Establishing the germline in spiralian embryos

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ABSTRACT  Elucidating the origin of germ cells in embryos and larvae is often obscured by the fact that the typical germ cell markers vasa, nanos and piwi are not exclusively expressed in primordial germ cells (PGCs), but are also commonly found in undifferentiated somatic tissues and stem cells as part of an evolutionary conserved ‘germline multipotency program’ (Juliano et al., 2010). Hidden in the crowd of undifferentiated cells, the PGCs have occasionally been overlooked and their formation during early embryogenesis was only revealed recently by new methodological approaches (e.g. Wu et al., 2011). Spiralians are excellent model organisms to deepen our understanding of PGC formation, given the highly stereotypical cleavage that occurs during embryogenesis. In these species, detailed cell lineage studies enable the tracing of single cells up to gastrulation stages. Here, I review our knowledge of the origin of PGCs in these invertebrates. Similarities in PGC formation among spiralian phyla as well as peculiarities of the highly derived clitellates are discussed with respect to developmental mode and evolution. Furthermore, the issue of gonad regeneration in platyhelminths and the asexually reproducing oligochaete Enchytraeus japonensis is addressed. An alternative strategy of compensating for caudal regeneration is presented for the polychaete Platynereis dumerilli. Finally, the molecular bases of PGC specification and the question of germ-plasm are discussed.

KEY WORDS: Spiralia, primordial germ cell, mesoblast, vasa, nanos, PIWI

Introduction

Sexual reproduction and thus the acquisition of new traits require heterozygous gametes, which arise by meiosis from oogonia or spermatogonia during gametogenesis. Gonia are descendants of the ‘primordial germ cells’ (PGCs), which in turn form during embryonic development by asymmetric cleavage from multipotent cells called ‘presumptive primordial germ cells’ (pPGCs). While the pPGCs contribute to both soma and germline, the PGCs are the first cells of a developing organism exclusively restricted to a germ cell fate (Extavour and Akam, 2003; Nieuwkoop and Satasurya, 1979; Wylie 1999). Following their formation, the PGCs often become transcriptionally and mitotically quiescent and migrate actively towards the developing somatic gonads (Seydoux & Brown 2006). In many species exhibiting a constant production of gametes, such as Drosophila, the PGCs are the first to cellularize after fertilization (Huettner, 1923). Similarly, the single germline progenitor P4 in C. elegans arises already at the fourth cleavage of the embryo (Seydoux and Fire, 1994). In many deuterostome and lophotrochozoan phyla, the PGCs are specified during blastula or gastrula stages, such as in the sea urchin Stronglocentrotus purpuratus, the ascidian Ciona intestinalis, the cephalochordate Branchiostoma floridae, the vertebrates Danio rerio, Gallus gal-
lus, Xenopus laevis, Mus musculus, as well as in the polychaete Platynereis dumerilii and the gastropod Crepidula fornicata (Lyons et al., 2012; Raz, McLaren, 2003; 2002; Rebscher et al., 2012; Shirai-Kurabayashi et al., 2011; Taguchi et al., 2012; Tsunekawa et al., 2000; Wu et al., 2011; Yajima and Wessel, 2011a). In species exhibiting high regenerative capacities, such as cnidarians and platyhelminths, the potential to develop PGCs from stem cells even is maintained lifelong (Mochizuki et al., 2001; Peter et al., 2004; Rink, 2013).

