

Mechanisms of germ cell specification across the metazoans: epigenesis and preformation

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Summary

Germ cells play a unique role in gamete production, heredity and evolution. Therefore, to understand the mechanisms that specify germ cells is a central challenge in developmental and evolutionary biology. Data from model organisms show that germ cells can be specified either by maternally inherited determinants (preformation) or by inductive signals (epigenesis). Here we review existing data

on 28 metazoan phyla, which indicate that although preformation is seen in most model organisms, it is actually the less prevalent mode of germ cell specification, and that epigenetic germ cell specification may be ancestral to the Metazoa.

Supplemental data available online

Introduction

Germ cell segregation is an important problem in developmental biology, as it addresses how the fundamental distinctions between germ cells and somatic cells are initiated and maintained throughout development. The timing and mechanism of this segregation are also important for our understanding of evolution, for these influence the selective pressures that act on germ cells prior to gametogenesis, and so have important consequences for the selection of heritable variation (Extavour and García-Bellido, 2001).

Primordial germ cells of many different species share intrinsic qualities that differentiate them from somatic cells, often long before the somatic gonads are formed. However, there has been a history of disagreement as to how germ cells may be identified, and when in development the germ line is specified. In this review, we examine descriptive and experimental data on the timing and mode of origin of the germ cell lineage throughout the animal kingdom.

There are at least two distinct modes of germ line segregation in animals, both of which are well documented from experimental studies in model systems. These modes are summarised in Box 1. In some species, germ cells can easily be identified very early in embryogenesis, when their differentiation as germ cells is assured by the localisation of maternally inherited determinants before, or immediately following, fertilisation ('preformation'). In other species, germ cells are not observed until later in development, and arise as a result of inductive signals from surrounding tissues ('epigenesis').

To avoid confusion, the terminology that we will use in this review for germ cells and their precursors follows the nomenclature of Nieuwkoop and Sutasurya (Nieuwkoop and Sutasurya, 1979). When germ cells become sexually differentiated and enter the first stages of gametogenesis, they are collectively termed gonidia (oogonia and spermatogonia). Through the processes of oogenesis and spermatogenesis,

gonidia become oocytes and spermatocytes, maturing finally into ova and spermatozoa, respectively. Many organisms generate their gonidia from cells capable of almost indefinite rounds of asymmetric, self-renewing mitotic divisions; these cells are called germ line stem cells. The first cells that will give rise exclusively to germ cells by clonal mitotic divisions are called primordial germ cells (PGCs). The precursors to the PGCs, which are often initially morphologically indistinguishable from the surrounding somatic cells, are called presumptive primordial germ cells (pPGCs). These divide mitotically to produce one PGC and one somatic cell.

Several aspects of germ cell morphology and function are clearly similar across many phyla of animals (Box 2). In spite of this, the mechanisms that generate germ cells appear to be highly variable, involving either prelocalised determinants or inductive processes. Previous monographs on comparative germ cell specification are now over 20 years old (Bounoure, 1939; Nieuwkoop and Sutasurya, 1979; Nieuwkoop and Sutasurya, 1981; Wolff, 1964). This review examines over 150 years worth of data on modes of germ cell specification in 28 metazoan phyla, expanding previous studies with the addition of recent molecular and experimental data. In this article we have also mapped the data onto a modern phylogeny of the Metazoa, to address the question of the ancestral mode and evolution of germ cell specification mechanisms. We conclude, in agreement with earlier surveys, that epigenesis is a more frequent mode of germ cell specification than preformation. This finding, together with data on germ cell origin in basal metazoans, suggests that epigenesis may have been the ancestral mechanism of early metazoan germ cell segregation. Our conclusion challenges a widely held view in the field of developmental biology (e.g. Wolpert, 1998) that epigenetic germ cell determination is an exception, and that most animals use localised cytoplasmic determinants to specify the germ line.

In the following sections, we review data on the earliest specification of germ cells in development, in both the

bilateral animals (see Box 3) and their outgroups. We first consider findings in the few well-studied model organisms, and then the much wider range of studies on non-model organisms. [As we present the conclusions of the extensive studies on model organisms only briefly, we refer the reader to other

reviews for further detail (Houston and King, 2000b; Matova and Cooley, 2001; Noce et al., 2001; Saffman and Lasko, 1999; Wylie, 2000)].

Germ cell specification in model systems

Preformation in germ cell development

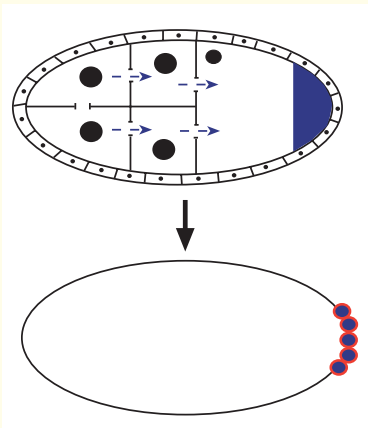
The most comprehensive data set on the molecular mechanisms of germ cell specification is that available for *Drosophila melanogaster*. Before blastoderm formation, precocious cellularisation at the posterior pole of the embryo creates four to five pole cells (Huettner, 1923), which are the exclusive progenitors of the germ line (Fig. 1A) (Technau and Campos-Ortega, 1986; Williamson and Lehmann, 1996). The pole cells acquire PGC identity through the inheritance of specialised pole plasm, which is assembled at the posterior pole of the oocyte before fertilisation (reviewed by Mahowald, 2001). Transplantation (Illmensee and Mahowald, 1974; Illmensee and Mahowald, 1976; Illmensee et al., 1976) or forced assembly of pole plasm in ectopic sites, such as at the anterior of the oocyte (Ephrussi and Lehmann, 1992), results in PGC formation at these sites, which indicates that the pole plasm is a true germ cell determinant, and not simply a germ cell marker. Germ cell specification in *D. melanogaster* is obviously driven by preformation. In fact, it seems that all Diptera (see Box 3) localise pole plasm and form pole cells, although this preformation with respect to germ line segregation is not representative of most insects (see discussion below).

Caenorhabditis elegans embryos contain electron-dense granules called P granules, which are scattered evenly throughout the cytoplasm before and just after fertilisation, but which then move to the posterior of the embryo during pronuclear fusion (Hird et al., 1996). These granules are asymmetrically segregated during the unequal early cleavages so that the small P₄ blastomere of the 16- to 24-cell embryo contains all of them and is the single PGC (Deppe et al., 1978; Strome and Wood, 1982). *C. elegans* thus provides a second example where germ cells are likely to be specified by preformation. In other nematodes that have been studied (see Table 2, a fully referenced version of which is available online at <http://dev.biologists.org/supplemental/>), the P₄ cell is always the PGC, although there are differences in the timing of P₄ formation relative to total embryonic developmental time and to the appearance of the other blastomeres.

Studies on anuran amphibian embryos (see Box 3) have provided some of the first experimental evidence of preformation and the role of germ plasm in vertebrate germ cell specification (Bounoure, 1939). During *Xenopus laevis* oogenesis, specialised cytoplasm is synthesized and localised to the vegetal subcortex. This vegetal plasm is characterised by an accumulation of mitochondria (sometimes called the mitochondrial cloud, see Box 3) that is associated with electron-dense granules, and specific proteins and RNAs (Heasman et al., 1984; Houston and King, 2000a; Kloc et al., 2001; Kloc et al., 2002; Zhou and King, 1996). Following fertilisation, the vegetal plasm forms patchy aggregates in the vegetal hemisphere, which are segregated unequally into cleavage cells and finally accumulate specifically in a few cells that become the PGCs (Whittington and Dixon, 1975). Experiments that compromise the vegetal plasm by physical

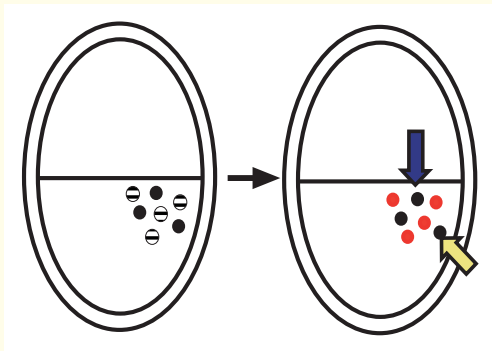
Box 1. Modes of germ cell specification: preformation and epigenesis

Preformation



During oogenesis in *Drosophila melanogaster*, RNAs and proteins are synthesised by the nurse cells (see Table 1). These products (blue) are transported through cytoplasmic bridges (blue arrows) to the oocyte. They become localised to the posterior of the ooplasm both by molecular anchoring at the posterior of the oocyte, and by posterior-specific translational and transcriptional regulation. This posterior ooplasm is the germ plasm, or germ line determinant. During early embryogenesis, cells which inherit the germ plasm become the primordial germ cells (PGCs; red).

Epigenesis



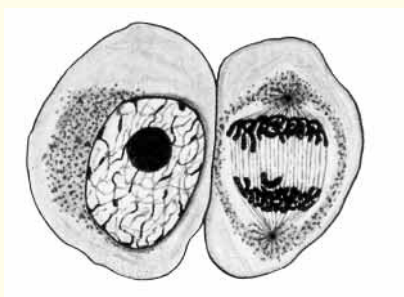
No maternally deposited germ plasm has been observed in the oocytes of the mouse *Mus musculus*. Instead, PGC determination takes place after the segregation of embryonic and extraembryonic tissues. A subpopulation of the pluripotent epiblast cells express 'germline competence genes' (striped). These cells are able to interpret the inductive signals that arrive from neighbouring tissues and differentiate into PGCs (red). The inductive signals come from the extraembryonic ectoderm (blue) and endoderm (yellow).

Box 2. Germ cell identification

Germ cells can often be distinguished from somatic cells during early development using histological and molecular characteristics. Studies to define the embryonic origin of germ cells should show that putative primordial germ cells (PGCs) satisfy as many of these identification criteria as possible. In laboratory organisms, descriptive techniques can be combined with experimental methods to provide conclusive proof of PGC identity. Although experimental data are not available for most non-model organisms considered here, often a combination of histological and molecular data can indicate the site and developmental timing of PGC formation.

Histological characteristics

Until the advent of molecular techniques, most cell types were identified by their histological characteristics. Germ cells were recognised by their characteristic large round nucleus, single large nucleolus, cytoplasm relatively clear of organelles, and granular



cytoplasmic material (called 'nuage', see below). These features are shown in the drawing of a resting and a dividing primordial germ cell from the genital ridge of the turtle *Sternotherus odoratus* (Risley, 1933). Modern studies using molecular criteria have generally confirmed PGC identifications made using older histological methods.

Electron-dense cytoplasmic bodies

Transmission electron microscopy (TEM) has revealed that electron-dense masses exist in the cytoplasm of germ cells of all phyla studied to date. These dense bodies are often called nuage or germ granules, and can be used to identify PGCs at early developmental stages. Germ cell-specific organelles (such as the mitochondrial cloud and Balbiani body) contain dense bodies.

Molecular markers

Enzyme markers, such as alkaline phosphatase, can be used to identify PGCs. However, because these markers are not always expressed by PGCs at all stages of development, they are usually only suitable for identifying germ cells at certain times of development. Modern studies often identify PGCs by



identifying the products of germ cell-specific genes (see Table 1 for genes and proteins involved in germ cell specification and identity, which are useful as germ cell markers in a range of species).

Products of the vasa gene family are the most widely used molecular PGC markers. *vasa* encodes a DEAD-box RNA helicase that is usually expressed specifically in the germ line. The high conservation of motifs in these genes have made them easy to clone from many phyla. The accompanying figure shows anti-Vasa antibody staining in PGCs of the crustacean *Parhyale hawaiiensis* (C.G.E., unpublished).

Transcriptional and translational regulation

When first specified, PGCs often remain transcriptionally quiescent, while the surrounding soma is usually transcriptionally active. Germ cell transcriptional repressors can be gene-specific (e.g. *germ cell-less* in *Drosophila*) or global (e.g. *pie-1* in *C. elegans*). Translational repression in the germ line has also been documented in *Drosophila* and *C. elegans*, but it is not clear how widely the mechanisms are shared.

removal (Buehr and Blackler, 1970; Nieuwkoop and Suminski, 1959) or by ultraviolet irradiation (Ikenishi et al., 1974; Smith, 1966; Tanabe and Kotani, 1974; Züst and Dixon, 1975), and the injection of irradiated embryos with purified fractions of vegetal plasm (Ikenishi et al., 1986), have confirmed that the vegetal plasm contains germ cell determinants. Preformation is also the mechanism that is used for germ line specification by all other anuran amphibians that have been studied (Table 2, Table S2).

The origin of PGCs in the zebrafish *Danio rerio* was unclear (Lin et al., 1992; Walker and Streisinger, 1983) until the identification of a *vasa*-like gene in 1997 (Olsen et al., 1997; Yoon et al., 1997). *vasa* mRNA is synthesized during oogenesis, localises to the cleavage furrows during the first embryonic cleavages, and seems to be thereby drawn into clumps that segregate into four cells by the 32-cell stage of embryogenesis (Yoon et al., 1997). These four cells become the PGCs. Cell lineage studies and *vasa* expression patterns in other fish (Braat et al., 1999) suggest that preformation may be

a common mechanism for the teleosts, but not necessarily for all fish (see Table 2, Table S2).

Chicken germ cells were thought to originate from the hypoblast (see Box 3) (Swift, 1914) until 1981, when experiments using chick-quail chimaeras made before primitive streak formation showed that they were of epiblastic origin (Eyal-Giladi et al., 1981). PGCs were then thought to arise through a gradual epigenetic process beginning at around stage X (an intrauterine early blastoderm stage) (Karagenc et al., 1996; Naito et al., 2001). However, the recent isolation of a chicken *vasa* homologue has made it possible to trace pPGCs as far back as cleavage stage embryos (Tsunekawa et al., 2000). Chicken *vasa* protein forms part of the mitochondrial cloud in chick oocytes, and localises to cleavage furrows until stage IV, when six to eight cells of the ~300-cell embryo contain *vasa* and are good candidates for the PGCs (Tsunekawa et al., 2000). These data suggest that preformation may be the mechanism for germ cell specification in chickens, although functional studies have yet to be carried out.

Box 3. Glossary of terms

4d CELL The mesentoblast cell of spirally cleaving animals; gives rise to both mesoderm and endoderm.

ALLANTOIS A mesoderm-derived structure that emerges from the posterior end of the embryo and attaches to the placenta. It gives rise to the placental blood vessels and the umbilical cord.

AGNATHA A grade of chordate, including hagfish and lampreys, characterized by the absence of jaws.

ANURA Amphibian order that includes those without a tail, such as frogs and toads.

BALBIANI BODY Found in oocytes of some species, this organelle contains mitochondria, Golgi vesicles, centrosomes and endoplasmic reticulum; also called the yolk nucleus or vitelline body; probably a condensed form of the mitochondrial cloud.

BASAL An evolutionary lineage, or animal within a lineage, that arises close to the root or base within a phylogeny.

BILATERIA Animals that show bilateral symmetry across a body axis.

CHAETOGNATHA The phylum of arrow worms, small transparent marine worms found both in the plankton and in the benthos.

CLADE A lineage of organisms that comprises an ancestor and all its descendants.

COLLEMBOLA The arthropod order of direct-developing, wingless hexapods, also known as springtails.

DERIVED Evolved to a state that is not like the primitive condition.

DEUTEROSTOME A bilaterian animal whose mouth forms as a secondary opening, separate from the blastopore.

DIPLOBLAST Animals with only two germ layers (ectoderm and endoderm), including the Cnidaria and Ctenophora.

DIPNOI The subclass of sarcopterygian fishes known as lungfishes, which breathe by a modified air bladder, as well as gills.

DIPTERA The insect order of true flies that bear only one pair of functional wings, such as *Drosophila melanogaster*, mosquitoes, gnats and midges.

ECDYSOZOA A protostome clade of moulting animals that includes both *C. elegans* and *D. melanogaster*, but not annelids.

ENTEROPNEUSTA The subphylum of hemichordates known as the acorn worms.

EPIBLAST The embryonic layer of vertebrate embryos from which the embryo proper arises during gastrulation; gives rise to all three germ layers of the embryo.

HOMOLOGOUS A character in two or more taxa with a unique origin in the common evolutionary ancestor of those taxa. A statement of homology is an evolutionary hypothesis, and relates to a particular attribute of a structure or process. For further discussion, see Bolker and Raff (Bolker and Raff, 1996).

HYPOBLAST Older term for the inner germ layer in bird and reptile embryos; the origin of the endoderm.

LOPHOTROCHOZOA A clade of protostomes supported by most molecular phylogenies, including spirally cleaving animals such as molluscs and annelids, as well as lophophorates such as brachiopods and phoronids.

METATHERIA Marsupials: mammals that give birth to live offspring and suckle young in maternal pouches.

MITOCHONDRIAL CLOUD An organelle composed of a high concentration of mitochondria, containing electron dense cytoplasm similar to germ plasm. Probably a diffuse form of the Balbiani body.

MONOTREMATA The egg-laying mammals (platypuses and echidnas).

OOPLASM The cytoplasm of the oocyte or unfertilised egg.

PROTOSTOME A bilaterian animal whose mouth and anus develop from the same invagination (the blastopore) during embryogenesis

PHYLUM The highest taxonomic category used to subdivide the animals or species of any other taxonomic kingdom.

SARCOPTERYGII The vertebrate group that includes lobe-finned fish and tetrapods, including lungfishes and coelacanth.

SAUROPSIDA A group of vertebrates including birds, dinosaurs and reptiles other than turtles.

TRIPLOBLAST An animal with three germ layers (ectoderm, mesoderm and endoderm).

URODELA An order of amphibians including axolotls, salamanders and newts.

Epigenesis in germ cell development

The time and site of origin of mammalian germ cells was a controversial issue for several decades (see Everett, 1945; Heys, 1931) (see also references in Table S2 online). This controversy continued until alkaline phosphatase activity was first used in 1954 as a marker for mouse germ cells (Chiquoine, 1954). This technique was later used to identify these cells in mouse embryos between 7 and 7.5 days post coitum (dpc) (Fig. 1B) (Ginsburg et al., 1990; Ozdzenski, 1967). In 1994, lineage tracing studies moved the time of origin of these cells to an even earlier stage of development, 6.5 dpc (Lawson and Hage, 1994). At this stage of development, these cells are found posterior to the primitive streak in the extraembryonic mesoderm, at the base of the allantois (see Box 3). They are incorporated into the hindgut epithelium, move into the dorsal mesentery, and from there, they colonise the genital ridges on the dorsal body wall, forming the gonad primordia (Chiquoine, 1954; Ginsburg et al., 1990; Gomperts et al., 1994).

