meiotic arrest coincides with that of the change of subcellular localization of MVH protein. MVH protein is distributed uniformly in the cytoplasm of cells from spermatogonia to leptotene spermatocytes and appears to aggregate gradually to granules in zygotene-diplotene spermatocytes. In the cytoplasm of round spermatids MVH protein localization is found in a perinuclear electron dense

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**Fig. 3.** Cytochemical staining of the adult testis. Sections of adult mouse testes were stained with anti-MVH using FITC conjugated secondary antibody (a, d, g) and stained simultaneously with mitotracker (specific for mitochondria; shown as pseudocolor-red; b, e) or rhodamine phalloidin (specific for F-actin, Molecular Probes; shown as pseudocolor-red; h). The merged images were showed in c, f, i. Arrows indicate the representative signal showing the co-localization of MVH protein and mitochondria cluster or F-actin in the chromatoid body. Bar; 20 μm for d-i.
granule, which is known as the chromatoid body (Fig. 3a and 3d). The subcellular localization of MVH in spermatocytes is coincident with the nuage-like structure in the interstices of mitochondrial clusters in spermatocytes, which is regarded as the precursor materials of the chromatoid body (Fawcett et al., 1970). These structural features are common to those of the germplasm in many animal species. Previous studies at electron microscopic level revealed that the chromatoid body is composed of several RNAs and proteins. So far, in addition to MVH protein, ribonucleoproteins (snRNPs, hnRNPs and ribosomal proteins; Biggiogera et al., 1993; Moussa et al., 1994), actin (Walt and Armbuster, 1984), two cytochrome-c isozymes (Hess et al., 1993), germ cell-specific RNA binding protein, p48/52 (Oko et al., 1996) and transition protein 2 (TP2) mRNA (Saunders et al., 1992) have been reported to localize in the chromatoid body. As shown in Fig. 3, the concentrations of F-actin and mitochondria into the chromatoid body are also visible by fluorescent staining with phalloidin and mitotracker, respectively.

In Drosophila germplasm (polar granule), several RNA-binding proteins such as VASA, NANOS and STAUHEN, and the mitochondrial components such as mtrRNAs are involved in the assembly of the granules. The polar granule appears to contain germ cell-specific translational machineries, in which VASA and Nanos have been demonstrated to function as translational regulators (Gavis et al., 1996; Dahanukar and Wharton 1996; Tomancak et al., 1998; Styhler et al., 1998). Analogous to the polar granules, one can speculate the possibility that the chromatoid body also functions as a translational apparatus specific for spermatogenesis. Indeed, it has been well known that majority of haploid-specific genes such as protamine (Prm-1: Braun et al., 1989; Prm-2: Kwon and Hecht, 1991) are transcribed in spermatocytes and controlled at the translational level until haploid cells begin to differentiate (Eddy and Deborah, 1998). The finding that mRNA for one of the haploid genes, TP-2, was located to the chromatoid body may be supporting evidence for the possibility. However, since it has been demonstrated that mRNAs for TP-1 and protamines showed no accumulation to the chromatoid body has another physiological role such as a scaffold for the storage of RNA-related factors. Moreover, the close relationship with mitochondrial clouds and findings of mitochondrial components, such as mtrRNAs in germ plasm and cytories in chromatoid body, may indicate the attractive possibility that both of the germ cell-specific nuage-like structures serve as a reconfirmation mechanism for the symbiotic interaction between mitochondria and the nuclear genome transmitted to the next generation. We believe further studies on the molecular basis of the chromatoid body including VASA-related proteins could be informative for understanding germ cell development and the meiotic process.

References


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