Two distinct mechanisms of PGC specification exist in the animal kingdom: in Drosophila, C. elegans, or Xenopus laevis, the future PGCs inherit germ plasm containing maternal determinants early in development (Ikenishi et al., 1986; Mahowald, 2001; Strome and Wood, 1982). Alternatively, the PGCs are specified from undifferentiated cells by inductive signals, as it has been described from such diverse species as the cnidarian Nemastostella and the mouse (Extavour et al., 2005; Tsang et al., 2001). Examples for both mechanisms can be found throughout the animal kingdom, but the latter mechanism, sometimes referred to as ‘epigenesis’, has been proposed to constitute the ancestral mode of PGC specification in metazoans (Extavour and Akam, 2003). This assumption is supported by the finding that the PGCs often become detectable only late in development within undifferentiated tissue expressing the typical germ cell markers vasa, nanos and piwi (reviewed in Extavour and Akam, 2003). Experimental evidences for specific inductive cues such as BMP2/4, as well as a demonstration of the full totipotency of their putative founder cells however are so far restricted to few species, i.e. mouse, axolotl and the cricket Gryllus bimaculatus (Donouge et al., 2014; Johnson et al., 2003; Ying et al., 2001). Recently, the hypothesis of an evolutionary ancestry of late PGC specification by inductive signals has been challenged by the use of additional molecular markers as well as new methodological approaches including transgenic lines, lineage tracing, live imaging, and EdU pulse/chase experiments, which allows for the identification of PGCs at much earlier stages (Fischer and Arendt, 2013; Lyons et al., 2012; Raz, 2002; Rebscher et al., 2012). It became evident by these studies, that in some species, vasa, nanos and piwi expressing cells do not form a homogeneous population of undifferentiated cells, from which the PGCs are specified by inductive signals, but rather constitute a mix of PGCs and undifferentiated somatic cells. In the cephalochordate Branchiostoma floridae for example, the PGCs were previously assumed to emerge late in development from the growth zone (Nieuwkoop and Satabury, 1979). Recently however, it has been shown that the first two PGCs are already detectable at the onset of gastrulation, while the nanos, piwi, and vasa expressing cells of the posterior growths zone form later around the transition from mid- to late gastrula stages (Wu et al., 2011). During neurulation, the PGCs merge with the growth zone, and are indistinguishable from it until larval stages. Similarly, the PGCs of the polychaete Platynereis dumerilii are initially indistinguishable from the cells of the mesodermal posterior growth zone by both morphology and gene expression pattern. However, they arise several hours earlier in development and are mitotically quiescent, in contrast to the proliferating somatic stem cells (Rebscher et al., 2012). Putative PGCs forming a subpopulation within larger clusters of vasa expressing cells are also found in the gastropod Halitosis asinina (Kranz et al., 2010). In the platyhelminths Dugesia japonica, Schmidtea mediterranea and Schistosoma mansoni, the expression of the germ cell marker nanos is limited to a subpopulation within the piwi/argonaute-positive neoblasts (Handberg-Thorsager and Saló, 2007; Sato et al., 2006; Shibata et al., 2010; Wang et al., 2013).

Taken together, the presence of PGCs as a distinct subpopulation within vasa, nanos, and piwi positive cells indicates, that the PGCs not necessarily arise rise from these stem cells during ontogenesis. Rather, the co-expression of these genes might be explained by the common ontogenetic and evolutionary origin of these two cell types, which is reflected by a conserved molecular ‘germline multipotency program’ (Gazave et al., 2013; Juliano et al., 2010; Seydoux and Strome, 1999).

A second obstacle when investigating the origin of the germ cells is the reliance on maternal vasa, nanos, and piwi transcripts as germ cell markers. These mRNAs are found broadly distributed in embryos of many species as maternal transcripts, and only the zygotic transcripts can be considered to be truly germ cell-specific (Lasko, 2011; Pilon and Weisblat, 1997 Rebscher et al., 2007; Shimomiya et al., 2000). Maternal transcripts might be furthermore be

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**Fig. 1. Generalized cell-lineage of the D-quadrant in spiraline embryos.**

The zygote (black) divides twice, yielding four large blastomeres; the macromeres A-D (white and grey), corresponding to the four quadrants of the embryos. For simplification, only the fate of the D-quadrant (grey) is shown from here on. The D-macromere bud of generations of micromeres called 1d, 2d, 3d… (grey) towards the animal pole during spiral cleavage. The micromeres continue dividing in animal/vegetal orientation (not shown), with exception of the somatoblast 2d (blue) and the mesoblast 4d (red), which both divide bilaterally, forming the precursors of trunk ectoderm and mesoderm, respectively. The 4d-micromere divides bilaterally into the left and right mesoblast (MR and ML, yellow). The mesoblasts in turn produce small blast cells (green) named 1m, 2m, 3m… according to their birth rank, while they maintain their stem cell characteristics (1M, 2M, 3M…). The mesodermal blast cells are the founders of diverse cell types and tissues, such as the primordial germ cells, the mesodermal posterior growth zone, the trunk mesoderm and other structures (see in text). Blastomere nomenclature according to Lyons et al., 2012. Cell lineage modified after (Fischer and Arendt, 2013; Lyons et al., 2012; Rebscher et al., 2012; Schneider and Bowerman, 2007).
initially subject to translational repression (Vasudevan et al., 2006), while PGC specification might be mediated in the meantime by maternal proteins. Focusing on the analysis of maternal transcripts alone may therefore provoke misleading conclusions regarding the timing and mode of PGCs specification in some species. Unfortunately, specific antibodies allowing the detection of these proteins are often not available in non-model organisms. Therefore the localization of these proteins in early forming PGCs, hidden between vasa, nanos, and piwi expressing somatic cells, might have been overlooked.

