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MINIREVIEW

The mechanisms of primordial germ cell determination during embryogenesis in molluscan species

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Abstract

Primordial germ cells (PGCs) are the first germ cells distinguishable from surrounding somatic cells during embryogenesis. In many animal species PGC specification is generally classified into two modes: preformation, in which PGCs are determined by maternally inherited components in early development, and epigenesis, in which PGCs arise from proximal somatic cells by inductive signal in late development. In this review we focused on the process of PGC formation in molluscan bivalves and gastropods and compared the PGC specification modes of these two classes. Several reports indicated that bivalves tend to adopt preformation, as maternally inherited germline-specific genes are transcribed in specific blastomeres differentiating into PGCs. In gastropods, maternal germline-specific genes are transcribed in mesodermal stem cells. PGCs seem to be epigenetically determined from mesodermal stem cells by inductive signals after the veliger larval stage, which indicates that PGC precursor cells not only generate germline tissues but also generate mesodermal somatic tissues. The common origin of germline and mesodermal somatic tissues is observed in the annelids Platyhelminthes and Cnidaria, and is considered to be an ancient mode of germ cell determination. We suggest that gastropods retain the ancient PGC specification mode, while bivalves switch their PGC specification mode to preformation.

Key Words: primordial germ cell; *vasa*; early development, preformation; epigenetic; mollusc

Introduction

Stem cells are defined by the abilities to self-renew and to differentiate into other cell types by asymmetric division (Lin, 1997). They are classified by their differentiating abilities as unipotent, multipotent, pluripotent and totipotent stem cells. Germinal stem cells (GSCs) are pluripotent stem cells that can differentiate into almost every cell type except extraembryonic tissues. Indeed, GSCs are able to generate gametes and transmit the individual's DNA to the next generation. Primordial germ cells (PGCs) are germline cells that can be genetically and morphologically distinguished from somatic tissues during early embryogenesis. The origin of the PGCs and the timing of PGC segregation from somatic cells during development have been reported for many animal species (Extavour and Akam, 2003). Several studies have also been conducted on the origins of germline cells in molluscs, especially in bivalves and gastropods (Woods, 1931; Fabioux *et al.*, 2004; Swartz *et al.*, 2008; Kranz *et al.*, 2010).

In this paper we review the existing literature on germ cells determination during development in various animal species, in particular in molluscan bivalves and gastropods. First, we introduce two general mechanisms of germline specification during embryogenesis: preformation and epigenesis. Second, we list genes that are important in molluscan GSCs. Finally, we compare the PGC specification modes in bivalves and gastropods.

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Mechanisms of germline segregation from somatic tissues during embryogenesis

Preformation

Data from many previous studies on PGC origins have suggested that there are two distinct modes of PGC specification: preformation and epigenesis (Extavour and Akam, 2003). In some species, PGCs can be identified during early development by morphological features such as a large round nucleus, a single large nucleolus, relatively clear cytoplasmic organelles and granular cytoplasmic material. Granular cytoplasmic material in particular is a useful PGC marker for many animal

species. It is known under various synonyms like germ plasm, nuage, mitochondrial cloud and chromatoid body. In this study, we use the term "germ plasm", as this is the most commonly used term in many animal species (Saffman and Lasko, 1999).

Germ plasm can be observed as electron-dense granules containing mitochondria, proteins and RNA. Transcripts of many genes have been identified as germ plasm components. Among these, the germline markers *vasa* and *nanos* are conserved among a wide range of animal classes, and these genes can thus be used as molecular markers for PGC identification. In some species, germ plasm is stored in oocytes before fertilization, and the maternally inherited cytoplasm is transmitted to the PGC precursor cells. This means that in some animal species the origin of PGCs is determined by maternal factors. This PGC specification mode is called preformation. Preformation is reported in *Drosophila melanogaster* (Sonnenblick, 1950), anuran species (Bounoure, 1939), most teleosts (Yoon *et al.*, 1997) and *Caenohabditis elegans* (Deppe *et al.*, 1978; Strome and Wood, 1982).

In *D. melanogaster*, germ plasm is assembled at the posterior poles of the oocytes before fertilization (reviewed by Mahowald, 2001). It is then transmitted to the five posterior pole cells by unequal cleavage before blastoderm formation (Huettner, 1923). These pole cells exclusively become progenitors of PGCs. Transplanted germ plasm can induce PGC formation at ectopic sites (Illmensee and Mahowald, 1974; Illumensee *et al.*, 1976). Moreover, disassembly of germ plasm prevents PGC formation (Rongo and Lehman, 1996). These results indicate that germ plasm is essential for germline formation in *Drosophila*.