In contrast to the studies in chick and zebrafish, the isolation of a mouse *vasa* homologue has not resulted in the identification of pPGCs at even earlier stages of development (Fujiwara et al., 1994; Noce et al., 2001; Toyooka et al., 2000). Although mouse *vasa* homologue protein is expressed in

oocytes, it is not localised to a specific subcellular region, and no germ plasm is formed (Toyooka et al., 2000). Instead, a true epigenetic mechanism for germ line specification has been demonstrated by both descriptive and experimental evidence (Tsang et al., 2001). Cells of the distal epiblast (see Box 3), which normally differentiate into ectodermal derivatives, can differentiate as PGCs when transplanted into the proximal epiblast, the region from which the PGCs normally derive. Conversely, proximal epiblast cells will not differentiate as PGCs when transplanted to distal sites (Tam and Zhou, 1996). These experiments suggested that inductive signals might be required for germ cell specification in the mouse. At least some of these inductive signals have been identified as members of the bone morphogenetic protein (BMP) class of TGF β superfamily intercellular signaling proteins (Hogan, 1996). The expression of *Bmp4* (Lawson et al., 1999) and *Bmp8b* (Ying et al., 2000) in the extraembryonic ectoderm, and *Bmp2* in the endoderm (Ying and Zhao, 2001), is required for the induction of germ cell fate among proximal epiblast cells. A study of gene expression at the single cell level has indicated that the genes *fragilis* and *stella* are upregulated in a subset of the proximal epiblast cells. The expression of these two genes appears to make the cells competent to respond to BMP

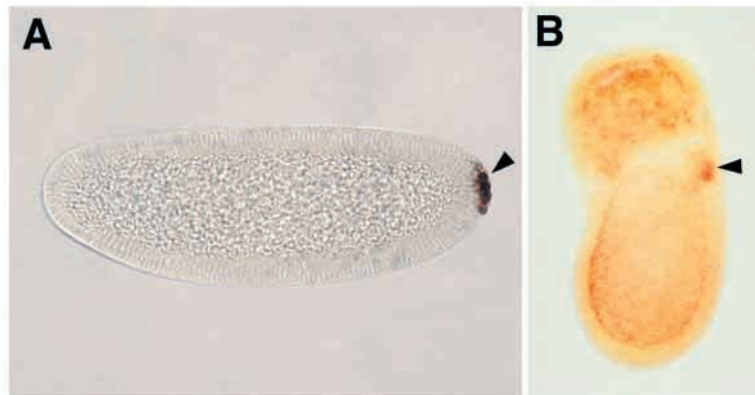


Fig. 1. Germ cell specification in model systems. (A) A cellular blastoderm stage *D. melanogaster* embryo stained with anti-Vasa antibody. The pole cells (arrowhead), located at the posterior pole of the embryo, are the primordial germ cells (PGCs), and express vasa protein. (B) Mouse embryo at 7 dpc stained with alkaline phosphatase. Enzymatic activity is high in the PGCs (arrowhead), which are located in the proximal epiblast at the base of the allantois [Reproduced with permission from McLaren (McLaren, 2003)]. Anterior is to the left in both panels.

signals, which direct them to differentiate into PGCs (Saitou et al., 2002). However, even cells of the distal epiblast, which do not normally express *fragilis* or *stella*, can be induced to differentiate into PGCs if placed next to the source of the BMP signals (Tam and Zhou, 1996). These results tell us that germ cell specification in mice is clearly epigenetic and does not depend on maternally localised determinants.

The only other unequivocal evidence for inductive germ cell specification has arisen from studies on urodele amphibians (see Box 3). Germ cells were first identified in the lateral plate mesoderm (LPM) of many urodele species (Humphrey, 1925; Humphrey, 1929; Ikenishi and Nieuwkoop, 1978). Careful explant and grafting experiments have shown that the LPM is not merely the place in which germ cells could first be unambiguously identified, but also that these cells actually arose there as a result of inductive signals from the ventral endoderm (Boterenbrood and Nieuwkoop, 1973; Nieuwkoop, 1947). These signals induce both PGCs and other somatic cell types. Kotani showed that presumptive epidermal cells placed at the site of the LPM can give rise to PGCs (Kotani, 1957), and later studies demonstrated that any part of the animal half of the blastula can give rise to PGCs under the inductive influence of the ventral endoderm (Sutasurya and Nieuwkoop, 1974). Recent studies in the axolotl *Ambystoma mexicanum* have confirmed that both a mitochondrial cloud and localised molecular determinants are absent in oocytes of this organism (Johnson et al., 2001) (A. D. Johnson, M. Drum and R. Bachvarova, unpublished). The products of germ cell-specific genes, such as *Dazl* and *vasa*, are not localised in the oocytes or early embryos of this axolotl, and are not zygotically transcribed in PGCs until they approach the gonadal ridges (Johnson et al., 2001; Johnson et al., 2003). Although no data are available yet on the molecular nature of the endodermal signal that induces PGC and LPM differentiation in urodeles, BMP4 is known to induce ventral mesoderm in *X. laevis* (Dale et al., 1992; Jones et al., 1992), and it is therefore possible that this signal plays a role in axolotl PGC specification.

Germ cell specification in non-model systems

The laboratory models we have considered thus far are members of only three bilaterian phyla (Arthropoda, Nematoda and Chordata), and cannot be considered to represent the diversity of the Metazoa. To evaluate the distribution of preformation and epigenesis as modes of germ cell specification, we now summarise what is known about the

mode of germ cell formation for each metazoan phylum. Most of these data do not provide conclusive evidence, but the fulfillment of multiple criteria for the identification of PGCs, together with experimental evidence, strongly indicate the mode of PGC determination in many such phyla.

In Table 2, we present recent molecular data, and older descriptive and experimental literature, on comparative germ cell specification (for a fully referenced version of this table, see Table S2 online at <http://dev.biologists.org/supplemental/>). This table lists the phyla that we have reviewed; the observed location, developmental timing and presumed mode of germ cell specification; and whether functional experiments have been carried out to distinguish between epigenesis and preformation. The criteria used to identify pPGCs and PGCs are also indicated. In the following section, specific references are given only for a few examples of each major clade (see Box 3); references for all other statements can be found in Table S2 at <http://dev.biologists.org/supplemental/>.

Origin of germ cells in basal animal lineages

Porifera (sponges) and Cnidaria (corals, jellyfish, hydra) are the most basal (see Box 3) branches of the Metazoa. In these phyla, germ cells arise from a stem cell population that also generates other cell types. Thus, the boundary between germ line and soma is a fluid one. For this reason, these basal groups are sometimes omitted from comparative discussions of germ cell origin (e.g. Dixon, 1994; Ransick et al., 1996). However, these organisms can produce haploid gametes and reproduce sexually, and in that sense their germ line serves the same function as it does in bilaterian animals. In hydrozoan cnidarians, pluripotent cells called interstitial cells (I cells) contain electron-dense cytoplasmic bodies similar to those associated with germ cells in all phyla (Eddy, 1975). These bodies become more numerous in I cells that develop into germ cells, and decrease in number in I cells that differentiate into nematocytes. In Porifera, archaeocytes are pluripotent cells that are capable of both germ line and somatic stem cell divisions.

Ctenophores (comb jellies) also probably diverged from other Metazoa before the origin of the Bilateria (Fig. 2A). Ctenophore germ cells have been described as arising epigenetically, from the meridional canal endoderm (Fig. 2B), but their having an extragonadal origin, followed by their migration to the meridional canal primordium, cannot be ruled out.

Table 1. Genes required by germ cells for development*

Gene (common name) [†]	Species with homologues [‡] (homologue names) [§]						Gene product	Germ cell function [¶]
	Fly (D)	Worm (C)	Frog (X)	Fish (Dr)	Mouse (M)	Other [‡]		
<i>boule</i>	yes		yes		yes	A (<i>Axdazl</i>), Cb, Hs (<i>DAZ</i>), Ma, Mm, Pt, Pa	RNP-type RNA binding protein with DAZ repeats	Meiosis; PGC differentiation (Hs, M, X)
<i>aubergine</i>	yes						Similar to eIFC2 (translation initiation factor)	Pole cell formation; translational regulation of <i>osk</i>
<i>bruno</i>	yes	yes	yes			Hs	RNP-type binding domains	Translational regulation of <i>osk</i> and <i>grk</i> (D)
<i>capuccino</i>	yes						Actin binding protein	<i>osk</i> & <i>stau</i> localisation in oocyte (D)
<i>DEADSouth</i>			yes				eIF4A-like helicase	Localised to germ granules (X)
<i>fragilis</i>					yes		IFN inducible TM family member	Confers PGC competence (M)
<i>germ-cell-less</i>	yes	yes			yes		Nuclear pore associated protein	Transcriptional repression (D)
<i>gld-1</i>		yes					KH motif RNA binding protein	Translational repression (C)
<i>gp130</i>					yes		Cytokine receptor	Mutant has fewer PGCs (M)
<i>gurken</i>	yes						EGFR ligand	Oocyte patterning and germ plasm assembly (D)
<i>gustavus</i>	yes						Novel protein	VAS localisation in oocyte
<i>homeless</i>	yes						RNA-dependent ATPase	G plasm component localisation (D)
<i>mago nashi</i>	yes	yes	yes		yes	Hs	Novel protein	Germ plasm assembly (C, D)
<i>mes-2</i>		yes					Similar to <i>E(z)</i> (D polycomb gene)	Transcriptional repression (C)
<i>mes-3</i>		yes					Novel protein	MES-2 and MES-6 localisation (C)
<i>mes-4</i>		yes					Novel protein	GC survival (C)
<i>mes-6</i>		yes					Novel protein	Transcriptional repression, MES-2 localisation (C)
<i>mex-1</i>		yes					Zinc finger protein	PIE-1 and P granule segregation (C)
<i>mex-3</i>		yes					KN domain RNA binding protein	Blastomere identity; mutation leads to ectopic GCs (C)
<i>mtlrRNA</i>	yes		yes				Mitochondrial ribosomal RNA	Localisation of mitochondrial ribosomes on P granules (D)
<i>nanos</i>	yes	yes	yes	yes	yes	Ch, Dv, Gd, H (<i>Cnnos1</i> , <i>Cnnos2</i>), Hr (<i>Hmos</i>), S, Md	CCHC Zn-finger protein	Translational and transcriptional repression (C, Ch, D, Dv, Md)
<i>orb</i>	yes						RNA binding protein	<i>osk</i> localisation (D)
<i>oskar</i>	yes					Dv	Novel protein	Germ plasm assembly (D)
<i>par-1</i>	yes	yes			yes	Hs, R	Ser/Thr kinase	OSK phosphorylation, germ plasm assembly (C, D)
<i>pgc-1</i>	yes						Non-coding RNA	PC migration (D)
<i>pie-1</i>		yes					Zinc finger protein	Transcriptional repression (C)
<i>pog</i>					yes		Plant homeodomain motifs	PGC proliferation (M)
<i>pumilio</i>	yes	yes			yes	Hs (CUG-BP) S	Novel RNA binding domains	Translational repression (D, C)
<i>spire</i>	yes						Novel protein	<i>osk</i> and <i>stau</i> localisation in oocyte (D)
<i>staufen</i>	yes					Hs	dsRNA binding protein	Germ plasm assembly (D)
<i>stella</i>					yes		Novel protein	Confers PGC competence (M)
<i>tropomyosin II</i>	yes						Actin binding protein	<i>osk</i> and <i>stau</i> localisation in oocyte (D)

Table 1. Continued

Gene (common name) [†]	Species with homologues [‡] (homologue names) [§]						Gene product	Germ cell function [¶]
	Fly (D)	Worm (C)	Frog (X)	Fish (Dr)	Mouse (M)	Other [‡]		
<i>tudor</i>	yes					Hs (<i>tudor domain protein</i>)	Novel 'tudor domain' repeats	Germ plasm assembly; <i>nos</i> localisation (D)
<i>valois</i>	yes						Novel protein	Germ plasm assembly (D)
<i>vasa</i>	yes	yes	yes	yes	yes	**	DEAD-box RNA helicase; eIF4A (translation initiation factor) homology	Germ plasm assembly; translational regulation (D)
<i>Xlirts</i>			yes			Hs (<i>HumXist</i>)	Non-coding RNA	mRNA localisation to vegetal cortex (X)
<i>Xpat</i>			yes				Novel protein	Localised to germ plasm (X)

*Data compiled from 143 references, which are available in the online version of this table (see Table S1 at <http://dev.biologists.org/supplemental/>).

[†]Usually the name of the first gene in the family to be identified.

[‡]Abbreviations for species names are as follows: A, *Ambystoma mexicanum* (axolotl); Aa, *Aurelia aurita* (moon jellyfish); Ad, *Acropora digitifera* (staghorn coral); B, *Bombyx mori* (silkworm); C, *Caenorhabditis elegans* (nematode); Ca, *Carassius auratus* (goldfish); Cb, *Cebus sp.* (capuchin monkey); Cc, *Cyprinus carpio* (carp); Ch, *Chironomus samoensis* (midge); Ci, *Ciona intestinalis* (ascidian); Cp, *Cynops pyrrhogaster* (newt); Cr, *Craspedacusta sowerbyi* (freshwater jellyfish); Cs, *Ciona savignyi* (ascidian); D, *Drosophila melanogaster* (fruit fly); Dd, *Dugesia dorotocephala* (flatworm); Dj, *Dugesia japonica* (flatworm); Dr, *Danio rerio* (zebrafish); Dv, *Drosophila virilis* (fruit fly); E, *Ephydatia fluviatilis* (sponge); Ec, *Equus caballus* (horse); G, *Gallus gallus* (chicken); Gd, *Gryllus domesticus* (cricket); H, *Hydra magnipapillata* (hydra); He, *Hydractinia echinata* (colonial hydroid); Hr, *Helobdella robusta* (leech); Hs, *Homo sapiens* (human); Hy, *Hyphessobrycon ecuadoriensis* (Columbian tetra); L, *Leucopsarion petersii* (ice goby); M, *Mus musculus* (mouse); Ma, *Macaca fascicularis* (crab-eating macaque); Md, *Musca domestica* (housefly); Mf, *Melanotaenia fluviatilis* (rainbowfish); Mm, *Macaca mulatta* (rhesus monkey); O, *Oryzias latipes* (medaka); Om, *Oncorhynchus mykiss* (rainbow trout); On, *Oreochromis niloticus* (Ukuobu); P, *Pantodon buchholzi* (butterfly fish); Pa, *Papio anubis* (baboon); Pt, *Pan troglodytes* (chimp); R, *Rattus norvegicus* (rat); S, *Schistocerca americana* (grasshopper); Sa, *Sanderia malayaensis* (Malaysian jellyfish); Sg, *Schistocerca gregaria* (locust); Sm, *Schmidtea mediterranea* (flatworm); Sp, *Sparus aurata* (gilthead bream); Sq, *Squalus acanthias* (spiny dogfish); T, *Tetranychus urticae* (spider mite); Tf, *Tima formosa* (elegant jellyfish); X, *Xenopus laevis* (clawed frog).

[§]Note that many homologues are not given new names, but may be called 'x-like gene', where 'x' is the name of the first gene in the family to be identified.

[¶]Species for which functional information is available are in parentheses.

**Aa, Ad, B, Ca, Cc, Ci (*CiDEAD1b*), Cp, Cr, Cs (*CsDEAD1a*, *CsDEAD1b*), Dd (*Plvas1*), Dj (*Djvlga*, *Djvlgb*), Dv, E (*PoVAS1*), Ec, G (*Cvh*), H (*CnVAS1*, *CnVAS2*), He, Hs, Hy, L, Mf, O (*olvas*), Om, On, P, R (*RVLG*), Sa, Sg, Sm, Sp, Sq, T, Tf.

Only one other group of animals is now thought to have diverged from the bilaterian stem before the split between protostomes and deuterostomes. These are the acoelomorph flatworms (acoels and nemertodermatids) (Ruiz-Trillo et al., 2002; Telford et al., 2003). Several molecular datasets suggest that they are basal to the Bilateria, and not closely related to the other flatworms in the phylum Platyhelminthes. Germ cells in acoels are derived from a population of pluripotent cells called neoblasts. Neoblasts can also give rise to somatic cells, and are the cells that make regeneration possible in these animals.

There is no evidence for germ line determination by preformation in any of these basal animal lineages.

Germ cell specification in bilaterian animals

Recent metazoan phylogenies based on molecular characters suggest that, with the exception of the basal animal groups mentioned above, all animals fall within one of three great lineages, each of which includes both simple and complex animals (Adoutte et al., 2000; Peterson and Eernisse, 2001). These three clades are the deuterostomes (which include the chordates), and two clades of protostomes, the ecdysozoans (which include *C. elegans* and *Drosophila*) and the lophotrochozoans (for which there are no well-studied laboratory models) (see Box 3). In Table 2, the phyla are organised into these groupings, although in the text we consider the protostomes as a whole (see supplemental Data 1 at <http://dev.biologists.org/supplemental/> for a guide to the taxonomic groupings used in Table 2). Within each of these clades, the relationships between phyla are poorly resolved, so

at present it is not easy to predict which phyla are most likely to retain ancestral characteristics.

PGCs in protostomes

Drosophila and *C. elegans* developmental studies have provided us with so much molecular genetic information on germ cell specification that it is easy to forget how little is known about the other protostomes, which include at least 20 phyla and make up the vast majority of animal species (Brusca and Brusca, 2003). A few remarkable cases of germ plasm segregation have indeed been observed outside of fruit flies and nematode worms. For example, fertilized eggs of the bivalve mollusc *Sphaerium striatinum* contain an asymmetrically localised dense matter, which is segregated during unequal cleavages to the 4d cell (Woods, 1931; Woods, 1932). The 4d cell (see Box 3) then gives rise to the PGCs. However, although it is tempting for developmental biologists to assume that germ plasm localisation is a universal mechanism for protostomian germ line determination, our survey of published data suggests that this is actually an unusual derived (see Box 3) feature of nematodes, dipterans and a few other animals [for a summary of older literature see Nieuwkoop and Sutasurya (Nieuwkoop and Sutasurya, 1981)].