Taken together, increasing evidence suggests that the PGCs might be specified early in more species than previously expected, with maternal proteins orchestrating this process. In the absence of specific antibodies for early PGCs, lineage analysis is a powerful tool to address this question in depth. The highly stereotypical cleavage pattern of spiralian embryos (Lambert, 2010) renders them perfect model organisms for the investigation of the origin of the PGCs. Here, I will review the current knowledge on PGC formation in this interesting, yet so far rarely investigated group of invertebrates.

**Origin of the germ cells in spiralian embryos**

Cell lineage has been studied extensively in spiralian embryos: at the end of the 19th century pioneering studies were conducted in the gastropod *Crepidula fornicata* (Conklin, 1897), as well as in the polychaetes *Nereis limbata* (succinea) and *Platynereis megalops* (Wilson, 1892). Spiralians share a highly conserved early cleavage pattern and fate map (Gline et al., 2011; Henry and Martindale, 1998; Lambert, 2010). A common feature is the formation of the large micromere 4d (also called M in *Nereis spp.* or DM in the leech), which occurs by asymmetric cleavage during the 6th cleavage round (Fig. 1 and Fischer and Arendt, 2013; Gline et al., 2011). This blastomere then divides bilaterally, yielding a pair of stem cells called mesoblasts or mesoteloblasts, respectively, depending on their size. In some spiralians, the 4d descendants additionally contribute to ectodermal and endodermal tissue, and are therefore occasionally referred to as mesectoblasts or mesendoblasts, respectively. The paired mesoblasts have previously been addressed by various names in different species such as MR/L in the leeches *Helobdella austsinensis*, and *H. robusta*, M1/2 in *Nereis spp.*, 4d in *Platynereis*, 4dR/L in *Crepidula*, and Mr/L in *Ilyanassa obsoleta* (Dorrestijn, 1990; Ackermann et al., 2005; Henry et al., 2010; Lambert, 2010; Wilson, 1892). However, a uniform spiralian nomenclature was recently devised (Lyons et al., 2012) and will be used throughout this review in order to facilitate cross species comparisons.

The right and left mesoblasts MR/L undergo asymmetrical stem-cell-like divisions, producing small, undifferentiated cells called ‘blast cells’. While in the course of this review, only the mesodermal blast cells are discussed, it has to be pointed out the ‘blast cells’ also occur in other lineages e.g. in the ciliatedtates (Weisblat and Shankland, 1985). The blast cells are numbered 1mR/L, 2mR/L and so on, according to their birth rank (Fischer and Arendt, 2013; Gline et al., 2011; Lyons et al., 2012 and Fig. 1). In ciliatedtates, the blast cells are exclusively produced towards the anterior pole of the developing worm, forming regular, elongating germ bands (Gline et al., 2011; Weisblat and Shankland, 1985). Instead, the blast cells of the gastropods *Crepidula fornicata* and *Ilyanassa obsoleta* are budded off in alternating directions, thus forming a blast cell cluster with the mesoblasts lying in the center (Lyons et al., 2012; Rabinowitz et al., 2008; Swartz et al., 2008). In the trochophore larva of the polychaete *Platynereis dumerilii*, the blast cells also form a cluster, but since they bud forth in an anterior direction only, the cluster lays anterior of the mesoblasts (Fischer and Arendt, 2013; Rebscher et al., 2012).