Many genes, such as *vasa*, *oskar*, *tudor* and *gurken*, are required for posterior germ plasm formation in *Drosophila* (Rongo and Lehman, 1996). In particular, *oskar* has a critical role in germ plasm assembly at the posterior of the oocyte, as it is required for the posterior localization of the germ plasm components *vasa, nanos* and *tudor* (Rongo and Lehman, 1996). *Vasa* acts as a translational regulator of *gurken* and *oskar* (Mahowald, 2001).

Epigenesis

In some species germ cells cannot be identified until late development and PGCs arise by inductive signals from surrounding somatic tissues. This PGC specification mode is called epigenesis. Epigenesis is observed in mammals (Saffman and Lasko, 1999), urodeles (Ikenishi and Nieuwkoop, 1978), sea urchins (Juliano *et al.*, 2006) and Cnidaria (Noda and Kanai, 1977). In mouse embryos, no maternally derived germ plasm has been found (Eddy, 1975). Unlike *vasa* transcripts in preformation species, mouse *vasa* orthologs cannot be used to identify PGCs in early development, because its protein is not localized to a specific subcellular region (Toyooka *et al.*, 2000). Germ cells can first be identified by alkaline phosphatase staining at 6.5 days post conception (Lawson and Hage, 1994). PGC determination at the proximal epiblast is induced by signals from the extraembryonic ectoderm and endoderm. It is reported that Bmp4, Bmp8b and Bmp2, members of the bone morphogenetic proteins (BMP) of the transforming growth factor β (TGFβ) superfamily, function as the inductive signals (Hogan, 1996; Lawson *et al.*, 1999; Ying *et al*., 2000; Ying and Zhao, 2001).

Extavour and Akam (2003) reviewed the PGC determination modes of 28 Metazoan phyla. In 23 of the 28 phyla it was suggested that PGCs are determined by epigenesis. This indicates that epigenesis is an ancient mode of PGC determination in Metazoa.

Transcripts that play an important role in molluscan GSCs

Germline-specific transcripts have been observed in many animal species, and their functions in PGCs have been described. In molluscs some gene transcripts have been isolated from germline cells and are used to identify the localization of PGCs and elucidate the mechanism of gametogenesis. *Vasa* is an ATP-dependent RNA helicase of the DEAD-box family of proteins. It unwinds double-strand RNA to promote the translation of target genes (Hay *et al.*, 1988; Lasko and Ashburner, 1988; Carrera *et al.*, 2000; Raz, 2000; Johnstone and Lasko, 2001). One of the target genes of *vasa* translational control is *nanos,* which in turn regulates the translation of other genes (Johnstone and Lasko, 2001). *Vasa* is widely used as a molecular marker for germlines including PGCs in many animal species (Extavour and Akam, 2003). In molluscs, *vasa* orthologs have also been isolated and are used for PGC identification and germline observation (Fabioux *et al.*, 2004; Swartz *et al*., 2008; Kranz *et al.*, 2010; Obata *et al.*, 2010). Fabioux *et al.* (2009) performed RNA interference on the oyster *vasa* ortholog *oyvlg* in *Crassostrea gigas* gonads and suggested that *oyvlg* has an important role in germ cell proliferation and early meiosis.

Nanos is a translational regulator in germline cells. Its own translation is controlled by *vasa*. In insects it is also required for somatic patterning (Lehman and Nusslein-Vorhard, 1991; Lall *et al.*, 2003). In molluscs, *nanos* ortholog transcription has been observed in *Ilyanassa obsoleta* and *Haliotis asinina* embryos. In *I. obsoleta*, *nanos* orthologs were transmitted to the 4d blastomere. *Nanos* ortholog transcriptions remained in 4d derivatives including germline and somatic tissues at later stages of development (Rabinowitz *et al.*, 2008). Inhibition of *nanos* orthologs by morpholino oligo injection resulted in larvae lacking all 4d derivatives. These larvae had no hearts and abnormal retractor muscles and intestines. In *H. asinina* embryos, *nanos* orthologs were transcribed in second quartet micromeres (2a-2d blastomeres), which give rise to the foot and ectoderm anterior. These results suggest that *nanos* functions in both germline and somatic tissue development in gastropod embryos.