One might hope that *D. melanogaster* would be representative of the arthropods (see Box 3), at least, with respect to germ line specification mechanisms. In reality, the diversity in the temporal and spatial origin of arthropod germ cells is extreme (Anderson, 1973; Kumé and Dan, 1968; Nelsen, 1934). However, a few generalisations can be made concerning PGC origin in the major arthropod subphyla.

Table 2. Determining the mode of germ cell specification across the Metazoa*

	PGC origin [†]		Mode of PGC specification [‡]	Experimental evidence [§]	PGC identification criteria [¶]
	Stage	Location/derivation			
BASAL LINEAGES					
Porifera	Gastrulation	Mesenchymal cells	E	–	LM, TEM, MM
Cnidaria					
<i>Anthozoa</i>	Post-embryonic	In coelom from gastrodermal cells of mesentery or endocoelic epithelial cells	E	–	TEM, LM
<i>Scyphozoa</i>	Post-embryonic	Within ovaries from endodermally derived gastrodermis	E	–	TEM
<i>Hydrozoa</i>	Gastrulation	Endodermal core	E	+	LM, TEM, MM
Ctenophora	Early larval stage	Endoderm	E	–	LM
BILATERIA (Triploblasts)					
Acoelomorpha	Late embryogenesis	Mesenchymal	E	–	LM, TEM
Lophotrochozoa (Protostomes)					
Platyhelminthes					
<i>Turbellaria</i>	Late embryogenesis	Mesenchymal	E	+	LM, TEM, MM
<i>Trematoda</i>	First cleavage	First cleavage	P	–	LM
<i>Cestoda</i>	Late embryogenesis	Mesenchymal	E	–	LM, TEM
Rotifera	Before gastrulation	4d cell	P	–	LM
Entoprocta	nd	nd	nd	–	nd
Ectoprocta	Post-embryonic	Mesenchyme: gonadal epithelium	E	–	LM
Nemertea	Late embryogenesis	Mesodermally derived cells of parenchyma or gonadal epithelium	E	–	LM, TEM
Phoronida	Late embryogenesis	Peritoneal epithelium	E	–	LM
Brachiopoda	Late embryogenesis	Ileo-parietal epithelium	E	–	LM, TEM
Gnathostomulida	nd	nd	nd	–	nd
Pogonophora	Post-embryonic	Gonadal epithelium	E	–	LM
Echiura	Larval stage	Mesoderm	E	–	LM, TEM
Sipunculida	Larval stage	Gonadal epithelium	E	–	LM
Mollusca					
<i>Aplacophora</i>	Post-larval	Mesodermal?	E	–	LM
<i>Polyplacophora</i>	Post-embryonic	Gonadal epithelium	E	–	TEM
<i>Cephalopoda</i>	Blastoderm stage	Blastoderm superficial layer	P	–	LM
<i>Gastropoda</i>	Late embryogenesis/early cleavage?	Mesodermal/early cleavage blastomere?	E/P	–	LM, TEM
<i>Bivalvia</i>	Early cleavage	4d cell	P	–	LM
Annelida					
<i>Polychaeta</i>	Early cleavage/post-larval	4d cell/peritoneal vascular epithelium/	E/P	–	LM, TEM
<i>Oligochaeta</i>	Early cleavage/late embryogenesis	4d cell/unknown source before mesoderm formation/unknown source late in development	E/P	+	LM, TEM
<i>Hirudinea</i>	Early cleavage	D blastomere	P	–	LM, MM
Ecdysozoa (Protostomes)					
Arthropoda					
<i>Collembola</i>	Early cleavage	Early cleavage blastomeres	P	–	LM, TEM
<i>Insecta</i>	Early cleavage/late embryogenesis	Early cleavage blastomere/mesoderm	E/P	+	LM, TEM, SEM, EM, MM, LI
<i>Crustacea</i>	Early cleavage/late embryogenesis	Early cleavage blastomere/mesoderm	E/P	–	LM, TEM, MM, LI
<i>Chelicerata</i>	Early cleavage/late embryogenesis	Inner blastoderm cells/primary cumulus/secondary cumulus/mesoderm	E/P	–	LM, TEM, SEM, MM
<i>Myriapoda</i>	Late embryogenesis	Mesoderm: coelomic sacs	E	–	LM
Tardigrada	Late embryogenesis	Mesoderm: coelomic sacs	E	–	LM
Onychophora	Gastrulation/late embryogenesis	Blastopore/endoderm/mesoderm	E/P	–	LM
Nematoda	First cleavage	First cleavage blastomere	P	+	LM, TEM, SEM, MM, LI
Priapulida	nd	nd	nd	–	nd
Gastrotricha	Late embryogenesis	Base of proctodeum	E	–	LM
Kinorhyncha	nd	Apical cells of gonad	E	–	LM
Deuterostomes					
Chaetognatha	First cleavage	First cleavage blastomere	P	+	LM, TEM, MM, LI
Hemichordata	Late embryogenesis	Ectoderm/mesoderm	E	–	LM

Table 2. Continued

	PGC origin [†]		Mode of PGC specification [‡]	Experimental evidence [§]	PGC identification criteria [¶]
	Stage	Location/derivation			
Echinodermata					
<i>Crinoidea</i>	Metamorphosis	Wall of stomatocoel	E	–	LM
<i>Asteroidea</i>	Metamorphosis	Wall of stomatocoel	E	–	LM, TEM
<i>Holothuroidea</i>	Post-larval	Gonadal epithelium	E	+	LM, TEM
<i>Echinoidea</i>	Metamorphosis/16-cell stage?	Wall of stomatocoel/small micromeres?	E/P	+	LM, TEM, MM
Chordata					
<i>Urochordata</i>	64-cell stage/post-metamorphosis	B7.6 cells: posterior of embryo/hemocytetes	E/P	+	LM, TEM, MM, LI
<i>Cephalochordata</i>	Cleavage stages/larval stages	Mesoderm of myocoel/gonadal epithelium/single cleavage stage blastomere?	E/P	–	LM, TEM
<i>Agnatha</i>	Gastrulation	Unclear	E	–	LM
<i>Chondrichthyes</i>	Late cleavage stages/late embryogenesis	Blastoderm/mesoderm	E/P	–	LM
<i>Actinopterygii</i>	Cleavage stages/late embryogenesis	Cleavage blastomeres/endoderm	E/P	+	LM, TEM, MM, LI
<i>Dipnoi</i>	Late embryogenesis	Unclear	E	–	MM
<i>Caudata</i>	Late embryogenesis	Lateral plate mesoderm	E	+	LM, TEM, MM
<i>Anura</i>	Cleavage stages	Cleavage blastomeres/endoderm	P	+	LM, TEM, MM, LI
<i>Archosauria</i>	Cleavage stages	Cleavage stages	P	+	LM, TEM, EM, MM
<i>Lepidosauria</i>	Primitive streak formation	Extraembryonic endoderm	E	–	LM, MM
<i>Testudines</i>	Primitive streak formation	Extraembryonic endoderm	E	–	LM, TEM, MM
<i>Mammalia</i>	Primitive streak formation	Proximal epiblast	E	+	LM, TEM, EM, MM, LI

*Data compiled from 292 references, which are available in the online version of this table (see Table S2 at <http://dev.biologists.org/supplemental/>).

[†]As comparing the duration of stages of development in different species is often confusing, we describe relative developmental stages rather than absolute time.

nd, no data.

[‡]P, preformation; E, epigenesis.

[§]+, yes; –, no.

[¶]LM, light microscopic histological analysis, of either whole mounts or sections; TEM, transmission electron microscopy; SEM, scanning electron microscopy; EM, enzymatic markers; MM, molecular markers, usually in situ hybridization or antibody staining; LI, cell lineage studies.

Among the hexapods, most of the basal insect orders for which data are available do not appear to have early segregated germ cells (Fig. 2C,D) (e.g. Heymons, 1891). The collembolans (see Box 3) are an exception, showing segregation of electron dense granules to PGCs in early embryonic cleavages (Klag, 1982; Klag and Swiatek, 1999; Tamarelle, 1979), but these animals may not be closely related to other hexapods (Nardi et al., 2003). Clear examples of preformation are generally found in some, but not all, species of higher insect orders such as Diptera (flies) (e.g. Lassmann, 1936), Lepidoptera (moths and butterflies) (e.g. Berg and Gassner, 1978) and Hymenoptera (ants, bees and wasps) (e.g. Gatenby, 1917). The PGCs of most crustaceans appear to form late in development from the mesodermal cells of the coelomic cavities, although early segregation has been observed in some copepods (Fig. 2E,F) (Amma, 1911) and cladocerans (Kühn, 1913). Various authors have claimed that in some members of the chelicerate order Arachnida, the PGCs are segregated early in embryogenesis, forming a clump of cells between the yolk and the embryonic primordium (e.g. Juberthie, 1964). However, most studies of both chelicerate and myriapod embryogenesis show no evidence for early segregated cytoplasmic determinants, and instead report a late mesodermal origin of PGCs (e.g. Heymons, 1901; Kautzsch, 1910). In summary, it is not at all clear what the ancestral mechanism of arthropod germ line specification might have been, but epigenesis appears to be more frequent than preformation.

Nematodes are the only protostome phylum in which all members that have been studied exhibit preformation in

PGC development; all other cases of preformation in the protostomes have been observed in only a few derived species within phyla for which epigenesis is prevalent and likely ancestral. For example, most species of the Platyhelminthes derive their germ cells from neoblasts, a pluripotent cell type that gives rise to different types of somatic cells, as well as to germ cells (Gustafsson, 1976; Ladurner et al., 2000). However, the trematode flatworms are a derived group within the Platyhelminthes that segregate their germ cells by preformation at the beginning of embryogenesis (Bednarz, 1973).

Most other protostomes develop their germ cells from a subpopulation of mesodermal cells at an advanced stage of embryogenesis during the differentiation of specialised mesodermal cell types. Among lophotrochozoan protostomes with canonical spiral cleavage (such as some molluscs and some annelids), this mesodermal subpopulation is derived from one of the products of the division of the 4d mesendoblast cell. With only two documented exceptions (among the molluscs) (Dohmen and Lok, 1975; Dohmen and Verdonk, 1974; Verdonk, 1973), no putative cytoplasmic determinants have been observed in precursors of this cell, hence there is currently no evidence for preformation in most annelids and molluscs. The germ line in other groups (such as nemerteans, brachiopods and some arthropods) develops during larval stages, or continuously throughout adult development, from the mesodermally derived cells of the gonadal epithelium.

The phylogenetic position of chaetognaths (see Box 3) has been contested for many decades. Because recent studies have questioned their traditional classification as deuterostomes

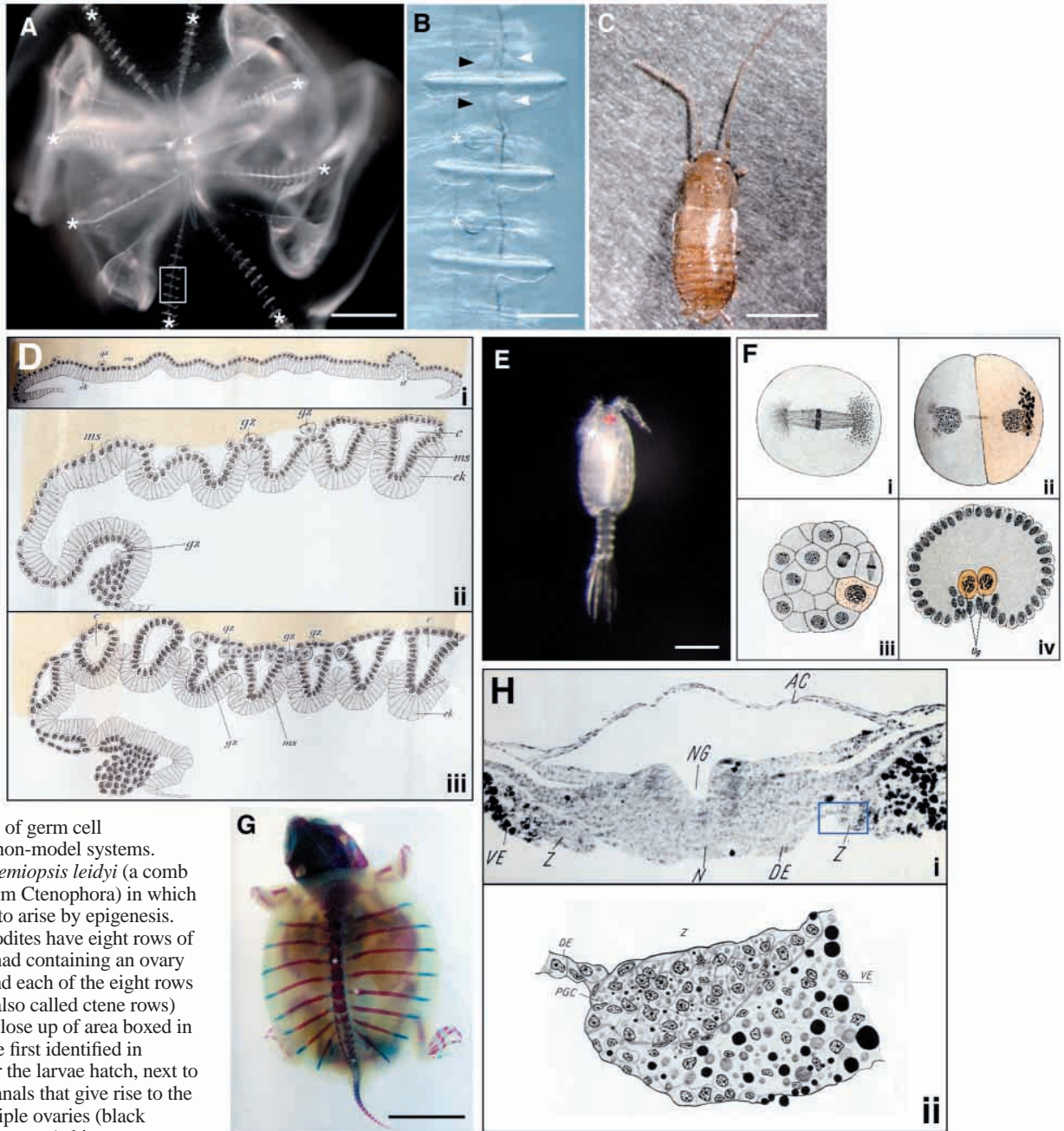


Fig. 2. Examples of germ cell identification in non-model systems.

(A) An adult *Mnemiopsis leidyi* (a comb jelly of the phylum Ctenophora) in which germ cells seem to arise by epigenesis.

These hermaphrodites have eight rows of gonads, each gonad containing an ovary and a testis behind each of the eight rows of comb plates (also called ctene rows) (asterisks). (B) Close up of area boxed in A. Germ cells are first identified in ctenophores after the larvae hatch, next to the meridional canals that give rise to the ctene rows. Multiple ovaries (black arrowheads) and testes (white arrowheads) develop on either side of the canals. In this panel, eggs (asterisks) are being extruded through the gonoducts. (C) Juvenile *Blatta germanica* cockroach (phylum Arthropoda). Germ cells in these insects do not appear to be determined by preformation. (D) The embryonic rudiment of *B. germanica* forms on the surface of the yolk (yellow). (D, part i) Germ cells (gz) are first identified at the posterior of the germ band, after formation of the mesoderm (ms). (ii) As development proceeds, germ cells continue to arise from the mesoderm of the coelomic sacs (c), which are being formed in each segment in an anteroposterior progression. (iii) The number of germ cells increases, and they populate the coelomic sacs of the segments from which the gonad will form. c, coelomic sac; ek, ectoderm; gz, germ or reproductive cells (genitalzellen); ms, mesoderm; st, stomodaeum (Heymons, 1891). (E) A copepod of the genus *Cyclops* (phylum Arthropoda). All copepods that have been studied segregate germ cells by preformation. (F) Embryonic cleavages of *Cyclops fuscus* are holoblastic and equal. (i) In the first cleavage, dense granular material associates with only one of the centrosomes. (ii) The resulting two-cell stage has the granular material in only one of the blastomeres (orange). (iii) The granular material continues to be asymmetrically segregated to a single blastomere (orange) in subsequent cleavages. (iv) At the time of gastrulation, the cell containing the granular cytoplasm has divided to give rise to two cells that are located at the tip of the invaginating archenteron, which are the PGCs (Ug: Urgeschlechtszellen) (Amma, 1911). (G) Late stage embryo of the turtle *Trachemys scripta* (phylum Chordata), stained with Alcian Blue for cartilage and Alizarin Red for bone. Reptiles seem to segregate germ cells epigenetically. (H, part i) Section of an embryo of the turtle *Sternotherus odoratus* at the three somite stage. Germ cells are first identified at this stage of development, in two zones (Z) lateral to the neural groove (NG). (ii) Close up of area boxed in i. PGCs in the germ cell zone (Z) are distinguishable from somatic cells of the ventral ectoderm (VE) as large cells with round nuclei and granular cytoplasm. AC, amnion and chorion; DE, definitive endoderm; N, notochord; NG, neural groove; VE, vitelline endoderm; Z, germ cell zone. Reproduced with permission from Risley (Risley, 1933). Scale bars: 3 mm in A; 250 μ m in B; 150 μ m in C; 50 μ m in E; 5 mm in G.

(Matus et al., 2002; Telford and Holland, 1993), we consider them here along with the other protostomes. Elpatievsky was the first to recognise that, in chaetognaths, a specific cytoplasmic structure was assembled after fertilisation and asymmetrically segregated into a single cell at the 32-cell stage. He traced the fate of this cell and found that its four descendants were the PGCs of the juvenile gonad (Elpatievsky, 1909). Blastomere ablation and cytoplasmic disruption experiments (Ghirardelli, 1954; Ghirardelli, 1955), combined with recent data showing that this cytoplasmic structure contains a *Vasa*-like protein, support the idea that it may be not only a marker, but also a determinant of germ cells (Carré et al., 2002). Given these findings, there is little doubt that the mode of chaetognath PGC specification is preformation.

Protostomes are a hugely diverse group, with few shared embryological characteristics (Nielsen, 2001). However, the present survey suggests that most protostomes use epigenesis to specify germ cells. Preformation appears in few groups, but was unlikely to have been used to specify the germ cells of the last common ancestor of either ecdysozoans or lophotrochozoans. We therefore suggest that the ancestral protostomian mechanism for germ line specification was epigenetic, and that germ plasm specification by preformation evolved as a derived character several times in diverse groups.