In most spiralian species, for which cell lineage has been analyzed to this level, the blast cells or their descendants, respectively, are the source of the PGCs (Cho et al., 2013; Fischer and Arendt, 2013; Kato et al., 2013; Kranz et al., 2010; Lyons et al., 2012; Rebscher et al., 2012; Schneider and Bowerman, 2007; Wilson, 1892 and Table 1). In the bivalve *Sphaerium striatium* instead, the mesoblasts themselves have been proposed to cease dividing after 3 rounds of asymmetric cleavage and to develop into a pair

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**Fig. 2. Location of primordial germ cells (PGCs) in spiralian larvae and juveniles.** (A) In the gastropod *Crepidula fornicata*, the right PGC (white asterisk) is labelled upon injection into the blast cell 2mR² in this veliger larva, lateral view. The crystal cell (black asterisk), a somatic descendant of 2mR², is also labelled (image courtesy of Jonathan Henry). (B) Piwi expressing PGCs in the leech *Helobdella austsinensis* in the midbody segments M6 (female genital segment, green asterisk) and M8 – 17 (future male genital segments, red asterisk) in a stage 10 juvenile. Nanos expression (red) is lacking in the future oogonia (image modified after Cho et al. 2013 with permission from MBE). (C) Vasa positive PGCs (black asterisks) in the future female (X) and male (XI) genital segments of *Tubifex tubifex* (image by Takashi Shimizu, with permission from the author). (D) Four single PGCs (white asterisks) leaving the mesodermal posterior growth zone (arrow) in a *Platynereis dumerilii* three segmented young worm. Immunohistochemical detection of Vasa protein. (E) Nanos expressing PGCs (white asterisks) in the location of the future testes in the planarian *Schmidtea mediterranea* (image courtesy of Emili Saló).
of large, quiescent PGCs (3MR/L or M and M1,2,2.2., Woods, 1932). Similarly, Swartz et al. 2008 suggested that the corresponding cells in the gastropod Ilyanassa (here called 4dRL) might constitute the founders of the germline based on their prolonged vasa expression. However, the latter authors do not rule out that vasa expression in the mesoblasts might rather reflect a function in the somatic lineage. Being more in line with the notion, that generally the blast cells are the founders of the germline in spiralians, the Ilyanassa Nanos protein has been found to persist longest in the nuclei of the blast cells 2mRL (here called 4dL/R112, Rabinowitz et al., 2008), which are homologous to the direct PGC precursors in the gastropod Crepidula (Lyons et al., 2012). In the current absence of markers for PGCs in mollusk larvae, the identity of the PGCs in Ilyanassa and Sphaerium cannot be unambiguously answered at the moment. Modern lineage tracing methods or the use of species-specific Vasa antibodies in larval stages and beyond would be suitable approaches to address this open question.

In the nemertean Cerebratulus lacteus, the planarian Helobdella robusta, the sipunculan Phascolosoma esculenta and the myzostomid Myzostoma cirriferum, current cell lineage studies unfortunately do not extend beyond 4d, therefore the precise origin of their germline remain to be elucidated (Boyer et al., 1998; Henry and Martindale, 1998; Weigert et al., 2013; Ying et al., 2009 and Table 1).

In clitellates, the PGCs form directly at the site of the future gonads in their respective segments (Cho et al., 2013; Kato et al., 2013 and Fig. 2). Similarly, nanos expressing putative male germ cells are first detectable at the location of the future testes in the planarian Schmidtea mediterranea (Handberg-Thorsager and Saló, 2007 and Fig. 2). In the oyster Crassostrea gigas, the gastropod Haliotis asinina or the polychaete Capitella telata, the PGCs initially form transient, bilateral clusters in the trunk region of the larvae (Dill and Seaver, 2008; Kranz et al., 2010; Fabioux et al., 2004 and Fig. 2). In other species, such as in the mollusks Crepidula fornica and Sphaerium striatum or the polychaete Platynereis dumerilii, the PGCs reside in the larval mesoderm as single cells (Lyons et al., 2012; Rebscher et al., 2007; Woods, 1931 and Fig. 2). In Platynereis dumerilii, the four PGCs migrate actively as single cells from the posterior growth zone along the mesoderm towards the neck region of the larva, which acts as a transient gonad (Rebscher et al., 2007). PGCs migration most likely also occurs in the polychaete Capitella telata (Giani et al., 2011). Whether active migration of PGCs occurs in other spiralian species as well, or whether the PGCs are rather passively transported during gastrulation movements, so far remains