The TGFβ family is structurally conserved and plays an important role in cell proliferation and differentiation during development (ten Dijke *et al.*, 2000). In *Drosophila*, TGFβ signal transduction pathways affect germline stem cell numbers and the

size of the stem cell niche (Schulz *et al.*, 2004). In rainbow trout, TGFβ increased spermatogonium proliferation and oocyte maturation (Sawatari *et al.*, 2007). In the bivalve *C. gigas,* a TGFβ ortholog (*og-TGFβ*) is found in the somatic tissues surrounding the spermatogonia and oogonia (Fleury *et al.*, 2008). Results from an *in vivo* RNA interference study on in male and female gonad in *C. gigas* suggested that *og-TGFβ* is required for spermatogonial and oogonial proliferation and oocyte maturation (Huvet *et al.,* 2012). The TGFβ superfamily may play an important role in embryogenesis and germ cell maintenance in many molluscan species.

Cell lineage and PGC location in spiral cleavage embryos

In molluscs, the cleavage mode of early development is spiral cleavage. This form of cleavage is observed in many phyla like annelids, entoprocts, nemertines and Platyhelminthes (Nielsen, 2010). The spiral cleavage pattern was first shown more than 100 years ago (Conklin, 1897). In spiral cleavage, embryos are divided into AB and CD blastomeres at the two-cell stage, and into A, B, C and D blastomeres at the four-cell stage. In many annelids and molluscs, a polar lobe is produced during each of the first two cleavages (Figs 1, A1, A3). The two polar lobes are absorbed into the CD blastomere at the two-cell stage and the D blastomere at the four-cell stage (Figs 1, A2, A4). At the third cleavage (which corresponds with the first quartet cleavage), A, B, C and D blastomeres divide synchronously and produce four micromeres toward the animal pole (1a, 1b, 1c and 1d) and four macromeres at the vegetative pole (1A, 1B, 1C and 1D) (Henry *et al.*, 2006) (Fig. 1, A5). The following quartets (second, third and fourth) are oblique with alternating directions of spindles, resulting in the spiral cleavage pattern (Figs 1, A6-A8).

There are two types of spiral cleavage: early equal cleavage and unequal cleavage (Henry *et al.*, 2006). In early equal cleavage, the first four quadrants have an equal cleavage pattern until the 32-cell stage (Van den Biggelaar and Guerrier, 1979; Gonzales *et al.*, 2007) (Fig. 1, B). Early equal cleavage is considered to be an ancestral condition of spiral cleavage (Freeman and Lundelius, 1992; Henry, 2002). In unequal cleavage, the first cleavage is asymmetrical, which is caused by biased mitotic spindle orientation and polar lobe absorption. Unequal cleavage results in larger blastomeres at the vegetative pole side of the embryo, which is derived from D blastomeres at the four-cell stage, and smaller blastomeres at the animal pole.

Spiral cleavage patterns and cell lineages are conserved among various animal species (Nielsen, 2004, 2005; Lambert, 2007). 3D Blastomeres arise from D blastomeres through three quartet cleavages, dividing into large 4D and small 4d blastomeres at the fourth quartet cleavage (Figs 1, A8, B6). The 4d blastomere differentiates into most mesodermal tissues, including germline cells (Conklin, 1897). The specification of mesodermal lineages from 4d blastomeres by asymmetric cleavage is a common phenomenon in animal species with spiral cleavage patterns (Wilson, 1892; Conklin, 1897; Woods, 1931).

PGC segregation during embryogenesis in bivalves

PGC specification modes have been reported for some molluscan species. The process and mode of PGC specification is different in each class, although PGCs commonly arise from 4d blastomeres. Woods (1931) studied *Sphaerium striatum* oocytes under light microscope and noted that the 4d blastomere divided equally into M and M1 blastomeres and that PGCs were generated from these blastomeres. He also reported that the germ plasm was localized at the vegetative pole in unfertilized eggs. Germ plasm was specifically transmitted to D blastomere derivatives and finally segregated in germ cells in the late gastrula. Recently, transcripts of the germline-specific gene *vasa* have been used as a germline molecular marker in some bivalve species (Fabioux *et al.*, 2004; Kakoi *et al*., 2008; Obata *et al.*, 2010). Fabioux *et al.* (2004) observed *C. gigas vasa* ortholog (*oyvlg*) transcription at the vegetative pole of fertilized eggs. The transcripts were specifically transmitted to one blastomere during early development. At the morula stage, *oyvlg* was observed in the 4d blastomere only. The 4d blastomere equally divided into M and M1 blastomeres, which then differentiated into PGCs. The *oyvlg* transcription pattern is identical to the location of germ plasm in *S. striatum*, which suggests that *oyvlg* is a general component of mollusc germ plasm, like in *Drosophila*. Kakoi *et al*. (2008) reported on the localization of the *vasa* ortholog *Sk-vasa* in *Sakkostrea kegaki* embryos. *Sk-vasa* was transcribed throughout the embryo until the eight-cell stage. At the 50-cell stage, the transcripts were limited to the 2d descendant cell and a pair of cells located posteriorly, which were likely to be 4d blastomeres. In the late gastrula, strong *Sk-vasa* expression was observed in the posterior mesoderm. Germline-specific localization of *Sk-vasa* could not be observed until the veliger larval stage.