PGCs in non-chordate deuterostomes

The deuterostomes include three major phyla, the echinoderms, the hemichordates and the chordates. In the non-chordate deuterostome phyla, modes of germ cell specification are hard to classify. The only studies available on hemichordates are early histological analyses of enteropneust (see Box 3) development, and opinion was divided among those researchers as to whether the PGCs were of mesodermal or ectodermal origin (Bateson, 1885; Morgan, 1894; Spengel, 1893). There is no suggestion that PGCs are specified early in this group.

Echinoderm gonidia are presumed to originate epigenetically from the gonadal epithelium in juveniles and throughout adult life. Regeneration of PGCs, presumably from mesenchymal cells, has been observed even in fragments of animals without gonads. The small micromeres of the 16-cell echinoid embryo seem to share some mitotic characteristics with dipteran pole cells (Pehrson and Cohen, 1986), but removal of these cells does not alter the fertility of the adult urchins (Ransick et al., 1996). However, intriguing data showing the specific accumulation in the small micromeres of molecules usually associated with PGC fate, such as mitochondrial rRNA (Ogawa et al., 1999) and *Vasa* protein (C.G.E., unpublished), suggest that the role of the small micromeres as potential pPGCs should be re-evaluated.

PGCs in the chordates

The phylum Chordata includes two invertebrate groups, urochordates (e.g. sea squirts) and cephalochordates (e.g. *Amphioxus*), as well as the vertebrates. The origin of the germ line in urochordates is best understood in solitary ascidians like *Halocynthia roretzi*, in which detailed cell lineage studies have paved the way for contemporary molecular studies (Nishida, 1987; Nishida and Satoh, 1983; Nishida and Satoh, 1985), and *Ciona intestinalis*, which has recently joined the ranks of the 'genomic' Metazoa (Dehal et al., 2002). Some descriptive

evidence (such as *vasa* mRNA and protein localisation, transcriptional repression of somatic genes) has suggested that two small blastomeres (B7.6 cells) at the 64-cell stage of *C. intestinalis* and *H. roretzi* are the pPGCs (Fujimura and Takamura, 2000; Takamura et al., 2002; Tomioka et al., 2002). An organelle whose ultrastructure resembles that of germ plasm, called the centrosome-attracting body (CAB), has been identified in *H. roretzi* embryos (Iseto and Nishida, 1999; Nishikata et al., 1999). The CAB is formed in the posterior vegetal cytoplasm of the two-cell stage embryo, is inherited by the B7.6 cells during early cleavages, and has been observed to co-localise with specific mRNAs (Nakamura et al., 2003). This observation raises the possibility that somatic and/or germ cell determinants may be transmitted to putative PGCs via the CAB. Among the colonial ascidians, it is known that individual zooids of the colony can exchange germ cells, such that the PGCs from a single zooid can give rise to almost all of the offspring of the colony (Stoner and Weissman, 1996). However, the embryological origin of the PGCs is unknown.

In cephalochordates, the first morphological identification of germ cells is very late in development in the region of the gonad anlagen, suggesting that they are epigenetically determined. Interestingly, an electron-dense region of ooplasm has been reported to localise to a single blastomere at early cleavage stages (Holland and Holland, 1992). Further studies will be necessary to establish whether this blastomere gives rise to the germ cells, which would provide another example of germ cell segregation by preformation.

As the evolution of germ cell origin in vertebrates has been recently reviewed (Johnson et al., 2003), we summarise only briefly here the general patterns of vertebrate epigenesis and preformation. Among the Agnatha (see Box 3), lamprey germ cells are first distinguished at the time of gastrulation, although their germ layer of origin is uncertain (Beard, 1902a; Okkelberg, 1921). Few data are available on the embryology of hagfish, but germ cells in this group have been reported to arise from the gonadal epithelium (Walvig, 1963). In cartilaginous fishes, most researchers have first identified germ cells at late stages of development, and have presumed that they were of mesodermal origin, although in 1900 John Beard suggested that their yolky nature meant that they derived from the blastoderm before mesoderm formation (Beard, 1900; Beard, 1902b). Extensive studies in zebrafish and some other teleosts have shown that germ cells form by preformation, but the examination of other bony fish using a variety of markers leaves it unclear whether germ cell segregation by preformation is common to all teleosts, let alone to all ray-finned fish. Thus it is uncertain whether preformation is the ancestral mechanism of germ cell formation for all fish.

Very little embryological information is available on sarcopterygians (see Box 3) other than tetrapods, but in dipnoans (see Box 3), Andrew Johnson and colleagues have failed to detect a mitochondrial cloud in oocytes of the lungfish *Protopterus annectans*, which suggests that germ cell determinants are not localised in this animal before the onset of embryogenesis. This leads Johnson and colleagues to favour an epigenetic origin of germ cells in this group (Johnson et al., 2002).

The living tetrapods include the Amphibia (frogs, salamanders, newts) and the Amniota (birds, reptiles, mammals). We have already discussed the descriptive and

experimental evidence on germ cell formation in *A. mexicanum* and other urodeles, and in the anuran *X. laevis*. The evidence provided by *A. mexicanum* and *X. laevis* seems to hold true generally for urodeles and anurans, respectively. The urodeles employ epigenetic mechanisms late in development to specify germ cells, whereas anurans clearly specify their germ cells by preformation.

Few amniotes, other than birds and mammals, have been studied in detail. The studies on vasa protein distribution throughout chick development have shown that germ cells are specified during cleavage stages, and are not induced from a

subset of epiblast cells around the time of primitive streak formation, as had been thought previously. Most studies on reptiles, including turtles (Fig. 2G), suggest that PGCs in these organisms originate in the extraembryonic endoderm, presumably epigenetically as there is no evidence for a predetermined subset of extraembryonic cells that later differentiate to become PGCs (Fig. 2H) (Risley, 1933). Notwithstanding these data, bird PGCs were also considered to be induced epigenetically from extraembryonic tissue before the convincing quail-chick chimaera experiments on uterine stage chick embryos, which showed that chick PGCs were

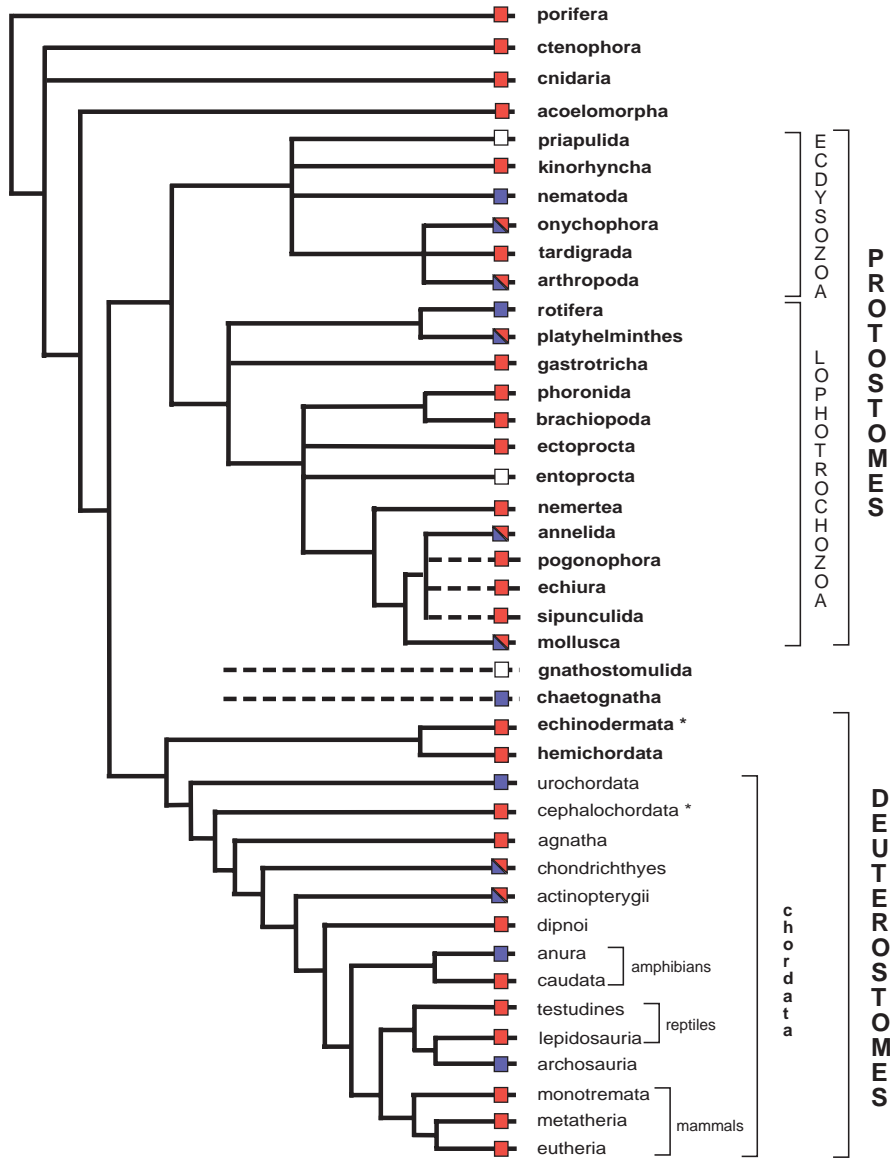


Fig. 3. Modes of germ cell specification across the Metazoa. Boxes refer to modes of germ cell specification as described in the existing literature: red, epigenesis; blue, preformation; half red, half blue, groups in which some species show preformation and others epigenesis; white, no data. Asterisks indicate phyla in which epigenesis has been claimed, but recent data suggest preformation (see discussion in main text). Phylogeny is modified from Peterson and Eernisse (Peterson and Eernisse, 2001), but many relationships within the Ecdysozoa and Lophotrochozoa remain unresolved by molecular data (Adoutte et al., 2000). The phylogenetic positions of the Chaetognatha and Gnathostomulida are particularly uncertain (dotted lines).

derived from the epiblast well before primitive streak formation (Eyal-Giladi et al., 1981), were published. Subsequent examination of Vasa protein localisation was necessary to establish that chick PGCs are probably specified by preformation. The availability of *vasa* as a molecular marker may allow the origin of PGCs in other sauropsid species (see Box 3) to be clarified. The cross-reacting chicken Vasa antibody developed by Tsunekawa and coworkers (Tsunekawa et al., 2000) also offers exciting possibilities for further study of germ cell origin in reptiles.

The data available on the Eutheria (placental mammals) strongly suggests that the epigenetic segregation of germ cells, which has been so well characterised in mice, is common to all placental mammals. However, embryological studies carried out in the past on the monotremes (see Box 3) and metatherians (see Box 3) have not been able to determine the time or place of germ cell origin in these animals. The possibility that localised determinants may play a role in embryonic pattern formation in marsupials cannot be ruled out (Selwood, 1968), but there is nothing to suggest that these determinants have anything to do with germ cells, whose earliest reported visualisation is at the 12 somite stage using alkaline phosphatase as a marker (Ullmann et al., 1997). The extreme difficulty of obtaining monotreme specimens for study (Caldwell, 1887) means that even modern studies of development in these animals often rely on histological preparations that are ~100 years old (Hughes and Hall, 1998). Overall, it seems likely that, with few or no exceptions, mammals rely upon epigenetic mechanisms to specify germ cells.

Fig. 3 summarises the published data on germ cell specification mechanisms in the 28 metazoan phyla discussed above. In the following discussion, we present interpretations and predictions arising

from this summary on the ancestry and evolution of germ cell specification mechanisms.

Similarities and differences in the germ line across the Metazoa

In species that segregate PGCs by preformation, the germ line is immortal and continuous from generation to generation, and this makes it tempting to speculate that preformation has a common origin and continuous history. However, closer inspection makes it clear that in only three cases are entire phyla characterised by germ plasm-driven PGC specification (rotifers, nematodes and chaetognaths), and none of these phyla can be considered to be basal to the Metazoa (Fig. 3). Other clades that show PGC segregation via preformation (e.g. dipteran insects, anuran amphibians, archosaurian reptiles) are derived lineages within phyla for which epigenetic specification is likely to be a basal mechanism (Fig. 3).

The data we have reviewed here suggest that PGCs can be segregated at almost any point during embryogenesis: before blastoderm formation; after embryonic rudiment formation but before germ layer separation; after germ layer separation but before gonadogenesis; or after gonadogenesis and continuously throughout adult life. Although many studies are not experimental, and are therefore not conclusive, for most phyla we have been able to combine observations based on the distinctive morphology of germ cells with those based on molecular techniques of PGC identification. In members of 23 out of 28 phyla, PGCs are first observed after embryonic rudiment formation. These observations imply that inductive signals are probably responsible for germ line segregation in these groups. The alternative hypothesis is that, in these groups, a germ line is segregated early, but is not distinguished cytologically, and has not yet been identified. Although this is certainly likely to be true for some groups, there are others where the data argue strongly against it (e.g. nemertean, holothuroids, acoelomorphs). On balance, we believe that epigenesis is likely to be the mode used to segregate germ cells in most animals, including all animals basal to the Bilateria. This suggests that epigenesis is probably the basal mode of germ cell specification for the Metazoa. However, the variability in timing and site of germ cell origin suggests that the specific molecular mechanisms used for inductive signaling are unlikely to be the same in all cases.

Evolutionary origin of germ cells

The most obvious similarity of PGCs across phyla is the presence of some kind of aggregate of electron-dense, basophilic bodies in the cytoplasm of germ cells. Such aggregates are widely accepted as markers of germ cells, and in some cases have been shown to confer germ cell fate autonomously on the cells that contain them. These aggregates are variously called dense bodies, nuage, mitochondrial clouds, chromatoid bodies, yolk nuclei or Balbiani bodies, and have been observed at some stage during the development of the germ cells of all phyla examined by electron microscopy (see references in Table S2 at <http://dev.biologists.org/supplemental/>) (see also Eddy, 1975). The exact relationship between all of these differently named structures has not been determined, but it is possible that they are all different morphological manifestations of the same germ line-specific body. The pluripotent cell types of several basal

phyla also contain these dense bodies, and gonidia in these phyla are derived from such pluripotent cells.

Several convincing studies have shown that the composition of the electron-dense aggregates found in germ cells is similar in widely divergent phyla. They always contain a combination of RNAs, proteins, endoplasmic reticulum and mitochondria, and may sometimes contain other organelles (such as microtubules) as well. Where studied, the proteins and RNAs localised to these aggregates are products of germ cell-specific genes that are often conserved across divergent phyla (e.g. Bradley et al., 2001). The dynamics of organelle movement during the assembly of these aggregates also shows striking similarity between different animals (Carré et al., 2002; Heasman et al., 1984; Holland and Holland, 1992). Thus primordial germ cells, as a specialised cell type, may well be homologous across all Metazoa, by the criterion that they have retained an ancestral suite of molecular characteristics that define the germ cell lineage.

We suggest that this complex suite of molecular characters, including several gene expression profiles, the subcellular architecture of germ cells and possibly molecular mechanisms of regulating gene activity, is likely to have evolved only once, and thus may constitute a homologous cell identity 'program'. However, this suite of germ cell characters may be turned on in cells of different germ layer origin, at different times and places during development. This means that neither the mechanisms that trigger germ cell formation, nor the cells in which the 'program' is elicited, are homologous.

In bilaterian outgroups and basal Bilateria, the induction of germ cells probably occurred in a population of pluripotent somatic stem cells (similar to the archaeocytes of Porifera, the I cells of Cnidaria and the neoblasts of Acoelomorpha). In higher bilaterian lineages, the same germ cell fate may be elicited at different times and from different cells during development, by a variety of mechanisms. In some derived animal lineages, this mechanism may be maternal segregation of determinants, which include components of the molecular assembly that characterise germ cells. If this view is correct, then we might expect that future investigations on the molecular aspects of germ cell differentiation will continue to reveal conservation of the gene products and cell biological characteristics of germ cells, whereas studies on the mechanisms of PGC segregation in non-model organisms may provide experimental evidence for a diversity of mechanisms that trigger germ cell formation, including epigenetic induction, as well as the segregation of determinants.

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References

- Adoutte, A., Balavoine, G., Lartillot, N., Lespinet, O., Prud'homme, B. and de Rosa, R. (2000). The new animal phylogeny: reliability and implications. *Proc. Natl. Acad. Sci. USA* **97**, 4453-4456.