**TABLE 1**

ORIGIN OF PRIMORDIAL GERM CELLS IN SPIRALIAN EMBRYOS

<table>
<thead>
<tr>
<th>Phylum/Class</th>
<th>Species</th>
<th>Un-known</th>
<th>Proposed 4d origin</th>
<th>4d origin</th>
<th>PGC identity</th>
<th>References</th>
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<tr>
<td>Annelida*</td>
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<td></td>
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<td>11mRL</td>
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<td>10mRL</td>
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<tr>
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<tr>
<td></td>
<td>Platynereis dumerilii</td>
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<td>1mRL</td>
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<tr>
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<tr>
<td></td>
<td>Schmidtea mediterranea</td>
<td>X</td>
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</table>

The table summarizes data on the PGC lineage from different taxa. In all spiralian species, in which this issue has been studied so far, the PGCs are known or are proposed to descend from the mesoblast 4d. (*) Please note: although a recent phylogeny groups the Citellata within the Sedentaria (Struck et al., 2011), the classical annelid classes Citellata, Polychaeta, Spiculida (Phascolosomatoidea) and Myzostomida are used in the course of this review. (**) The studies on leech development were performed on two closely related species, Helobdella robusta (Kang et al., 2002) and Helobdella austiniensis (Cho et al., 2013). (***) This finding has not been verified by molecular markers, yet. (****) The identity of the PGCs in Ilyanassa is still under debate. While Swartz et al. 2008 propose that the mesoblasts 3MR/L develop into PGCs, Nanos protein expression pattern suggest that they are descendants of the blast cells 2mRL (Rabinowitz et al., 2008), similar to the situation found in Crepidula (Lyons et al., 2012). Blastomere nomenclature according to Lyons et al., 2012. Evidence for PGCs: t = transcript (vasa, nanos or piwi), p=protein (vasa or nanos), l=ligation tracing, h=histology.
From early to late: a heterochronic shift in germline segregation

In the polychaetes Platynereis dumerilii and Salmacina dysteri well as in the mollusks Crepulida fornicata, the PGCs either constitute, or are derived from the first two pairs of blast cells 1mR/L and 2mR/L (Lyons et al., 2012; Malaquin, 1925; Rebscher et al., 2012; and Fig. 3). A different situation is found in citellates: in Helobdella spp., and Tubifex tubifex only the blast cells from the the 10th or 16th birth rank on, respectively, contribute to the germline (Cho et al., 2013; Kato et al., 2013; Kang et al., 2002 and Fig. 3). In Tubifex tubifex, the birth rank of the blast cell directly corresponds to the number of their future adult body segment. Thus the PGCs derived from 10mR/L and 11mR/L will later end up in the genital segments X and XI, which harbor the female and male gonads of this hermaphroditic species. Additional vasa expressing blast cell descendants of unknown fate, therefore carefully called ‘vasa-expressing cells’ or ‘presumptive PGCs’, transiently occur in the adjacent segments VIII, IX, and XIII. However, these cells do not contribute to the germline and become undetectable in juveniles (Kato et al., 2013; Oyama et al., 2007). In the leeches Helobdella austinensis and H. robusta, the first midbody segments are formed by the pair of blast cells 11mR/L (= ‘sm5’), while earlier blast cells contribute either to non-segmental structures (1mR/L to 6mR/L = ‘sm1-6’) or head structures (7mR/L to 10mR/L = ‘sm1-4’). Therefore, the descendants of the blast cells 16mR/L (= ‘sm10’) that will produce the most anterior PGCs of the leech, are located in midbody segment M6 (Gline et al., 2011, Cho et al., 2013 and Fig. 3). As in Tubifex tubifex, additional PGCs are specified transiently in Helobdella spp., in segments flankng the genital segments, i.e. in the midbody segments M14-M17 in H. robusta and in M14 –M18 H. austinensis (Cho et al., 2013).