PGC segregation during embryogenesis in gastropods

In *I. obsoleta*, the *vasa* ortolog *loVasa* accumulates during early development (Swartz *et al.*, 2008). The transcripts were observed ubiquitously throughout the first seven cleavage cycles, while the RNA became more abundant in the D quadrant as the cleavage cycle progresses. Maternally inherited *loVasa* accumulated at 4d specifically between the 28-cell and the 35-cell stage. At the 70-cell stage, *loVasa* transcription was limited to $4d^{L121}$ and $4d^{R121}$. Swartz *et al.* (2008) suggested that $4d^{L121}$ and $4d^{R121}$ are likely to be homologous to the PGC lineage founder cells identified in *S. striatum* (Woods, 1931). However, *loVasa* could not be detected after the 108-cell stage.

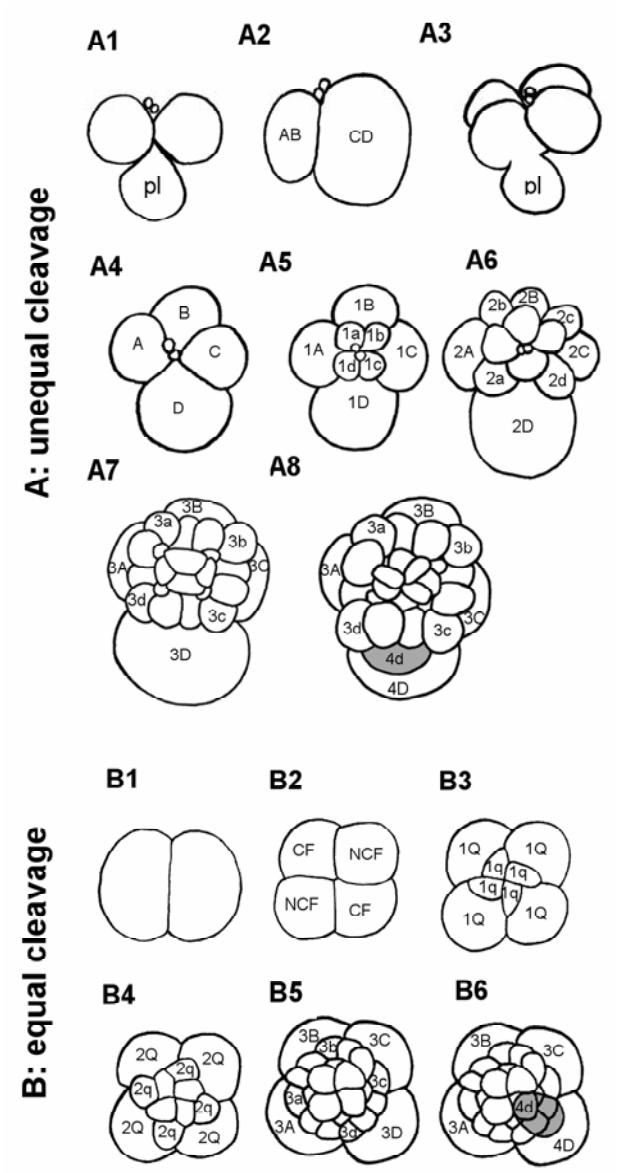

Fig. 1 Diagram of unequal and equal spiral cleavage. A) Unequal spiral cleavage of *Ilyanassa obsoleta*. A1) Polar lobe formation at first cleavage. A2) Two cell stage. A3) Polar lobe formation at second cleavage. A4) Four cell stage. A5) First quartet blastomeres at the 8 cell stage. A6) Second quartet blastomeres at the 12 cell stage. A7) Third quartet blastomeres at the 24 cell stage. A8) 4d Blastomere formation at fourth quartet cleavage. B) Equal spiral cleavage of *Crepidula fornicata*. B1) Two cell stage. B2) Four cell stage. B3) First quartet blastomeres at the 8 cell stage. B4) Second quartet blastomeres at the 12 cell stage. B5) Third quartet blastomeres at the 24 cell stage. B6) 4d Blastomere formation at fourth quartet cleavage. After third quartet cleavage, 4d blastomeres arise from 3D blastomeres by unequal cleavage in both spiral cleavages. 4d blastomere is shown as dark gray area.Polar lobe (pl).