- Amma, K.** (1911). Über die Differenzierung der Keimbahnzellen bei den Copepoden. *Arch. Zellforsch.* **6**, 497-576.
- Anderson, D. T.** (1973). *Embryology and Phylogeny in Annelids and Arthropods*. Oxford: Pergamon.
- Bateson, W.** (1885). The later stages in the development of *Balanoglossus kowalevskii*, with a suggestion as to the affinities of the Enteropneusta. *Quart. J. Microscop. Sci.* **25**, 81-122.
- Beard, J.** (1900). The morphological continuity of the germ cells in *Raja batis*. *Anat. Anz.* **18**, 465-485.
- Beard, J.** (1902a). The germ cells of *Pristiurus*. *Anat. Anz.* **21**, 50-61.
- Beard, J.** (1902b). The germ cells. I. *Raja batis*. *Zool. Jahrb. Abt. Anat. Ontogenie Tiere* **16**, 615-702.
- Bednarz, S.** (1973). The developmental cycle of the germ cells in several representatives of Trematoda (Digenera). *Zool. Pol.* **23**, 279-326.
- Berg, G. J. and Gassner, G.** (1978). Fine structure of the blastoderm embryo of the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: gelechiidae). *Int. J. Insect Morphol. Embryol.* **1**, 81-105.
- Bolker, J. A. and Raff, R. A.** (1996). Developmental genetics and traditional homology. *BioEssays* **18**, 489-494.
- Boterenbrood, E. C. and Nieuwkoop, P. D.** (1973). The formation of the mesoderm in the urodelan amphibians. V. Its regional induction by the endoderm. *Wilhelm Roux' Archiv. Entwickl. Org.* **173**, 319-332.
- Bounoure, L.** (1939). *L'origine des Cellules Reproductrices et le Problème de la Lignée Germinale*. Paris: Gauthier-Villars.
- Braat, A. K., Speksnijder, J. E. and Zivkovic, D.** (1999). Germ line development in fishes. *Int. J. Dev. Biol.* **43**, 745-760.
- Bradley, J. T., Kloc, M., Wolfe, K. G., Estridge, B. H. and Bilinski, S. M.** (2001). Balbiani bodies in cricket oocytes: development, ultrastructure, and presence of localized RNAs. *Differentiation* **67**, 117-127.
- Brusca, G. J. and Brusca, R. C.** (2003). *Invertebrates*. Sunderland, MA: Sinauer Associates.
- Buehr, M. and Blackler, A. W.** (1970). Sterility and partial sterility in the South African clawed toad following the pricking of the egg. *J. Embryol. Exp. Morphol.* **23**, 375-384.
- Caldwell, W. H.** (1887). The Embryology of Monotremata and Marsupialia. Part I. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **178**, 463-486.
- Carré, D., Djediat, C. and Sardet, C.** (2002). Formation of a large Vasa-positive granule and its inheritance by germ cells in the enigmatic Chaetognaths. *Development* **129**, 661-670.
- Chiquoine, A. D.** (1954). The identification, origin, and migration of the primordial germ cells in the mouse embryo. *Anat. Rec.* **2**, 135-146.
- Dale, L., Howes, G., Price, B. M. and Smith, J. C.** (1992). Bone morphogenetic protein 4: a ventralizing factor in early *Xenopus* development. *Development* **115**, 573-585.
- Dehal, P., Satou, Y., Campbell, R. K., Chapman, J., Degnan, B., De Tomaso, A., Davidson, B., Di Gregorio, A., Gelpke, M., Goodstein, D. M. et al.** (2002). The draft genome of *Ciona intestinalis*: insights into chordate and vertebrate origins. *Science* **298**, 2157-2167.
- Deppe, U., Schierenberg, E., Cole, T., Krieg, C., Schmitt, D., Yoder, B. and von Ehrenstein, G.** (1978). Cell lineages of the embryo of the nematode *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **75**, 376-380.
- Dixon, K. E.** (1994). Evolutionary aspects of primordial germ cell formation. *CIBA Found. Symp.* **182**, 92-120.
- Dohmen, M. R. and Lok, D.** (1975). The ultrastructure of the polar lobe of *Crepidula fornicata*. *J. Embryol. Exp. Morphol.* **34**, 419-428.
- Dohmen, M. R. and Verdonk, N. H.** (1974). The structure of a morphogenetic cytoplasm, present in the polar lobe of *Bithynia tentaculata* (Gastropoda, Prosobranchia). *J. Embryol. Exp. Morphol.* **31**, 423-433.
- Eddy, E. M.** (1975). Germ plasm and the differentiation of the germ cell line. *Int. Rev. Cytol.* **43**, 229-280.
- Elpatievsky, W.** (1909). Die Urgeschlechtszellenbildung bei *Sagitta*. *Anat. Anz.* **35**, 226-239.
- Ephrussi, A. and Lehmann, R.** (1992). Induction of germ cell formation by *oskar*. *Nature* **358**, 387-392.
- Everett, N. B.** (1945). The present status of the germ-cell problem in vertebrates. *Biol. Rev.* **20**, 45-55.
- Extavour, C. and García-Bellido, A.** (2001). Germ cell selection in genetic mosaics in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **98**, 11341-11346.
- Eyal-Giladi, H., Ginsburg, M. and Farbarov, A.** (1981). Avian primordial germ cells are of epiblastic origin. *J. Embryol. Exp. Morphol.* **65**, 139-147.
- Fujimura, M. and Takamura, K.** (2000). Characterization of an ascidian DEAD-box gene, *Ci-DEAD1*: specific expression in the germ cells and its mRNA localization in the posterior-most blastomeres in early embryos. *Dev. Genes Evol.* **210**, 64-72.
- Fujiwara, Y., Komiya, T., Kawabata, H., Sato, M., Fujimoto, H., Furusawa, M. and Noce, T.** (1994). Isolation of a DEAD-family protein gene that encodes a murine homolog of *Drosophila vasa* and its specific expression in germ cell lineage. *Proc. Natl. Acad. Sci. USA* **91**, 12258-12262.
- Gatenby, J. B.** (1917). The segregation of the germ-cells in *Trichogramma evanescens*. *Quart. J. Microscop. Sci.* **62**, 149-187.
- Ghirardelli, E.** (1954). Studi sul determinante germinale (d.g.) nei Chetognati: Ricerche sperimentali su *Spadella cephaloptera* Busch. *Pubbls. Staz. zool. Napoli* **25**, 444-453.
- Ghirardelli, E.** (1955). Studi sul determinante germinale (d.g.) nei Chetognati: Effetti della centrifugazione delle uova e azione del LiCl ed NaSCN. *Atti Acad. naz. Lincei Rc.* **19**, 498-502.
- Ginsburg, M., Snow, M. H. L. and McLaren, A.** (1990). Primordial germ cells in the mouse embryo during gastrulation. *Development* **110**, 521-528.
- Gomperts, M., García-Castro, M., Wylie, C. and Heasman, J.** (1994). Interactions between primordial germ cells play a role in their migration in mouse embryos. *Development* **120**, 135-141.
- Gustafsson, M. K. S.** (1976). Studies on cytodifferentiation in neck region of *Diphyllobothrium dendriticum* Nitzsch, 1824. *Z. Parasitenk.* **50**, 323-329.
- Heasman, J., Quarmby, J. and Wylie, C. C.** (1984). The mitochondrial cloud of *Xenopus* oocytes: the source of germinal granule material. *Dev. Biol.* **105**, 458-469.
- Heymons, R.** (1891). Die Entwicklung der Weiblichen Geschlechtsorgane von *Phyllostromia* (Blatta) *germanica*. *Z. Wiss. Zool.* **53**.
- Heymons, R.** (1901). Entwicklungsgeschichte der Scolopender. *Zoologica* **33**, 1-244.
- Heys, F.** (1931). The problem of the origin of germ cells. *Q. Rev. Biol.* **6**, 1-45.
- Hird, S. N., Paulsen, J. E. and Strome, S.** (1996). Segregation of germ granules in living *Caenorhabditis elegans* embryos: cell-type-specific mechanisms for cytoplasmic localisation. *Development* **122**, 1303-1312.
- Hogan, B. L.** (1996). Bone morphogenetic proteins: multifunctional regulators of vertebrate development. *Genes Dev.* **10**, 1580-1594.
- Holland, L. Z. and Holland, N. D.** (1992). Early development in the lancelet (=Amphioxus) *Branchiostoma floridae* from sperm entry through pronuclear fusion: presence of vegetal pole plasm and lack of conspicuous ooplasmic segregation. *Biol. Bull.* **182**, 77-96.
- Houston, D. W. and King, M. L.** (2000a). A critical role for *Xdazl*, a germ plasm-localized RNA, in the differentiation of primordial germ cells in *Xenopus*. *Development* **127**, 447-456.
- Houston, D. W. and King, M. L.** (2000b). Germ plasm and molecular determinants of germ cell fate. *Curr. Top. Dev. Biol.* **50**, 155-181.
- Huettner, A. F.** (1923). The origin of the germ cells in *Drosophila melanogaster*. *J. Morphol.* **2**, 385-422.
- Hughes, R. L. and Hall, L. S.** (1998). Early development and embryology of the platypus. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **353**, 1101-1114.
- Humphrey, R. R.** (1925). The primordial germ cells of *Hemidactylum* and other Amphibia. *J. Morphol. Physiol.* **41**, 1-43.
- Humphrey, R. R.** (1929). The early position of the primordial germ cells in Urodeles: evidence from experimental studies. *Anat. Rec.* **42**, 301-314.
- Ikenishi, K. and Nieuwkoop, P. D.** (1978). Location and ultrastructure of primordial germ cells (PGCs) in *Ambystoma mexicanum*. *Dev. Growth Diff.* **20**, 1-9.
- Ikenishi, K., Kotani, M. and Tanabe, K.** (1974). Ultrastructural changes associated with UV irradiation in the 'germinal plasm' of *Xenopus laevis*. *Dev. Biol.* **36**, 155-168.
- Ikenishi, K., Nakazato, S. and Okuda, T.** (1986). Direct evidence for the presence of germ cell determinant in vegetal pole cytoplasm of *Xenopus laevis* and in a subcellular fraction of it. *Dev. Growth Diff.* **28**, 563-568.
- Illmensee, K. and Mahowald, A. P.** (1974). Transplantation of posterior polar plasm in *Drosophila*. Induction of germ cells at the anterior pole of the egg. *Proc. Natl. Acad. Sci. USA* **4**, 1016-1020.
- Illmensee, K. and Mahowald, A. P.** (1976). The autonomous function of germ plasm in a somatic region of the *Drosophila* egg. *Exp. Cell Res.* **97**, 127-140.
- Illmensee, K., Mahowald, A. P. and Loomis, M. R.** (1976). The ontogeny of germ plasm during oogenesis in *Drosophila*. *Dev. Biol.* **49**, 40-65.
- Iseto, T. and Nishida, H.** (1999). Ultrastructural studies on the centrosome-attracting body: electron-dense matrix and its role in unequal cleavages in ascidian embryos. *Dev. Growth Diff.* **41**, 601-609.

- Johnson, A. D., Bachvarova, R. F., Drum, M. and Masi, T. (2001). Expression of axolotl *DAZL* RNA, a marker of germ plasm: widespread maternal RNA and onset of expression in germ cells approaching the gonad. *Dev. Biol.* **234**, 402-415.
- Johnson, A. D., Drum, M., Bachvarova, R. F., Masi, T., White, M. E. and Crother, B. I. (2003). Evolution of predetermined germ cells in vertebrate embryos: implications for macroevolution. *Evol. Dev.* **5**, 414-431.
- Jones, C. M., Lyons, K. M., Lapan, P. M., Wright, C. V. and Hogan, B. L. (1992). DVR-4 (bone morphogenetic protein-4) as a posterior-ventralizing factor in *Xenopus* mesoderm induction. *Development* **115**, 639-647.
- Juberthie, C. (1964). Recherches sur la biologie des Opilions. *Ann. Spél.* **19**, 1-237.
- Karagenc, L., Cinnamon, Y., Ginsburg, M. and Petite, J. N. (1996). Origin of primordial germ cells in the prestreak chick embryo. *Dev. Genet.* **19**, 290-301.
- Kautzsch, G. (1910). Über die Entwicklung von *Agelena labyrinthica* Clerck. II. Teil. *Zool. Jarhb. Anat.* **30**, 535-602.
- Klag, J. (1982). Germ line of *Tetradontophora bielensis* (Insecta, Collembola). Ultrastructural study on the origin of primordial germ cells. *J. Embryol. Exp. Morphol.* **72**, 183-195.
- Klag, J. and Swiatek, P. (1999). Differentiation of primordial germ cells during embryogenesis of *Allacma fusca* (L.) (Collembola: Symphyleona). *Int. J. Insect Morphol. Embryol.* **28**, 161-168.
- Kloc, M., Bilinski, S., Chan, A. P. and Etkin, L. D. (2001). Mitochondrial ribosomal RNA in the germinal granules in *Xenopus* embryos revisited. *Differentiation* **67**, 80-83.
- Kloc, M., Dougherty, M. T., Bilinski, S., Chan, A. P., Brey, E., King, M. L., Patrick, C. W., Jr and Etkin, L. D. (2002). Three-dimensional ultrastructural analysis of RNA distribution within germinal granules of *Xenopus*. *Dev. Biol.* **241**, 79-93.
- Kotani, M. (1957). On the formation of the primordial germ cells from the presumptive ectoderm of *Triturus gastrulae*. *J. Inst. Polytech. Osaka City Univ. D.* **8**, 145-159.
- Kühn, A. (1913). Die Sonderung der Keimesbezirke in der Entwicklung der Sommererier von *Polyphemus pediculus* de Geer. *Zool. Jahrb. Abt. Anat. Ontogenie Tiere* **35**, 243-340.
- Kumé, M. and Dan, K. (1968). *Invertebrate Embryology*. Belgrade: Prosveta.
- Ladurner, P., Rieger, R. and Bagnuà, J. (2000). Spatial distribution and differentiation potential of stem cells in hatchlings and adults in the marine platyhelminth *Macrostomum sp.*: a bromodeoxyuridine analysis. *Dev. Biol.* **226**, 231-241.
- Lassmann, G. W. P. (1936). The early embryological development of *Melophagus ovinus* L., with special reference to the development of the germ cells. *Ann. Entomol. Soc. Am.* **29**, 397-413.
- Lawson, K. A., Dunn, N. R., Roelen, B. A., Zeinstra, L. M., Davis, A. M., Wright, C. V., Korving, J. P. and Hogan, B. L. (1999). *Bmp4* is required for the generation of primordial germ cells in the mouse embryo. *Genes Dev.* **13**, 424-436.
- Lawson, K. A. and Hage, W. J. (1994). Clonal analysis of the origin of primordial germ cells in the mouse. *CIBA Found. Symp.* **182**, 68-91.
- Lin, S., Long, W., Chen, J. and Hopkins, N. (1992). Production of germ-line chimeras in zebrafish by cell transplants from genetically pigmented to albino embryos. *Proc. Natl. Acad. Sci. USA* **89**, 4519-4523.
- Mahowald, A. P. (2001). Assembly of the *Drosophila* germ plasm. *Int. Rev. Cytol.* **203**, 187-213.
- Manton, S. M. (1928). On the embryology of a mysid crustacean, *Hemimysis lamornae*. *Phil. Trans. R. Soc. Lond. Ser. B. Biol. Sci.* **216**, 363-463.
- Matova, N. and Cooley, L. (2001). Comparative aspects of animal oogenesis. *Dev. Biol.* **231**, 291-320.
- Matus, D. S., Huber, J. L., Halanych, K. M. and Martindale, M. Q. (2002). The phylogenetic position of the Chaetognaths: a molecular approach using developmental regulatory genes. *Integr. Comp. Biol.* **42**, 1274.
- McLaren, A. (2003). Primordial germ cells in the mouse. *Dev. Biol.* (in press).
- Morgan, T. H. (1894). The development of *Balanoglossus*. *J. Morphol.* **9**, 1-86.
- Naito, M., Sano, A., Matsubara, Y., Harumi, T., Tagami, T., Sakurai, M. and Kuwana, T. (2001). Localization of primordial germ cells or their precursors in stage X blastoderm of chickens and their ability to differentiate into functional gametes in opposite-sex recipient gonads. *Reproduction* **121**, 547-552.
- Nakamura, Y., Makabe, K. W. and Nishida, H. (2003). Localization and expression pattern of type I postplasmic mRNAs in embryos of the ascidian *Holocynthia roretzi*. *Gene Expr. Patterns* **3**, 71-75.
- Nardi, F., Spinsanti, G., Boore, J. L., Carapelli, A., Dallai, R. and Frati, F. (2003). Hexapod origins: monophyletic or paraphyletic? *Science* **299**, 1887-1889.
- Nelsen, O. E. (1934). The segregation of the germ cells in the grasshopper, *Melanoplus differentialis* (Acrididae: Orthoptera). *J. Morphol.* 545-575.
- Nielsen, C. (2001). *Animal Evolution: Interrelationships of the Living Phyla*. Oxford: Oxford University Press.
- Nieuwkoop, P. D. (1947). Experimental observations on the origin and determination of the germ cells, and on the development of the lateral plates and germ ridges in the urodeles. *Arch. Neerl. Zool.* **8**, 1-205.
- Nieuwkoop, P. D. and Suminski, E. H. (1959). Does the so-called 'germinal plasm' play an important role in the development of the primordial germ cells. *Arch. Anat. Microsc. Morphol. Exp.* **48**, 189-198.
- Nieuwkoop, P. D. and Sutasurya, L. A. (1979). *Primordial Germ Cells in the Chordates*. Cambridge: Cambridge University Press.
- Nieuwkoop, P. D. and Sutasurya, L. A. (1981). *Primordial Germ Cells in the Invertebrates: from epigenesis to preformation*. Cambridge: Cambridge University Press.
- Nishida, H. (1987). Cell lineage analysis in ascidian embryos by intracellular injection of a tracer enzyme. III. Up to the tissue restricted stage. *Dev. Biol.* **121**, 526-541.
- Nishida, H. and Satoh, N. (1983). Cell lineage analysis in ascidian embryos by intracellular injection of a tracer enzyme. I. Up to the eight-cell stage. *Dev. Biol.* **99**, 382-394.
- Nishida, H. and Satoh, N. (1985). Cell lineage analysis in ascidian embryos by intracellular injection of a tracer enzyme. II. The 16- and 32-cell stages. *Dev. Biol.* **110**, 440-454.
- Nishikata, T., Hibino, T. and Nishida, H. (1999). The centrosome-attracting body, microtubule system, and posterior egg cytoplasm are involved in positioning of cleavage planes in the ascidian embryo. *Dev. Biol.* **209**, 72-85.
- Noce, T., Okamoto-Ito, S. and Tsunekawa, N. (2001). *Vasa* homolog genes in mammalian germ cell development. *Cell Struct. Funct.* **26**, 131-136.
- Ogawa, M., Amikura, R., Akasaka, K., Kinoshita, T., Kobayashi, S. and Shimada, H. (1999). Asymmetrical distribution of mitochondrial rRNA into small micromeres of sea urchin embryos. *Zool. Sci.* **16**, 445-451.
- Okkelberg, P. (1921). The early history of the germ cells in the Brook Lamprey, *Entosphenus wilderi* (Gage). *J. Morphol.* **35**, 1-152.
- Olsen, L. C., Aasland, R. and Fjose, A. (1997). A *vasa*-like gene in zebrafish identifies putative primordial germ cells. *Mech. Dev.* **66**, 95-105.
- Ozdzenski, W. (1967). Observations on the origin of primordial germ cells in the mouse. *Zool. Pol.* **117**, 367-379.
- Pehrson, J. R. and Cohen, L. H. (1986). The fate of the small micromeres in sea urchin development. *Dev. Biol.* **113**, 522-526.
- Peterson, K. J. and Eernisse, D. J. (2001). Animal phylogeny and the ancestry of bilaterians: inferences from morphology and 18S rDNA gene sequences. *Evol. Dev.* **3**, 170-205.
- Ransick, A., Cameron, R. A. and Davidson, E. H. (1996). Postembryonic segregation of the germ line in sea urchins in relation to indirect development. *Proc. Natl. Acad. Sci. USA* **93**, 6759-6763.
- Risley, P. L. (1933). Contributions on the development of the reproductive system in *Sternotherus odoratus* (Latreille). I. The embryonic origin and migration of the primordial germ cells. *Zeit. Zellforsch. mikrosk. Anat.* **18**, 459-492.
- Ruiz-Trillo, I., Paps, J., Loukota, M., Ribera, C., Jondelius, U., Bagnuà, J. and Riutort, M. (2002). A phylogenetic analysis of myosin heavy chain type II sequences corroborates that Coela and Nemertodermatida are basal bilaterians. *Proc. Natl. Acad. Sci. USA* **99**, 11246-11251.
- Saffman, E. E. and Lasko, P. (1999). Germline development in vertebrates and invertebrates. *Cell. Mol. Life Sci.* **55**, 1141-1163.
- Saitou, M., Barton, S. C. and Surani, M. A. (2002). A molecular programme for the specification of germ cell fate in mice. *Nature* **418**, 293-300.
- Selwood, L. (1968). Interrelationships between developing oocytes and ovarian tissues in the chiton *Sypharochiton septentriones* (Ashby) (Mollusca, Polyplacophora). *J. Morphol.* **125**, 71-103.
- Smith, L. D. (1966). The role of a 'germinal plasm' in the formation of primordial germ cells in *Rana pipiens*. *Dev. Biol.* **14**, 330-347.
- Spengel, J. W. (1893). *Die Enteropneusten des Golfes von Neapel*. Napoli: Fauna Flora Golfes con Neapel Monogr.
- Stoner, D. S. and Weissman, I. L. (1996). Somatic and germ cell parasitism in a colonial ascidian: possible role for a highly polymorphic allorecognition system. *Proc. Natl. Acad. Sci. USA* **93**, 15254-15259.
- Strome, S. and Wood, W. B. (1982). Immunofluorescence visualization of germ-line-specific cytoplasmic granules in embryos, larvae, and adults of *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **79**, 1558-1562.