It is tempting to speculate, that the heterochronic shift observed in PGC formation in citellates might be correlated with their successive, lineage-dependent segmentation process: in these species, the teloblasts located in the posterior growth zone continuously bud off bandlets of blast cells towards the anterior pole of the embryo. By lineage tracing experiments, it has been shown that blast cells of a given birth rank contribute to distinct segments in invariant patterns (Rivera and Weisblat, 2008; Weisblat and Shankland, 1985). Notably, in both Tubifex tubifex and Helobdella spp. the PGCs never cross the boundaries of the segments in which they are born (Cho et al., 2013; Kato et al., 2013). Thus, by delaying PGC formation, this process seems to be synchronized with the formation of the future gonadal segments in citellates.

Germ cells, asexual reproduction, and regeneration

Many spiralian species are able to regenerate large parts of their body, including regions harboring the gonads. This raises the question of how these species maintain their fertility throughout regeneration or asexual reproduction. Regeneration of body regions initially lacking germ cells is best known from platyhelminths. The extraordinary regenerative capacities of these spiralian rely on multipotent stem cells called neoblasts distributed within the trunk mesenchyme (Rink, 2013; Wang Zaya et al., 2007). Recent investigations in the planarians Dugesia japonica and Schmidtea mediterranea have revealed that at least two different populations of neoblasts exist: while all neoblasts express the stem cell marker piwi, the germ cell marker nanos is restricted to a subpopulation of so called ‘germinal stem cells’ (Handberg-Thorsager and Saló, 2007; Sato et al., 2006, Shibata et al., 2010 and Fig. 2E). In the trematodes Schistosoma mansoni, which exhibits multiple generations of asexually propagating larval stages, clusters of so called ‘germinal cells’ exist which share high similarities in terms of morphology and gene expression pattern with planarian neoblasts: besides the piwi related piRNA interacting protein agonauta, three vasa-like transcripts are up-regulated in these
As in free living platyhelminths, only a subpopulation of these cells expresses nanos and exhibits a strongly prolonged cell cycle typical for PGCs in this parasite (Wang et al., 2013). Within the annelids, the asexually reproducing clitellate Enchytraeus japonensis is able to regenerate fully functional gonads from any segment due to the life-long presence of so-called ‘germline stem cells’ scattered throughout the trunk as a PGC reservoir (Sugio et al., 2008; Tadokoro et al., 2006). Similarly, in both Helobdella spp. and Tubifex tubifex, additional vasa/nanos/piwi positive blast cell descendants are transiently detectable in non-genital segments (Oyama and Shimizu, 2007, Cho et al. 2013 Kato et al., 2013, and Fig. 3). However, unlike in Enchytraeus japonensis, these cells have not been reported to contribute to the germline. Whether they are evolutionary remnants of a PGC reservoir as found in Enchytraeus japonensis, or rather constitute purely somatic stem cells, remains to be elucidated. So far nothing is known about regeneration of germ cells in mollusks or nemerteans.

Besides replacing lost germ cells by stem cells, a different strategy to cope with regeneration is found in the polychaete Platynereis dumerilii. Here, the PGCs migrate within a few days away from the posterior growth zone towards an anterior location, thus far away from the tail, which can autotomize when the worm is threatened by a predator (Rebscher et al., 2007 and Fig. 4). Therefore, it comes as no surprise that Platynereis dumerilii can easily regenerate all but the first 5 of its trunk segments whilst still remaining fertile (Hofmann, 1966).

**Fig. 4. Anterior location of primordial germ cells (PGCs) and caudal regeneration in Platynereis dumerilii.** (A) The four PGCs (green) reside just anterior to the mesodermal posterior growth zone in the 48 hpf trochophore larva. (B) At the end of larval development at day 4, the PGCs leave the growth zone and migrate anteriorly, until they reach a transient ‘niche’ in the neck region of the young worm (C). Loss of caudal segments by autotomy or amputation does not affect fertility from then on, since PGCs reside in a more anterior location. The worms are able to regenerate lost body segments as long as the head and at least 5 trunk segments remain (red dotted line, Hofmann, 1966). (D) At the onset of gametogenesis in juveniles > 20 segments, the PGCs start to proliferate, migrate posteriorly into the trunk mesoderm and form gonial clusters. (E) The gonial clusters enter the coelomic cavity, where they develop freely floating into gametes (here: oocytes).