Kranz *et al.* (2010) observed transcription of *vasa*, *nanos* and *PL10* orthologs in *Haliotis asinina* embryos. The *vasa* ortholog *HasVasa* was ubiquitously expressed throughout the embryo until the 16-cell stage. Strong transcription of *HasVasa* was observed in 4d blastomere at the 60–64-cell stage (3.5 hours post-fertilization (hpf)). At the gastrula stage, *HasVasa* was apparently distributed at the mesodermal band until the trochophore stage. *HasVasa* and *HasPL10* were colocalized in early veliger larvae. It was hypothesized that PL10 is transcribed in mesodermal cells, which also produce PGCs (Rebscher *et al*., 2007). From these results, Kranz *et al*. (2010) suggested that *HasVasa* is transcribed in undifferentiated multipotent cells that are precursor cells for PGCs and mesodermal somatic tissues. Germline-specific transcription of *HasVasa* could not be observed until the veliger larval stage. In *Viviparus viviparus*, PGCs have also not been identified in early development and

germline cells arise from the mesodermal pericardial epithelium (Griffond, 1977). Thus, in gastropods, maternally inherited germ plasm or mRNA is not reported to be transmitted to PGCs, which is in contrast with the findings in bivalves.

Comparison of PGC specification modes in bivalves and gastropods

In the bivalves *C. gigas* and *S. striatum*, maternally inherited germ plasm is transmitted to specific blastomeres by unequal cleavage from the first cleavage to the 4d blastomere formation. At the gastrula stage, germ plasm is distributed at germline-specific blastomeres only. Thus it can be concluded that the PGC specification mode in these species is preformation, since maternally inherited germ plasm is specifically transmitted only to germlines in early development, like in *D. melanogaster and C. elegans*. In the case of *S. kegaki*, however, maternally inherited *Sk-vasa* was ubiquitously transcribed during early development. Although the signal became stronger in the 4d blastomere, germline-specific localization of *Sk-vasa* could not be observed until the veliger larval stage. This suggests that PGC specification mode of *S. kegaki* is epigenetic, meaning that not all bivalve species may use preformation as the mode of PGC specification. However, more studies are required to clarify the PGC specification mode of *S. kegaki*.

Like in *S. kegaki*, *vasa* transcripts in gastropods do not have a specific distribution pattern during early development. In *I. obsoleta*, *loVasa* is more strongly transcribed in 4d blastomeres and the signal was transmitted to $4d^{L121}$ and $4d^{R121}$, which presumably differentiate into PGCs. From these results, Swartz *et al.* (2008) suggested that maternal *loVasa* RNA may be involved in germ cell specification. However, the localization of PGC could not be identified until the veliger larval stage in *I. obsoleta*, because *loVasa* transcription disappeared after the 108-cell stage. In *H. asinina*, transcription of *HasVasa* was observed in 4d blastomeres at 3.5 hpf. However, the transcription was observed in multipotent mesodermal cells, which differentiate into PGCs and mesodermal somatic tissues like heart and retractor muscles in veliger larvae. This suggests that PGCs are not established until the veliger larval stage, although maternal *vasa* is specifically transmitted to 4d blastomeres in gastropods. Judging from these results, the PGC specification mode of gastropods appears to be epigenesis.

Rebscher *et al.* (2007) reported that the transcription pattern of the *vasa* ortholog *Pdu-vasa* of *P. dumerilli* that is similar to that of *H. asinina*; ubiquitous distribution in most blastomeres during early development and specific localization at the mesodermal posterior growth zone (MPGZ; mesodermal stem cells) that arises from the 4d blastomeres at the gastrula stage. Specification of germlines from the MPGZ occurred at the juvenile II stage in late development. Rebscher *et al.* proposed that PGC specification in *P. dumerilli* is a two-step process. First, a population of undifferentiated cells that can differentiate into both somatic tissues and PGCs is established in the MPGZ. This process apparently requires maternally inherited germ plasm, suggesting that the establishment of MPGZ is by preformation. PGC are then determined by induction signals from the MPGZ in late development, which is the epigenetic mode of PGC specification. We suggest that this PGC specification mechanism is similar to that of *H. asinina*.