- Sutasurya, L. A. and Nieuwkoop, P. D.** (1974). The induction of the primordial germ cells in the urodeles. *Wilhelm Roux' Archiv. Entwickl. Org.* **175**, 199-220.
- Swift, C. H.** (1914). Origin and early history of the primordial germ-cells of the chick. *Am. J. Anat.* **483-516**.
- Takamura, K., Fujimura, M. and Yamaguchi, Y.** (2002). Primordial germ cells originate from the endodermal strand cells in the ascidian *Ciona intestinalis*. *Dev. Genes Evol.* **212**, 11-18.
- Tam, P. P. and Zhou, S. X.** (1996). The allocation of epiblast cells to ectodermal and germ-line lineages is influenced by the position of the cells in the gastrulating mouse embryo. *Dev. Biol.* **178**, 124-132.
- Tamarelle, M.** (1979). Recherches ultrastructurales sur la ségrégation et le développement de la lignée germinale chez les embryons de quatre collemboles (Insecta: Apterygota). *Int. J. Insect Morphol. Embryol.* **8**, 95-111.
- Tanabe, K. and Kotani, M.** (1974). Relationship between the amount of the "germinal plasm" and the number of primordial germ cells in *Xenopus laevis*. *J. Embryol. Exp. Morphol.* **31**, 89-98.
- Technau, G. M. and Campos-Ortega, J. A.** (1986). Lineage analysis of transplanted individual cells in embryos of *Drosophila melanogaster* Part III. Commitment and proliferative capabilities of pole cells and midgut progenitors. *Roux's Arch. Dev. Biol.* **195**, 489-498.
- Telford, M. J. and Holland, P. W.** (1993). The phylogenetic affinities of the Chaetognaths: a molecular analysis. *Mol. Biol. Evol.* **10**, 660-676.
- Telford, M. J., Lockyer, A. E., Cartwright-Finch, C. and Littlewood, D. T. J.** (2003). Combined large and small subunit ribosomal RNA phylogenies support a basal position of the acoelomorph flatworms. *Proc. Roy. Soc. Lond. B.* **270**, 1077-1083.
- Tomioka, M., Miya, T. and Nishida, H.** (2002). Repression of zygotic gene expression in the putative germline cells in ascidian embryos. *Zool. Sci.* **19**, 49-55.
- Toyooka, Y., Tsunekawa, N., Takahashi, Y., Matsui, Y., Satoh, M. and Noce, T.** (2000). Expression and intracellular localization of mouse Vasa-homologue protein during germ cell development. *Mech. Dev.* **93**, 139-149.
- Tsang, T. E., Khoo, P. L., Jamieson, R. V., Zhou, S. X., Ang, S. L., Behringer, R. and Tam, P. P.** (2001). The allocation and differentiation of mouse primordial germ cells. *Int. J. Dev. Biol.* **45**, 549-555.
- Tsunekawa, N., Naito, M., Sakai, Y., Nishida, T. and Noce, T.** (2000). Isolation of chicken vasa homolog gene and tracing the origin of primordial germ cells. *Development* **127**, 2741-2750.
- Ullmann, S. L., Shaw, G., Alcorn, G. T. and Renfree, M. B.** (1997). Migration of primordial germ cells to the developing gonadal ridges in the tamar wallaby *Macropus eugenii*. *J. Reprod. Fertil.* **110**, 135-143.
- Verdonk, N. H.** (1973). Cytoplasmic localization in *Bithynia tentaculata* and its influence on development. *Malacol. Rev.* **6**, 57.
- Walker, C. and Streisinger, G.** (1983). Induction of Mutations by Gamma Rays in Pre-gonial Germ Cells of Zebrafish Embryos. *Genetics* **103**, 125-136.
- Walvig, F.** (1963). The Gonads and the Formation of the Sexual Cells. In *The Biology of Myxine* (ed. A. Brodal and R. Fange), pp. 530-580. Oslo: Universitetsforlaget.
- Whittington, P. M. and Dixon, K. E.** (1975). Quantitative studies of germ plasm and germ cells during early embryogenesis of *Xenopus laevis*. *J. Embryol. Exp. Morphol.* **33**, 57-74.
- Williamson, A. and Lehmann, R.** (1996). Germ Cell Development in *Drosophila*. *Ann. Rev. Cell Dev. Biol.* **12**, 365-391.
- Wolff, E.** (1964). *L'origine de la lignée germinale chez les vertébrés et chez quelques groupes d'invertébrés*. Paris: Hermann.
- Wolpert, L.** (1998). *Principles of development*. London/Oxford: Current Biology/Oxford University Press.
- Woods, F. H.** (1931). History of the germ cells in *Sphaerium striatinum* (Lam.). *J. Morphol. Physiol.* **51**, 545-595.
- Woods, F. H.** (1932). Keimbahn determinants and continuity of the germ cells in *Sphaerium striatinum* (Lam.). *J. Morphol.* **53**, 345-365.
- Wylie, C.** (2000). Germ cells. *Curr. Opin. Genet. Dev.* **10**, 410-413.
- Ying, Y. and Zhao, G. Q.** (2001). Cooperation of endoderm-derived BMP2 and extraembryonic ectoderm-derived BMP4 in primordial germ cell generation in the mouse. *Dev. Biol.* **232**, 484-492.
- Ying, Y., Liu, X. M., Marble, A., Lawson, K. A. and Zhao, G. Q.** (2000). Requirement of *Bmp8b* for the generation of primordial germ cells in the mouse. *Mol. Endocrinol.* **14**, 1053-1063.
- Yoon, C., Kawakami, K. and Hopkins, N.** (1997). 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**, 3157-3166.
- Zhou, Y. and King, M. L.** (1996). Localization of *Xcat-2* RNA, a putative germ plasm component, to the mitochondrial cloud in *Xenopus* stage I oocytes. *Development* **122**, 2947-2953.
- Züst, B. and Dixon, K. E.** (1975). The effect of U.V. irradiation of the vegetal pole of *Xenopus laevis* eggs on the presumptive primordial germ cells. *J. Embryol. Exp. Morphol.* **34**, 209-220.

Data S1. Key to taxonomic groupings

BILATERIAN OUTGROUPS

Porifera (sponges)

Ctenophora (comb jellies)

Cnidaria

Anthozoa (corals, sea anemones, sea pens)

Hydrozoa (hydroids and hydromedusae)

Scyphozoa (true jellyfish)

BILATERIA

Acoelomorpha (acoels and nemertodermatids)

PROTOSTOMATA (mouth and anus derive from the blastopore)

Ecdysozoa (clade of moulting animals)

Priapulida (microscopic burrowing worms; penis worms)

Kinorhyncha (microscopic spiny-headed worms)

Nematoda (roundworms)

Onychophora (velvet worms; soft bodies and unsegmented legs)

Tardigrada (water bears; clawed legs, exoskeleton)

Arthropoda (most speciose metazoan phylum)

Hexapoda (arthropods with six walking legs)

Collembola (direct developing, wingless; springtails)

Insecta

Diptera (true flies with one pair of functional wings)

Hymenoptera (membrane-winged insects: bees, wasps, ants)

Lepidoptera (scale-winged insects: moths, butterflies)

Crustacea

Cladocera (microscopic branchiopod crustaceans)

Copepoda (planktonic maxillopodan crustaceans)

Cheliceriformes (spiders, scorpions, horseshoe crabs, sea spiders)

Arachnida (eight walking legs)

Myriapoda (millipedes and centipedes)

Chaetognatha (arrow worms)

Lophotrochozoa (clade of animals with lophophores or trochophore larvae)

Rotifera (microscopic aquatic invertebrates with an oral crown of cilia)

Platyhelminthes

Cestoda (tapeworms)

Trematoda (flukes)

Turbellaria (free-living flatworms)

Gastrotricha (microscopic worms, marine and freshwater)

Phoronida (lophophorates known as horseshoe worms)

Brachiopoda (lamp shells)

Ectoprocta (also known as Bryozoa; moss animals)

Entoprocta (small animals superficially similar to some Ectoprocta and hydroids)

Nemertea (unsegmented ribbon or proboscis worms)

Annelida (segmented worms)

Hirudinea (leeches)

Oligochaeta (earthworms, segmented freshwater worms)

Polychaeta (marine worms including tube and fan worms)

Pogonophora (beard worms)

Echiura (unsegmented benthic coelomate worms)

Sipunculida (peanut worms: unsegmented, U-shaped gut, dorsal anus)

Mollusca

Aplacophora (shell-less marine molluscs)

Bivalvia (clams, mussels)

Cephalopoda (squid, octopi, cuttlefish)

Gastropoda (snails, slugs)

Gnathostomulida (microscopic vermiform animals with monociliated epidermis)

DEUTEROSTOMATA (mouth is not derived from blastopore)

Echinodermata

- Asteroidea (sea stars)
- Crinoidea (sea lilies, feather stars)
- Echinoidea (sea urchins)
- Holothuroidea (sea cucumbers)

Hemichordata

- Enteropneusta (acorn worms)
- Pterobranchia (colonial hemichordates)

Chordata

- Urochordata (tunicates or sea squirts)
- Cephalochordata (lancelets: *Amphioxus*)
- Vertebrata
 - Agnatha* (jawless vertebrates: hagfish and lampreys)
 - Gnathostomata (jawed vertebrates)
 - Chondrichthyes (sharks, rays and chimaeras)
 - Osteichthyes (bony fish and tetrapods)
 - Actinopterygii (ray-finned bony fish)
 - Teleostei (modern bony fish, including zebrafish and sticklebacks)
 - Sarcopterygii (lobe-finned fish and tetrapods)
 - Dipnoi (lungfish)
 - Tetrapoda
 - Amphibia
 - Anura (frogs and toads)
 - Urodela (newts, axolotls, and salamanders)
 - Amniota (terrestrial vertebrates whose eggs contain an amnion)
 - Sauropsida (living and extinct reptiles, dinosaurs and birds)
 - Testudines (turtles, terrapins and tortoises)
 - Squamata (snakes and lizards)
 - Archosauria (crocodiles, pterosaurs, dinosaurs and birds)
 - Aves (birds)
 - Synapsida (mammals and extinct mammal-like reptiles)
 - Mammalia
 - Monotremata (egg-laying mammals)
 - Metatheria (marsupials)
 - Eutheria (placental mammals)

Legend

BILATERIAN CLADES

Protostome clades

Phyla

Subphyla

Classes

Subclasses

Orders

Large monophyletic groups

*Paraphyletic group

Table S1. Genes required by germ cells for development

Gene (common name)*	Species with homologues [†] (homologue names) [‡]						Gene product	Germ cell function [§]	References
	Fly (D)	Worm (C)	Frog (X)	Fish (Dr)	Mouse (M)	Other [‡]			
<i>boule</i>	yes		yes		yes	A (<i>Axdazl</i>), Cb, Hs (<i>DAZ</i>), Ma, Mm, Pt, Pa	RNP-type RNA binding protein with DAZ repeats	Meiosis; PGC differentiation (Hs, M, X)	(Eberhart et al., 1996; Houston and King, 2000; Houston et al., 1998; Johnson et al., 2001; Ruggiu et al., 1997; Venables et al., 2001; Xu et al., 2001)
<i>aubergine</i>	yes						Similar to eIFC2 (translation initiation factor)	Pole cell formation; translational regulation of <i>osk</i>	(Harris and Macdonald, 2001; Schüpbach and Wieschaus, 1991; Wilson et al., 1996)
<i>bruno</i>	yes	yes	yes			Hs	RNP-type binding domains	Translational regulation of <i>osk</i> and <i>grk</i> (D)	(Castagnetti et al., 2000; Filardo and Ephrussi, 2003; Knecht et al., 1995; Timchenko et al., 1996; Webster et al., 1997)
<i>capuccino</i>	yes						Actin binding protein	<i>osk</i> and <i>stau</i> localisation in oocyte (D)	(Clark et al., 1994; Emmons et al., 1995)
<i>DEADSouth</i>			yes				eIF4A-like helicase	Localised to germ granules (X)	(MacArthur et al., 2000)
<i>fragilis</i>					yes		IFN inducible TM family member	Confers PGC competence (M)	(Saitou et al., 2002)
<i>germ-cell-less</i>	yes	yes			yes		Nuclear pore associated protein	Transcriptional repression (D)	(Jongens et al., 1992; Leatherman et al., 2002; Robertson et al., 1999)
<i>gld-1</i>		yes					KH motif RNA binding protein	Translational repression (C)	(Lee and Schedl, 2001; Schisa et al., 2001)
<i>gp130</i>					yes		Cytokine receptor	Mutant has fewer PGCs (M)	(Koshimizu et al., 1996)
<i>gurken</i>	yes						EGFR ligand	Oocyte patterning and germ plasm assembly (D)	(Filardo and Ephrussi, 2003; Gonzalez-Reyes et al., 1995; González-Reyes and St. Johnston, 1994; Neuman-Silberberg and Schupbach, 1993; Roth et al., 1995; Styhler et al., 1998; Tinker et al., 1998; Tomancak et al., 1998)
<i>gustavus</i>	yes						Novel protein	VAS localisation in oocyte	(Styhler et al., 2002)
<i>homeless</i>	yes						RNA-dependent ATPase	G plasm component localisation (D)	(Gillespie and Berg, 1995)
<i>mago nashi</i>	yes	yes	yes		yes	Hs	Novel protein	Germ plasm assembly (C, D)	(Li et al., 2000; Mohr et al., 2001; Newmark and Boswell, 1994; Newmark et al., 1997; Zhao et al., 1998)
<i>mes-2</i>		yes					Similar to <i>E(z)</i> (D polycomb gene)	Transcriptional repression (C)	(Capowski et al., 1991; Garvin et al., 1998; Holdeman et al., 1998; Kelly and Fire, 1998)
<i>mes-3</i>		yes					Novel protein	MES-2 and MES-6 localisation (C)	(Garvin et al., 1998; Holdeman et al., 1998)
<i>mes-4</i>		yes					Novel protein	GC survival (C)	(Capowski et al., 1991; Garvin et al., 1998)
<i>mes-6</i>		yes					Novel protein	Transcriptional repression, MES-2 localisation (C)	(Capowski et al., 1991; Garvin et al., 1998; Holdeman et al., 1998; Kelly and Fire, 1998)
<i>mex-1</i>		yes					Zinc finger protein	PIE-1 and P granule segregation (C)	(Guedes and Priess, 1997; Schisa et al., 2001)
<i>mex-3</i>		yes					KN domain RNA binding protein	Blastomere identity; mutation leads to ectopic GCs (C)	(Draper et al., 1996)
<i>mtlRNA</i>	yes		yes				Mitochondrial ribosomal RNA	Localisation of mitochondrial ribosomes on P granules (D)	(Amikura et al., 2001; Iida and Kobayashi, 1998; Kloc et al., 2001; Kobayashi et al., 1998; Kobayashi et al., 1995; Kobayashi and Okada, 1989)