The question of the PGC determinants: molecules, granules and germplasm

In species in which the germline is established early in development by the inheritance of maternal determinants, maternal transcripts and proteins are assembled into ribonucleotide particles (RNPs) also called germinal granules, polar granules, or ‘nuage’ (Ewen-Campen et al., 2010; Wylie, 1999). In many spiralian, the germinal granules are compounds of a perinuclear yolk free cytoplasm formed during oogenesis. Upon fertilization, the yolk free cytoplasm - also called ‘teloplasm’ in the clitellates – is localized towards one (Fabiose et al., 2004; Rebscher et al., 2007) or both (Kang et al., 2002; Kato et al., 2013) poles of the fertilized egg in the course of ooplasmic segregation (Fig. 5, red box). During the following unequal cleavages, the yolk free cytoplasm is then preferentially segregated into the D-quadrant and later enriched in 4d, the paired mesoblasts MRL/L, and the PGCs, respectively, suggesting an...
early specification of the PGCs by inheritance of these cytoplasmic compounds in *Crassostrea gigas*, *Helobdella sp.*, *Platynereis dumerilii* and *Tubifex tubifex*. In the gastropods *Crepidula fornicate*, *Ilyanassa obsoleta*, and *Haliotis asinina*, as well as in the polychaete *Capitella telata*, no asymmetric distribution of germ cell determinants in either fertilized eggs or early cleavage stages has been reported based on expression studies of *vasa*, *nanos* and *piwi*. Instead, these transcripts are found associated to the nuclei or centrosomes of all blastomeres of the cleaving embryos (Fig. 5B, blue box). A specific accumulation of *vasa*, *nanos* and *piwi* is only found later, when the mesoblasts MR/L form (Dill and Seaver, 2008; Giani et al., 2011; J. J. Henry et al., 2010; Swartz et al., 2008). However, it cannot be ruled out that future analyses at the protein level using specific antibodies will unravel the existence of asymmetrically segregating cytoplasmic determinants in these species.

In general, maternal proteins and transcripts have to be subject to a tight temporal and spatial regulation during development (Vasudevan et al.2006). In ecdysozoan and vertebrate species, Vasa is known to enhance the translation of *nanos*, *cyclin B* and *gurken* in PGCs by its RNA unwinding capacity (Raz, 2000; Yajima and Wessell, 2011). Nanos in turn acts as a translational repressor, especially of genes related to somatic differentiation. Furthermore, it is involved in the establishment of transcriptional and mitotic quiescence in the PGCs (Asaoka-Taguchi et al., 1999; Leatherman and Jorgens, 2003). The stability of maternal transcripts as well as their translation in the germline is additionally known to be regulated by miRNAs (Giraldez et al., 2006; Jin and Xie, 2006; Mishima et al., 2006). How the complex posttranscriptional networks operating in PGCs act to orchestrate PGC formation in spiralian species still remains to be elucidated.

**Conclusions**

Their highly stereotypic cleavage pattern in conjunction with an increasing availability of molecular tools render spiralian embryos excellently suited to study the cellular and molecular events leading to the formation of the PGCs. Our knowledge on function and expression pattern of developmentally relevant genes in spiralian species has greatly increased within the last decade. While an origin of specific trait, the molecules involved in PGC specification such as PGCs, their expression pattern of developmentally relevant genes in spiralians leading to the formation of the PGCs. Our knowledge on function and expression pattern of developmentally relevant genes in spiralian species has greatly increased within the last decade. While an origin of the PGCs from the mesoblast lineage seems to constitute a spiralian specific trait, the molecules involved in PGC specification such as Vasa, Nanos, and PIWI have turned out to be highly conserved between spiralians and other metazoans. Insights from spiralians as representatives of the superphylum Lophotrochozoa complement data obtained earlier from ecdysozoan and deuterostome species, and thus round up our understanding of the evolution of a distinct germline at the base of the animal kingdom.

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