The studies on *H. asinina* and *P. dumerilli* indicate that the germline-specific gene is distributed in the mesodermal stem cells, and not only the germlines. Moreover, PGCs and somatic tissues arise from a common precursor cell, the mesodermal stem cells. In fact, in some animal species such as annelids (Vincent *et al.*, 2011), Platyhelminthes (Shibata *et al.*, 1999) and Cnidaria (Noda and Kanai, 1977), germ plasm is not only observed in germlines but also in the somatic stem cells. Germline-specific genes like *vasa* and *Piwi* have an important role in the maintenance of somatic stem cells as well as in germlines in these species (Thomson and Lin, 2009). PGC specification by induction of multipotent somatic stem cells is considered to be an ancient mechanism of germline formation. Thus, *H. asinina* adopts an epigenetic mode for PGC specification, which may be the most ancient mode among molluscan species (Kranz *et al.*, 2010). On the other hand, the PGC specification mode used in some bivalve species like *C. Gigas* and *S. Striatum* is preformation. Extavour and Akam (2003) suggested that the basal PGC specification mode in Metazoa is epigenetic. Thus, we suggest that many bivalve species change their PGC specification mode from epigenetic to preformation. With this shift in germline specification mode, it is likely that germ plasm components like *vasa* lose their function in somatic stem cells. However, it is possible that not all bivalve species shift their germline specification mode to preformation, as *S. Kegaki* apparently adopts the epigenetic germline specification.

In conclusion, PGC specification modes are species-specific in molluscs, although 4d blastomeres are common precursor cells in both bivalves and gastropods. It is suggested that bivalves tend to use preformation while gastropods use epigenesis as their mechanism of PGC specification. Koop *et al*. (2007) suggested that equally cleaving gastropods tend to use inductive signals, while unequally cleaving gastropods tend to rely on inherited determinants. Kranz *et al.* (2010) hypothesized that this different strategy between equal and unequal spiral embryos may be correlated to the PGC specification mechanism. More information on PGC specification modes should be accumulated in other molluscan species to confirm this hypothesis.

References

Bounoure L. L'origine des cellules reproductrices et le probleme de la lignee germinale. The origin of the reproductive cells and the problem of the germ line. Gauthier-Villars, Paris, France, 1939.

Carrera P, Johnstone O, Nakamura A, Casanova J, Jäckle H, Lasko P. VASA mediates translation through Interaction with a *Drosophila* yIF2 homolog. Mol. Cell 5: 181-187, 2000.