<i>nanos</i>	yes	yes	yes	yes	yes	Ch, Dv, Gd, H (<i>Cnnos1</i> , <i>Cnnos2</i>), Hr (<i>Hrnos</i>), S, Md	CCHC Zn-finger protein	Translational and transcriptional repression (C, Ch, D, Dv, Md)	(Curtis et al., 1995; Deshpande et al., 1999; Forbes and Lehmann, 1998; Jaruzelska et al., 2003; Kang et al., 2002; Kobayashi et al., 1996; Kopranner et al., 2001; Lall et al., 2003; Lehmann and Nusslein-Volhard, 1991; Mochizuki et al., 2000; Mosquera et al., 1993; Pilon and Weisblat, 1997; Sonoda and Wharton, 1999; Subramaniam and Seydoux, 1999; Tsuda et al., 2002; Wang and Lehmann, 1991)
<i>orb</i>	yes						RNA binding protein	<i>osk</i> localisation (D)	(Christerson and McKearin, 1994; Lantz et al., 1992; Lantz et al., 1994)
<i>oskar</i>	yes					Dv	Novel protein	Germ plasm assembly (D)	(Castagnetti et al., 2000; Ephrussi and Lehmann, 1992; Kobayashi et al., 1995; Lehmann and Nusslein-Volhard, 1986; Markussen et al., 1995; Webster et al., 1994)
<i>par-1</i>	yes	yes			yes	Hs, R	Ser/Thr kinase	OSK phosphorylation, germ plasm assembly (C, D)	(Cox et al., 2001; Doring et al., 1993; Drewes et al., 1997; Guo and Kempfues, 1995; Inglis et al., 1993; Kempfues et al., 1988; Riechmann et al., 2002; Shulman et al., 2000; Tomancak et al., 2000)
<i>pgc-1</i>	yes						Non-coding RNA	PC migration (D)	(Nakamura et al., 1996)
<i>pie-1</i>		yes					Zinc finger protein	Transcriptional repression (C)	(Mello et al., 1996; Seydoux and Dunn, 1997; Seydoux et al., 1996; Tenenhaus et al., 2001)
<i>pog</i>					yes		Plant homeodomain motifs	PGC proliferation (M)	(AgoulNIK et al., 2002; Pellas et al., 1991)
<i>pumilio</i>	yes	yes			yes	Hs (CUG-BP) S	Novel RNA binding domains	Translational repression (D, C)	(Barker et al., 1992; Forbes and Lehmann, 1998; Jaruzelska et al., 2003; Kraemer et al., 1999; Lall et al., 2003; Lin and Spradling, 1997; Moore et al., 2003; Nakahata et al., 2001; Sonoda and Wharton, 1999; Spassov and Jurecic, 2003; White et al., 2001)
<i>spire</i>	yes						Novel protein	<i>osk</i> and <i>stau</i> localisation in oocyte (D)	(Clark et al., 1994)
<i>staufen</i>	yes					Hs	dsRNA binding protein	Germ plasm assembly (D)	(DesGroseillers and Lemieux, 1996; St Johnston et al., 1991; St Johnston et al., 1992)
<i>stella</i>					yes		Novel protein	Confers PGC competence (M)	(Saitou et al., 2002)
<i>tropomyosin II</i>	yes						Actin binding protein	<i>osk</i> and <i>stau</i> localisation in oocyte (D)	(Erdelyi et al., 1995)
<i>tudor</i>	yes					Hs (<i>tudor domain protein</i>)	Novel 'tudor domain' repeats	Germ plasm assembly; <i>nos</i> localisation (D)	(Boswell and Mahowald, 1985; Callebaut and Mornon, 1997; Wang et al., 1994)
<i>valois</i>	yes						Novel protein	Germ plasm assembly (D)	(Schüpbach and Wieschaus, 1989)
<i>vasa</i>	yes	yes	yes	yes	yes	Aa, Ad, B, Ca, Cc, Ci (<i>CiDEAD1b</i>), Cp, Cr, Cs (<i>CsDEAD1a</i> , <i>CsDEAD1b</i>), Dd (<i>Plvas1</i>), Dj (<i>Djvlga</i> , <i>Djvlgb</i>), Dv, E (<i>PoVAS1</i>), Ec, G (<i>Cvh</i>), H (<i>CnVAS1</i> , <i>CnVAS2</i>), He, Hs, Hy, L, Mf, O (<i>olvas</i>), Om, On,	DEAD-box RNA helicase; eIF4A (translation initiation factor) homology	Germ plasm assembly; translational regulation (D)	(Braat et al., 2000; Cardinali et al., 2002; Castrillon et al., 2000; Chang et al., 2002; Dearden et al., 2003; Fujiwara et al., 1994; Gruidl et al., 1996; Hay et al., 1988a; Hay et al., 1988b; Hay et al., 1990; Ikenishi and Tanaka, 2000; Ikenishi et al., 1996; Knaut et al., 2002; Kobayashi et al., 2000; Komiya et al., 1994; Komiya and Tanigawa, 1995; Lasko and Ashburner, 1988; Miyake et al., 2001; Mochizuki and Fujisawa, 2000; Mochizuki et al., 2001; Nakao, 1999; Olsen et al., 1997; Otani et al., 2002; Sánchez Alvarado et al., 2002; Sano et al., 2002; Schüpbach and Wieschaus, 1989; Shibata et al., 1999; Shinomiya et al., 2000; Styhler et al., 1998; Takamura et al., 2002; Tsunekawa et al., 2000; Tsunekawa et al., 2002; Wang and Callard, 2001; Wang et al., 1994; Woods et al., 2002; Yoon et al., 1997; Yoshizaki

						P, R (<i>RVLG</i>), Sa, Sg, Sm, Sp, Sq, T, Tf			et al., 2000)
<i>Xlsirts</i>			yes			Hs (<i>HumXist</i>)	Non-coding RNA	mRNA localisation to vegetal cortex (X)	(Kloc et al., 2002; Kloc et al., 1998; Kloc et al., 1993)
<i>Xpat</i>			yes				Novel protein	Localised to germ plasm (X)	(Hudson and Woodland, 1998; Kloc et al., 2002)
<p>*Usually the name of the first gene in the family to be identified.</p> <p>†Abbreviations for species names are as follows: A, <i>Ambystoma mexicanum</i> (axolotl); Aa, <i>Aurelia aurita</i> (moon jellyfish); Ad, <i>Acropora digitifera</i> (staghorn coral); B, <i>Bombyx mori</i> (silkworm); C, <i>Caenorhabditis elegans</i> (nematode); Ca, <i>Carassius auratus</i> (goldfish); Cb, <i>Cebus sp.</i> (capuchin monkey); Cc, <i>Cyprinus carpio</i> (carp); Ch, <i>Chironomus samoensis</i> (midge); Ci, <i>Ciona intestinalis</i> (ascidian); Cp, <i>Cynops pyrrhogaster</i> (newt); Cr, <i>Craspedacusta sowerbyi</i> (freshwater jellyfish); Cs, <i>Ciona savignyi</i> (ascidian); D, <i>Drosophila melanogaster</i> (fruit fly); Dd, <i>Dugesia dorotocephala</i> (flatworm); Dj, <i>Dugesia japonica</i> (flatworm); Dr, <i>Danio rerio</i> (zebrafish); Dv, <i>Drosophila virilis</i> (fruit fly); E, <i>Ephydatia fluviatilis</i> (sponge); Ec, <i>Equus caballus</i> (horse); G, <i>Gallus gallus</i> (chicken); Gd, <i>Gryllus domesticus</i> (cricket); H, <i>Hydra magnipapillata</i> (hydra); He, <i>Hydractinia echinata</i> (colonial hydroid); Hr, <i>Helobdella robusta</i> (leech); Hs, <i>Homo sapiens</i> (human); Hy, <i>Hyphessobrycon ecuadoriensis</i> (Columbian tetra); L, <i>Leucopsarion petersii</i> (ice goby); M, <i>Mus musculus</i> (mouse); Ma, <i>Macaca fascicularis</i> (crab-eating macaque); Md, <i>Musca domestica</i> (housefly); Mf, <i>Melanotaenia fluviatilis</i> (rainbowfish); Mm, <i>Macaca mulatta</i> (rhesus monkey); O, <i>Oryzias latipes</i> (medaka); Om, <i>Oncorhynchus mykiss</i> (rainbow trout); On, <i>Oreochromis niloticus</i> (Ukuobu); P, <i>Pantodon buchholzi</i> (butterfly fish); Pa, <i>Papio anubis</i> (baboon); Pt, <i>Pan troglodytes</i> (chimpanzee); R, <i>Rattus norvegicus</i> (rat); S, <i>Schistocerca americana</i> (grasshopper); Sa, <i>Sanderia malayaensis</i> (Malaysian jellyfish); Sg, <i>Schistocerca gregaria</i> (locust); Sm, <i>Schmidtea mediterranea</i> (flatworm); Sp, <i>Sparus aurata</i> (gilthead bream); Sq, <i>Squalus acanthias</i> (spiny dogfish); T, <i>Tetranychus urticae</i> (spider mite); Tf, <i>Tima formosa</i> (elegant jellyfish); X, <i>Xenopus laevis</i> (clawed frog).</p> <p>‡Note that many homologues are not given new names, but may be called 'x-like gene', where x is the name of the first gene in the family to be identified.</p> <p>§Species for which functional information is available are in parentheses.</p>									

References

- Agoulnik, A. I., Lu, B., Zhu, Q., Truong, C., Ty, M. T., Arango, N., Chada, K. K. and Bishop, C. E. (2002). A novel gene, *Pog*, is necessary for primordial germ cell proliferation in the mouse and underlies the germ cell deficient mutation, *gcd*. *Hum. Mol. Genet.* **11**, 3047-3053.
- Amikura, R., Kashikawa, M., Nakamura, A. and Kobayashi, S. (2001). Presence of mitochondria-type ribosomes outside mitochondria in germ plasm of *Drosophila* embryos. *Proc. Natl. Acad. Sci. USA* **98**, 9133-9138.
- Barker, D. D., Wang, C., Moore, J., Dickinson, L. K. and Lehmann, R. (1992). Pumilio is essential for function but not for distribution of the *Drosophila* abdominal determinant Nanos. *Genes Dev.* **6**, 2312-2326.
- Boswell, R. E. and Mahowald, A. P. (1985). *tudor*, a gene required for assembly of the germ plasm in *Drosophila melanogaster*. *Cell* **43**, 97-104.
- Braat, A. K., van de Water, S., Goos, H., Bogerd, J. and Zivkovic, D. (2000). Vasa protein expression and localization in the zebrafish. *Mech. Dev.* **95**, 271-274.
- Callebaut, I. and Mornon, J. P. (1997). The human EBNA-2 coactivator p100: multidomain organization and relationship to the staphylococcal nuclease fold and to the tudor protein involved in *Drosophila melanogaster* development. *Biochem. J.* **321**, 125-132.
- Capowski, E. E., Martin, P., Garvin, C. and Strome, S. (1991). Identification of grandchildless loci whose products are required for normal germ-line development in the nematode *Caenorhabditis elegans*. *Genetics* **129**, 1061-1072.
- Cardinali, M., Carnevali, O. and Yoshizaki, G. (2002). *Sparus aurata* vasa-like mRNA. NCBI Database Accession Number AF520608.
- Castagnetti, S., Hentze, M. W., Ephrussi, A. and Gebauer, F. (2000). Control of *oskar* mRNA translation by Bruno in a novel cell-free system from *Drosophila* ovaries. *Development* **127**, 1063-1068.
- Castrillon, D. H., Quade, B. J., Wang, T. Y., Quigley, C. and Crum, C. P. (2000). The human VASA gene is specifically expressed in the germ cell lineage. *Proc. Natl. Acad. Sci. USA* **97**, 9585-9590.
- Chang, C., Dearden, P. and Akam, M. (2002). Germ line development in the grasshopper *Schistocerca gregaria*: vasa as a marker. *Dev. Biol.* **252**, 100-118.
- Christerson, L. B. and McKearin, D. M. (1994). orb is required for anteroposterior and dorsoventral patterning during *Drosophila* oogenesis. *Genes Dev.* **8**, 614-628.
- Clark, I., Giniger, E., Ruohola-Baker, H., Jan, L. Y. and Jan, Y. N. (1994). Transient posterior localization of a kinesin fusion protein reflects anteroposterior polarity of the *Drosophila* oocyte. *Curr. Biol.* **4**, 289-300.
- Cox, D. N., Lu, B., Sun, T. Q., Williams, L. T. and Jan, Y. N. (2001). *Drosophila par-1* is required for oocyte differentiation and microtubule organization. *Curr. Biol.* **11**, 75-87.
- Curtis, D., Apfelt, J. and Lehmann, R. (1995). *nanos* is an evolutionarily conserved organizer of anterior-posterior polarity. *Development* **121**, 1899-1910.
- Dearden, P., Grbic, M. and Donly, C. (2003). Vasa expression and germ-cell specification in the spider mite *Tetranychus urticae*. *Dev. Genes Evol.* **212**, 599-603.
- DesGroseillers, L. and Lemieux, N. (1996). Localization of a human double-stranded RNA-binding protein gene (STAU) to band 20q13.1 by fluorescence in situ hybridization. *Genomics* **36**, 527-529.
- Deshpande, G., Calhoun, G., Yanowitz, J. L. and Schedl, P. D. (1999). Novel functions of *nanos* in downregulating mitosis and transcription during the development of the *Drosophila* germline. *Cell* **99**, 271-281.
- Doring, F., Drewes, G., Berling, B. and Mandelkow, E. M. (1993). Cloning and sequencing of a cDNA encoding rat brain mitogen-activated protein (MAP) kinase activator. *Gene* **131**, 303-304.
- Draper, B. W., Mello, C. C., Bowerman, B., Hardin, J. and Priess, J. R. (1996). MEX-3 is a KH domain protein that regulates blastomere identity in early *C. elegans* embryos. *Cell* **87**, 205-216.
- Drewes, G., Ebnet, A., Preuss, U., Mandelkow, E. M. and Mandelkow, E. (1997). MARK, a novel family of protein kinases that phosphorylate microtubule-associated proteins and trigger microtubule disruption. *Cell* **89**, 297-308.

- Eberhart, C. G., Maines, J. Z. and Wasserman, S. A. (1996). Meiotic cell cycle requirement for a fly homologue of human Deleted in Azoospermia. *Nature* **381**, 783-785.
- Emmons, S., Phan, H., Calley, J., Chen, W., James, B. and Manseau, L. (1995). *Cappuccino*, a *Drosophila* maternal effect gene required for polarity of the egg and embryo, is related to the vertebrate limb deformity locus. *Genes Dev.* **9**, 2482-2494.
- Ephrussi, A. and Lehmann, R. (1992). Induction of germ cell formation by *oskar*. *Nature* **358**, 387-392.
- Erdelyi, M., Michon, A. M., Guichet, A., Glotzer, J. B. and Ephrussi, A. (1995). Requirement for *Drosophila* cytoplasmic tropomyosin in *oskar* mRNA localization. *Nature* **377**, 524-527.
- Filardo, P. and Ephrussi, A. (2003). Bruno regulates *gurken* during *Drosophila* oogenesis. *Mech. Dev.* **120**, 289-297.
- Forbes, A. and Lehmann, R. (1998). *Nanos* and *Pumilio* have critical roles in the development and function of *Drosophila* germline stem cells. *Development* **125**, 679-690.
- Fujiwara, Y., Komiya, T., Kawabata, H., Sato, M., Fujimoto, H., Furusawa, M. and Noce, T. (1994). Isolation of a DEAD-family protein gene that encodes a murine homolog of *Drosophila vasa* and its specific expression in germ cell lineage. *Proc. Natl. Acad. Sci. USA* **91**, 12258-12262.
- Garvin, C., Holdeman, R. and Strome, S. (1998). The phenotype of *mes-2*, *mes-3*, *mes-4* and *mes-6*, maternal-effect genes required for survival of the germline in *Caenorhabditis elegans*, is sensitive to chromosome dosage. *Genetics* **148**, 167-185.
- Gillespie, D. E. and Berg, C. A. (1995). *homeless* is required for RNA localization in *Drosophila* oogenesis and encodes a new member of the DE-H- family of RNA-dependent ATPases. *Genes Dev.* **9**, 2495-2508.
- Gonzalez-Reyes, A., Elliott, H. and St Johnston, D. (1995). Polarization of both major body axes in *Drosophila* by *gurken-torpedo* signalling. *Nature* **375**, 654-658.
- González-Reyes, A. and St Johnston, D. (1994). Role of oocyte position in establishment of anterior-posterior polarity in *Drosophila*. *Science* **266**, 639-642.
- Gruidl, M. I., Smith, P. A., Kuznicki, K. A., McCrone, J. S., Kirchner, J., Roussell, D. L., Strome, S. and Bennett, K. L. (1996). Multiple potential germ-line helicases are components of the germ-line-specific P granules of *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **93**, 13837-13842.
- Guedes, S. and Priess, J. R. (1997). The *C. elegans* MEX-1 protein is present in germline blastomeres and is a P granule component. *Development* **124**, 731-739.
- Guo, S. and Kempthues, K. J. (1995). *par-1*, a gene required for establishing polarity in *C. elegans* embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. *Cell* **81**, 611-620.
- Harris, A. N. and Macdonald, P. M. (2001). *Aubergine* encodes a *Drosophila* polar granule component required for pole cell formation and related to eIF2C. *Development* **128**, 2823-2832.
- Hay, B., Ackerman, L., Barbel, S., Jan, L. Y. and Jan, Y. N. (1988a). Identification of a component of *Drosophila* polar granules. *Development* **103**, 625-640.
- Hay, B., Jan, L. Y. and Jan, Y. N. (1988b). A protein component of *Drosophila* polar granules is encoded by *vasa* and has extensive sequence similarity to ATP-dependent helicases. *Cell* **55**, 577-587.
- Hay, B., Jan, L. Y. and Jan, Y. N. (1990). Localization of *vasa*, a component of *Drosophila* polar granules, in maternal-effect mutants that alter embryonic anteroposterior polarity. *Development* **109**, 425-433.
- Holdeman, R., Nehrt, S. and Strome, S. (1998). MES-2, a maternal protein essential for viability of the germline in *Caenorhabditis elegans*, is homologous to a *Drosophila* Polycomb group protein. *Development* **125**, 2457-2467.
- Houston, D. W. and King, M. L. (2000). A critical role for *Xdazl*, a germ plasm-localized RNA, in the differentiation of primordial germ cells in *Xenopus*. *Development* **127**, 447-456.
- Houston, D. W., Zhang, J., Maines, J. Z., Wasserman, S. A. and King, M. L. (1998). A *Xenopus* DAZ-like gene encodes an RNA component of germ plasm and is a functional homologue of *Drosophila* boule. *Development* **125**, 171-180.
- Hudson, C. and Woodland, H. R. (1998). *Xpat*, a gene expressed specifically in germ plasm and primordial germ cells of *Xenopus laevis*. *Mech. Dev.* **73**, 159-168.
- Iida, T. and Kobayashi, S. (1998). Essential role of mitochondrially encoded large rRNA for germ-line formation in *Drosophila* embryos. *Proc. Natl. Acad. Sci. USA* **95**, 11274-11278.
- Ikenishi, K. and Tanaka, T. S. (2000). Spatio-temporal expression of *Xenopus vasa* homolog, XVLG1, in oocytes and embryos: the presence of XVLG1 RNA in somatic cells as well as germline cells. *Dev. Growth Diff.* **42**, 95-103.
- Ikenishi, K., Tanaka, T. S. and Komiya, T. (1996). Spatio-temporal distribution of the protein of *Xenopus vasa* homologue (*Xenopus vasa*-like gene 1, XVLG1) in embryos. *Dev. Growth Diff.* **38**, 527-535.
- Inglis, J. D., Lee, M. and Hill, R. E. (1993). Emk, a protein kinase with homologs in yeast maps to mouse chromosome 19. *Mamm. Genome* **4**, 401-403.
- Jaruzelska, J., Kotecki, M., Kusz, K., Spik, A., Firpo, M. and Reijo Pera, R. A. (2003). Conservation of a Pumilio-Nanos complex from *Drosophila* germ plasm to human germ cells. *Dev. Genes Evol.* **213**, 120-126.
- Johnson, A. D., Bachvarova, R. F., Drum, M. and Masi, T. (2001). Expression of axolotl *DAZZ* RNA, a marker of germ plasm: widespread maternal RNA and onset of expression in germ cells approaching the gonad. *Dev. Biol.* **234**, 402-415.
- Jongens, T. A., Hay, B., Jan, L. Y. and Jan, Y. N. (1992). The *germ cell-less* Gene Product: A Posteriorly Localized Component Necessary for Germ Cell Development in *Drosophila*. *Cell* **70**, 569-584.
- Kang, D., Pilon, M. and Weisblat, D. A. (2002). Maternal and zygotic expression of a *nanos*-class gene in the leech *Helobdella robusta*: primordial germ cells arise from segmental mesoderm. *Dev. Biol.* **245**, 28-41.
- Kelly, W. G. and Fire, A. (1998). Chromatin silencing and the maintenance of a functional germline in *Caenorhabditis elegans*. *Development* **125**, 2451-2456.
- Kempthues, K. J., Priess, J. R., Morton, D. G. and Cheng, N. S. (1988). Identification of genes required for cytoplasmic localization in early *C. elegans* embryos. *Cell* **52**, 311-320.
- Kloc, M., Bilinski, S., Chan, A. P. and Etkin, L. D. (2001). Mitochondrial ribosomal RNA in the germinal granules in *Xenopus* embryos revisited. *Differentiation* **67**, 80-83.