Conklin EG. Embryology of *Crepidula*: a contribution

to the cell lineage and early development of some marine gasterpods. J. Morphol. 13: 1-226, 1897.
Deppe U, Schierenberg E, Cole T, Krieg C, Schimitt D, Yoder B, *et al.* Cell lineages of the embryo of the nematode *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. 75: 376-380, 1978.
Eddy EM. Germ plasm and the differentiation of the germ cell line. Int. Rev. Cytol. 42: 229-280, 1975.
Ephrussi A, Lehmann R. Induction of germ cell formation by *oskar*. Nature 358: 387-392, 1992.
Extavour CG, Akam M. Mechanisms of germ cell specification across the metazoans: epigenesis and preformation. Development 130: 5869-5884, 2003.
Fabioux C, Huvet A, Lelong C, Robert R, Pouvreau S, Daniel JY. Oyster *vasa*-like gene as a marker of the germline cell development in *Crassostrea gigas*. Biochem. Biophys. Res. Commun. 320: 592-598, 2004.
Fabioux C, Corporeau C, Quillien V, Favrel P, Huvet A. *In vivo* RNA interference in oyster - *vasa* silencing inhibits germ cell development. FEBS 276: 2566-2573, 2009.
Fleury E, Fabioux C, Lelong C, Favrel P, Huvet A. Characterization of a gonad-specific transforming growth factor-β superfamily member differentially expressed during the reproductive cycle of the oyster *Crassostrea gigas.* Gene 410: 187-196, 2008.
Freeman G, Lundelius JW. Evolutionary implications of the mode of D quadrant specification in coelomates with spiral cleavage. J. Evol. Biol. 5: 205-247, 1992.
Gonzales EE, van der Zee M, Dictus WJAG, van den Biggelaar J. Brefeldin A or monensin inhibits the 3D organizer in gastropod, polyplacophoran, and scaphopod molluscs. Dev. Genes Evol. 217: 105-118, 2007.
Griffond B. Ultrastructural study of the first stages of ovarian post-embryonic differentiation of the paludin *Viviparus viviparus* L. (Mollusca, Gastropoda, Prosobranch of separate sexes). C. R. Acad. Sci. Hebd. Seances Acad. Sci. D. 284: 667-670, 1977.
Hay B, Jan LY, Jan YN. A protein component of *Drosophila* polar granules is encoded by *vasa* and has extensive sequence similarity to ATP-dependent helicases. Cell 55: 577-587, 1988.
Henry JJ. Conserved mechanism of dorsoventral axis determination in equal-cleaving spiralians. Dev. Biol. 248: 343-355, 2002.
Henry J, Perry KJ, Martindale MQ. Cell specification and the role of the polar lobe in the gastropod, *Crepidula fornicata*. Dev. Biol. 297: 295-307, 2006.
Hogan BL. Bone morphogenetic proteins: multifunctional regulators of vertebrate development. Genes Dev. 10: 1580-1594, 1996.
Huettner AF. The origin of the germ cells in *Drosophila melanogaster*. J. Morphol. 2: 385-422, 1923.
Huvet A, Fleury E, Corporeau C, Quillien V, Daniel JY, Riviere G, *et al. In vivo* RNA interference of a gonad-specific transforming growth factor-β in the Pacific oyster *Crassostrea gigas*. Mar. Biotech. 14: 402-410, 2012.
Ikenishi K, Nieuwkoop PD. Location and ultrastructure of primordial germ cells (PGCs) in *Ambystoma mexicanum*. Dev. Growth Diff. 20: 1-9, 1978.
Illumensee K, Mahowald AP. Transplantation of posterior polar plasm in *Drosophila*. Induction of germ cells at the anterior pole of the egg. Proc. Natl. Acad. Sci. 4: 1016-1020, 1974.
Illumensee K, Mahowald AP, Loomis MR. The ontogeny of germ plasm during oogenesis in *Drosophila*. Dev. Biol. 49: 40-65, 1976.
Johnstone O, Lasko P. Translational regulation and RNA localization in *Drosophila* oocytes and embryos. Annu. Rev. Genet. 35: 365-406, 2001.
Juliano CE, Veronina E, Stack C, Aldrich M, Cameron AR, Wessel GM. Germ line determinants are not localized early in sea urchin development, but do accumulate in the small micromere lineage. Dev. Biol. 300: 406-415, 2006.
Kakoi S, Kin K, Miyazaki K, Wada H. Early development of the Japanese spiny oyster (*Saccostrea kegaki*): Characterization of some genetic markers. Zool. Sci. 25: 455-464, 2008.
Koop D, Richard GS, Wanninger A, Gunter HM, Degnan BM. The role of MAPK signaling in patterning and establishing axial symmetry in the gastropod *Haliotis acinina*. Dev. Biol. 311: 200-212, 2007.
Kranz AM, Tollenaere A, Norris BJ, Degnan BM, Degnan SM. Identifying the germline in an equally cleaving mollusc: *Vasa* and *Nanos* expression during embryonic and larval development of the vetigastropod *Haliotis asinina*. J. Exp. Zool. 314: 267-279, 2010.
Lambert JD. Mesoderm in spiralians: the organizer and the 4d cell. Mol. Dev. Evol. 310: 15-23, 2007.
Lall S, Ludwig MZ, Patel NH. *Nanos* plays a conserved role in axial patterning outside of the Diptera. Curr. Biol. 13: 224-229, 2003.
Lasko P, Ashburner M. The product of the *Drosophila* gene *vasa* is very similar to eukaryotic initiation factor-4A. Nature 335: 611-617, 1988.
Lawson K, Hage WJ. Clonal analysis of the origin of primordial germ cells in the mouse. CIBA Found. Symp. 182: 68-91, 1994.
Lawson KA, Dunn NR, Roelen BAJ, Zeinstra LM, David AM, Wright CV, *et al.* Bmp4 is required for the generation of primordial germ cells in the mouse embryo. Genes Dev. 13: 424-436, 1999.
Lehman R, Nusslein-Vorhard C. The maternal gene *nanos* has a central role in posterior pattern formation of the *Drosophila* embryo. Development 112: 679-691, 1991.
Lin H. The TAO of stem cells in the germline. Annu. Rev. Genet. 31: 455-491, 1997.
Mahowald AP. Assembly of the *Drosophila* germ plasm. Int. Rev. Cytol. 203: 187-213, 2001.
Nielsen C. Trochophora larvae: Cell-lineages, ciliary bands, and body regions. 1. Annelida and mollusca. J. Exp. Zool. 302B: 35-68, 2004.

Nielsen C. Larval and adult brains. Evol. Dev. 7: 483-489, 2005.

Nielsen C. Some aspects of spiralian development. Acta Zool. 91: 20-28, 2010.

Noda K, Kanai C. An ultrastructural observation on *Pelmatohydra robusta* at sexual and asexual stages, with a special reference to “Germinal plasm”. J. Ultrastruct. Res. 61: 284-294, 1977.

Obata M, Sano N, Kimata S, Nagasawa K, Yoshizaki G, Komaru A. The proliferation and migration of immature germ cells in the mussel, *Mytilus galloprovincialis*: observation of the expression pattern in the *M. galloprovincialis vasa*-like gene (*Myvlg*) by in situ hybridization. Dev. Genes Evol. 220: 139-149, 2010.

Rabinowitz JS, Chan XY, Kingsley EP, Duan Y, Lambert JD. *Nanos* is required in somatic blast cell lineages in the posterior of a mollusk embryo. Curr. Biol. 18: 331-336, 2008.

Raz E. The function and regulation of *vasa*-like genes in germ-cell development. Genome Biol. 3: 1017, 2000.

Rebscher F, Banisch TU, Raible F, Arendt D. Vasa unveils a common origin of germ cells and of somatic stem cells from the posterior growth zone in the polychaete *Platynereis dumerilii.* Dev. Biol. 306: 599-611, 2007.

Rongo C, Lehman R. Regulated synthesis, transport and assembly of the *Drosophila* germ plasm. Genetics 12: 102-109, 1996.

Saffman EE, Lasko P. Germline development in vertebrates and invertebrates. Cell. Mol. Life Sci. 55: 1141-1163, 1999.

Sawatari E, Shikina S, Takeuchi T, Yoshizaki G. A novel transforming growth factor-β superfamily member expressed in gonadal somatic cells enhances primordial germ cell and spermatogonial proliferation in rainbow trout (*Oncorhynchus mykiss*). Dev. Biol. 301: 266-275, 2007.

Schulz C, Kiger AA, Tazuke SI, Yamashita Y, Pantalena-Filho LC, Jones DL, *et al*. A misexpression screen reveals effects of bag-of-marbles and TGF beta class signaling on the *Drosophila* male germ-line stem cell lineage. Genetics 167: 707-723, 2004.

Shibata N, Umesono Y, Orii H, Sakurai T, Watanabe K, Agata K. Expression of *vasa*(*vas*)-related genes in germline cells and totipotent somatic stem cells of planarians. Dev. Biol. 206: 73-87, 1999.

Sonnenblick BP. The early embryology of *Drosophila melanogaster*. In: Demerce M (ed), Biology of *Drosophila*, Wiley, New York, USA, pp. 62-167, 1950.

Strome S, Wood WB. Immunofluorescence visualization of germ-line-specific cytoplasmic granules in embryos, larvae, and adults of *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. 79: 1558-1562, 1982.

Swartz SZ, Chan XY, Lambert JD. Localization of *Vasa* mRNA during early cleavage of the snail *Ilyanassa*. Dev. Genes Evol. 218: 107-113, 2008.

ten Dijke P, Miyazono K, Heldin CH. Signaling inputs converge on nuclear effectors in TGF-β signaling. Trends Biochem. Sci. 25: 64-70, 2000.

Thomson T, Lin H. The biogenesis and function of PIWI proteins and piRNAs: progress and prospect. Annu. Rev. Cell Dev. Biol. 25: 355-376, 2009.

Toyooka Y, Tsunekawa N, Takahashi Y, Matsui Y, Satoh M, Noce T. Expression and intracellular localization of mouse *Vasa*-homologue protein during germ cell development. Mech. Dev. 93: 139-149, 2000.

Van den Biggelaar JAM, Guerrier P. Dorsoventral polarity and mesentoblast determination as concomitant results of cellular interactions in the mollusk *Patella vulgata*. Dev. Biol. 68: 462-471, 1979.

Vincent CG, Yamaguchi E, Boyle MJ, Seaver EC. Somatic and germline expression of *piwi* during development and regeneration in the marine polychaete annelid *Capitella teleta*. EvoDevo 2: 10-28, 2011.

Wilson EB. The cell-lineage of *Nereis*. J. Morphol. 6: 361-466, 1892.

Woods FH. History of the germ cells in *Sphaerium striatinum* (Lam.). J. Molphol. 51: 545-595, 1931.

Ying Y, Liu XM, Marble A, Lawson KA, Zhao GQ. Requirement of Bmp8b for the generation of primordial germ cells in the mouse. Mol. Endocrinol. 14: 1053-1063, 2000.

Ying Y, Zhao GQ. Cooperation of endoderm-derived BMP2 and extraembryonic ectoderm-derived BMP4 in primordial germ cell generation in the mouse. Dev. Biol. 232: 484-492, 2001.

Yoon C, Kawakami K, Hopkins N. Zebrafish *vasa* homologue RNA is localized to the cleavage planes of 2- and 4-cell-stage embryos and is expressed in the primordial germ cells. Development 124: 3157-3165, 1997.