- Kloc, M., Dougherty, M. T., Bilinski, S., Chan, A. P., Brey, E., King, M. L., Patrick, C. W., Jr and Etkin, L. D.** (2002). Three-dimensional ultrastructural analysis of RNA distribution within germinal granules of *Xenopus*. *Dev. Biol.* **241**, 79-93.
- Kloc, M., Larabell, C., Chan, A. P. and Etkin, L. D.** (1998). Contribution of METRO pathway localized molecules to the organization of the germ cell lineage. *Mech. Dev.* **75**, 81-93.
- Kloc, M., Spohr, G. and Etkin, L. D.** (1993). Translocation of repetitive RNA sequences with the germ plasm in *Xenopus* oocytes. *Science* **262**, 1712-1714.
- Knaut, H., Steinbeisser, H., Schwarz, H. and Nusslein-Volhard, C.** (2002). An evolutionarily conserved region in the *vasa* 3'UTR targets RNA translation to the germ cells in the zebrafish. *Curr. Biol.* **12**, 454-466.
- Knecht, A. K., Good, P. J., Dawid, I. B. and Harland, R. M.** (1995). Dorsal-ventral patterning and differentiation of *noggin*-induced neural tissue in the absence of mesoderm. *Development* **121**, 1927-1935.
- Kobayashi, S., Amikura, R. and Mukai, M.** (1998). Localization of mitochondrial large ribosomal RNA in germ plasm of *Xenopus* embryos. *Curr. Biol.* **8**, 1117-1120.
- Kobayashi, S., Amikura, R., Nakamura, A., Saito, H. and Okada, M.** (1995). Mislocalization of *oskar* product in the anterior pole results in ectopic localization of mitochondrial large ribosomal RNA in *Drosophila* embryos. *Dev. Biol.* **169**, 384-386.
- Kobayashi, S. and Okada, M.** (1989). Restoration of pole-cell-forming ability to u.v.-irradiated *Drosophila* embryos by injection of mitochondrial lrRNA. *Development* **107**, 733-742.
- Kobayashi, S., Yamada, M., Asaoka, M. and Kitamura, T.** (1996). Essential role of the posterior morphogen *nanos* for germline development in *Drosophila*. *Nature* **380**, 708-711.
- Kobayashi, T., Kajiura-Kobayashi, H. and Nagahama, Y.** (2000). Differential expression of *vasa* homologue gene in the germ cells during oogenesis and spermatogenesis in a teleost fish, tilapia, *Oreochromis niloticus*. *Mech. Dev.* **99**, 139-142.
- Komiya, T., Itoh, K., Ikenishi, K. and Furusawa, M.** (1994). Isolation and characterization of a novel gene of the DEAD box protein family which is specifically expressed in germ cells of *Xenopus laevis*. *Dev. Biol.* **162**, 354-363.
- Komiya, T. and Tanigawa, Y.** (1995). Cloning of a Gene of the Dead Box Protein Family Which Is Specifically Expressed in Germ-Cells in Rats. *Biochem. Biophys. Res. Commun.* **207**, 405-410.
- Koprunner, M., Thisse, C., Thisse, B. and Raz, E.** (2001). A zebrafish *nanos*-related gene is essential for the development of primordial germ cells. *Genes Dev.* **15**, 2877-2885.
- Koshimizu, U., Taga, T., Watanabe, M., Saito, M., Shirayoshi, Y., Kishimoto, T. and Nakatsuji, N.** (1996). Functional requirement of gp130-mediated signaling for growth and survival of mouse primordial germ cells in vitro and derivation of embryonic germ (EG) cells. *Development* **122**, 1235-1242.
- Kraemer, B., Crittenden, S., Gallegos, M., Moulder, G., Barstead, R., Kimble, J. and Wickens, M.** (1999). NANOS-3 and FBF proteins physically interact to control the sperm-oocyte switch in *Caenorhabditis elegans*. *Curr. Biol.* **9**, 1009-1018.
- Lall, S., Ludwig, M. Z. and Patel, N. H.** (2003). Nanos plays a conserved role in axial patterning outside of the Diptera. *Curr. Biol.* **13**, 224-229.
- Lantz, V., Ambrosio, L. and Schedl, P.** (1992). The *Drosophila orb* gene is predicted to encode sex-specific germline RNA-binding proteins and has localized transcripts in ovaries and early embryos. *Development* **115**, 75-88.
- Lantz, V., Chang, J. S., Horabin, J. I., Bopp, D. and Schedl, P.** (1994). The *Drosophila orb* RNA-binding protein is required for the formation of the egg chamber and establishment of polarity. *Genes Dev.* **8**, 598-613.
- Lasko, P. F. and Ashburner, M.** (1988). The product of the *Drosophila* gene *vasa* is very similar to eukaryotic initiation factor-4A. *Nature* **335**, 611-617.
- Leatherman, J. L., Levin, L., Boero, J. and Jongens, T. A.** (2002). *germ cell-less* acts to repress transcription during the establishment of the *Drosophila* germ cell lineage. *Curr. Biol.* **12**, 1681-1685.
- Lee, M. H. and Schedl, T.** (2001). Identification of in vivo mRNA targets of GLD-1, a maxi-KH motif containing protein required for *C. elegans* germ cell development. *Genes Dev.* **15**, 2408-2420.
- Lehmann, R. and Nusslein-Volhard, C.** (1991). The maternal gene *nanos* has a central role in posterior pattern formation of the *Drosophila* embryo. *Development* **112**, 679-691.
- Lehmann, R. and Nüsslein-Volhard, C.** (1986). Abdominal segmentation, pole cell formation, and embryonic polarity require the localized activity of *oskar*, a maternal gene in *Drosophila*. *Cell* **47**, 144-152.
- Li, W., Boswell, R. and Wood, W. B.** (2000). *mag-1*, a homolog of *Drosophila mago nashi*, regulates hermaphrodite germ-line sex determination in *Caenorhabditis elegans*. *Dev. Biol.* **218**, 172-182.
- Lin, H. and Spradling, A. C.** (1997). A novel group of *pumilio* mutations affects the asymmetric division of germline stem cells in the *Drosophila* ovary. *Development* **124**, 2463-2476.
- MacArthur, H., Houston, D. W., Bubunenko, M., Mosquera, L. and King, M. L.** (2000). *DEADSouth* is a germ plasm specific DEAD-box RNA helicase in *Xenopus* related to *eIF4A*. *Mech. Dev.* **95**, 291-295.
- Markussen, F. H., Michon, A. M., Breitwieser, W. and Ephrussi, A.** (1995). Translational control of *oskar* generates short OSK, the isoform that induces pole plasma assembly. *Development* **121**, 3723-3732.
- Mello, C. C., Schubert, C., Draper, B., Zhang, W., Lobel, R. and Priess, J. R.** (1996). The PIE-1 protein and germline specification in *C. elegans* embryos. *Nature* **382**, 710-712.
- Miyake, A., Saito, T., Kamimoto, M., Saito, T., Suzuki, T., Nakatsuji, N. and Nakatsuji, T.** (2001). The *vasa* mRNA expression and localization during embryogenesis of the shiro-uo (*Leucopsarion petersii*). In *14th International Congress of Developmental Biology*, Suppl. 43, pp. S121. Kyoto, Japan.
- Mochizuki, K. and Fujisawa, T.** (2000). *vasa*-related genes in Cnidaria. NCBI Database Accession Numbers AB048852, AB048853, AB048854, AB048856, AB048857, AB48858 and AB48859.
- Mochizuki, K., Nishimiya-Fujisawa, C. and Fujisawa, T.** (2001). Universal occurrence of the *vasa*-related genes among metazoans and their germline expression in *Hydra*. *Dev. Genes Evol.* **211**, 299-308.
- Mochizuki, K., Sano, H., Kobayashi, S., Nishimiya-Fujisawa, C. and Fujisawa, T.** (2000). Expression and evolutionary conservation of *nanos*-related genes in *Hydra*. *Dev. Genes Evol.* **210**, 591-602.
- Mohr, S. E., Dillon, S. T. and Boswell, R. E.** (2001). The RNA-binding protein Tsunagi interacts with Mago Nashi to establish polarity and localize *oskar* mRNA during *Drosophila* oogenesis. *Genes Dev.* **15**, 2886-2899.

- Moore, F. L., Jaruzelska, J., Fox, M. S., Urano, J., Firpo, M. T., Turek, P. J., Dorfman, D. M. and Pera, R. A. (2003). Human Pumilio-2 is expressed in embryonic stem cells and germ cells and interacts with DAZ (Deleted in AZoospermia) and DAZ-like proteins. *Proc. Natl. Acad. Sci. USA* **100**, 538-543.
- Mosquera, L., Forristall, C., Zhou, Y. and King, M. L. (1993). A mRNA localized to the vegetal cortex of *Xenopus* oocytes encodes a protein with a *nanos*-like zinc finger domain. *Development* **117**, 377-386.
- Nakahata, S., Katsu, Y., Mita, K., Inoue, K., Nagahama, Y. and Yamashita, M. (2001). Biochemical identification of *Xenopus* Pumilio as a sequence-specific cyclin B1 mRNA-binding protein that physically interacts with a Nanos homolog, Xcat-2, and a cytoplasmic polyadenylation element-binding protein. *J. Biol. Chem.* **276**, 20945-20953.
- Nakamura, A., Amikura, R., Mukai, M., Kobayashi, S. and Lasko, P. F. (1996). Requirement for a noncoding RNA in *Drosophila* polar granules for germ cell establishment. *Science* **274**, 2075-2079.
- Nakao, H. (1999). Isolation and characterization of a *Bombyx vasa*-like gene. *Dev. Genes Evol.* **209**, 312-316.
- Neuman-Silberberg, F. S. and Schupbach, T. (1993). The *Drosophila* dorsoventral patterning gene *gurken* produces a dorsally localized RNA and encodes a TGF alpha-like protein. *Cell* **75**, 165-174.
- Newmark, P. A. and Boswell, R. E. (1994). The *mago nashi* locus encodes an essential product required for germ plasm assembly in *Drosophila*. *Development* **120**, 1303-1313.
- Newmark, P. A., Mohr, S. E., Gong, L. and Boswell, R. E. (1997). *mago nashi* mediates the posterior follicle cell-to-oocyte signal to organize axis formation in *Drosophila*. *Development* **124**, 3197-3207.
- Olsen, C. E., Aasland, R. and Fjose, A. (1997). A *vasa*-like gene in zebrafish identifies putative primordial germ cells. *Mech. Dev.* **66**, 95-105.
- Otani, S., Maegawa, S., Inoue, K., Arai, K. and Yamaha, E. (2002). The germ cell lineage identified by *vas*-mRNA during the embryogenesis in goldfish. *Zool. Sci.* **19**, 519-526.
- Pellas, T. C., Ramachandran, B., Duncan, M., Pan, S. S., Marone, M. and Chada, K. (1991). *Germ-cell deficient (gcd)*, an insertional mutation manifested as infertility in transgenic mice. *Proc. Natl. Acad. Sci. USA* **88**, 8787-8791.
- Pilon, M. and Weisblat, D. A. (1997). A *nanos* homolog in leech. *Development* **124**, 1771-1780.
- Riechmann, V., Gutierrez, G. J., Filardo, P., Nebreda, A. R. and Ephrussi, A. (2002). Par-1 regulates stability of the posterior determinant Oskar by phosphorylation. *Nat. Cell Biol.* **4**, 337-342.
- Robertson, S. E., Dockendorff, T. C., Leatherman, J. L., Faulkner, D. L. and Jongens, T. A. (1999). *germ cell-less* is required only during the establishment of the germ cell lineage of *Drosophila* and has activities which are dependent and independent of its localization to the nuclear envelope. *Dev. Biol.* **215**, 288-297.
- Roth, S., Neuman-Silberberg, F. S., Barcelo, G. and Schupbach, T. (1995). *cornichon* and the EGF receptor signaling process are necessary for both anterior-posterior and dorsal-ventral pattern formation in *Drosophila*. *Cell* **81**, 967-978.
- Ruggiu, M., Speed, R., Taggart, M., McKay, S. J., Kilanowski, F., Saunders, P., Dorin, J. and Cooke, H. J. (1997). The mouse *Dazl* gene encodes a cytoplasmic protein essential for gametogenesis. *Nature* **389**, 73-77.
- Saitou, M., Barton, S. C. and Surani, M. A. (2002). A molecular programme for the specification of germ cell fate in mice. *Nature* **418**, 293-300.
- Sánchez Alvarado, A., Newmark, P. A., Robb, S. M. and Juste, R. (2002). The Schmidtea mediterranea database as a molecular resource for studying platyhelminthes, stem cells and regeneration. *Development* **129**, 5659-5665.
- Sano, H., Kobayashi, S. and Nakamura, A. (2002). *Drosophila virilis vasa (vas)* homolog. NCBI Database Accession Number AF513908.
- Schisa, J. A., Pitt, J. N. and Priess, J. R. (2001). Analysis of RNA associated with P granules in germ cells of *C. elegans* adults. *Development* **128**, 1287-1298.
- Schüpbach, T. and Wieschaus, E. (1989). Female Sterile Mutations on the Second Chromosome of *Drosophila melanogaster*. I. Maternal Effect Mutations. *Genetics* **121**, 101-117.
- Schüpbach, T. and Wieschaus, E. (1991). Female Sterile Mutations on the Second Chromosome of *Drosophila melanogaster*. II. Mutations Blocking Oogenesis or Altering Egg Morphology. *Genetics* **129**, 1119-1136.
- Seydoux, G. and Dunn, M. A. (1997). Transcriptionally repressed germ cells lack a subpopulation of phosphorylated RNA polymerase II in early embryos of *Caenorhabditis elegans* and *Drosophila melanogaster*. *Development* **124**, 2191-2201.
- Seydoux, G., Mello, C. C., Pettitt, J., Wood, W. B., Priess, J. R. and Fire, A. (1996). Repression of gene expression in the embryonic germ lineage of *C. elegans*. *Nature* **382**, 713-716.
- Shibata, N., Umesono, Y., Orii, H., Sakurai, T., Watanabe, K. and Agata, K. (1999). Expression of *vasa (vas)*-related genes in germline cells and totipotent somatic stem cells of planarians. *Dev. Biol.* **206**, 73-87.
- Shinomiya, A., Tanaka, M., Kobayashi, T., Nagahama, Y. and Hamaguchi, S. (2000). The *vasa*-like gene, *olvas*, identifies the migration path of primordial germ cells during embryonic body formation stage in the medaka, *Oryzias latipes*. *Dev. Growth Diff.* **42**, 317-326.
- Shulman, J. M., Benton, R. and St. Johnston, D. (2000). The *Drosophila* homolog of *C. elegans* PAR-1 organizes the oocyte cytoskeleton and directs *oskar* mRNA localization to the posterior pole. *Cell* **101**, 377-388.
- Sonoda, J. and Wharton, R. P. (1999). Recruitment of Nanos to *hunchback* mRNA by Pumilio. *Genes Dev.* **13**, 2704-2712.
- Spasov, D. S. and Jurecic, R. (2003). Mouse *Pum1* and *Pum2* genes, members of the Pumilio family of RNA-binding proteins, show differential expression in fetal and adult hematopoietic stem cells and progenitors. *Blood Cells Mol. Dis.* **30**, 55-69.
- St Johnston, D., Beuchle, D. and Nusslein-Volhard, C. (1991). *Staufen*, a gene required to localize maternal RNAs in the *Drosophila* egg. *Cell* **66**, 51-63.
- St Johnston, D., Brown, N. H., Gall, J. G. and Jantsch, M. (1992). A conserved double-stranded RNA-binding domain. *Proc. Natl. Acad. Sci. USA* **89**, 10979-10983.
- Styhler, S., Nakamura, A. and Lasko, P. (2002). VASA localization requires the SPRY-domain and SOCS-box containing protein, GUSTAVUS. *Dev. Cell* **3**, 865-876.

- Styhler, S., Nakamura, A., Swan, A. and Suter, B.** (1998). *vasa* is required for GURKEN accumulation in the oocyte, and is involved in oocyte differentiation and germline cyst development. *Development* **125**, 1569-1578.
- Subramaniam, K. and Seydoux, G.** (1999). *nos-1* and *nos-2*, two genes related to *Drosophila nanos*, regulate primordial germ cell development and survival in *Caenorhabditis elegans*. *Development* **126**, 4861-4871.
- Takamura, K., Fujimura, M. and Yamaguchi, Y.** (2002). Primordial germ cells originate from the endodermal strand cells in the ascidian *Ciona intestinalis*. *Dev. Genes Evol.* **212**, 11-18.
- Tenenhaus, C., Subramaniam, K., Dunn, M. A. and Seydoux, G.** (2001). PIE-1 is a bifunctional protein that regulates maternal and zygotic gene expression in the embryonic germ line of *Caenorhabditis elegans*. *Genes Dev.* **15**, 1031-1040.
- Timchenko, L. T., Miller, J. W., Timchenko, N. A., DeVore, D. R., Datar, K. V., Lin, L., Roberts, R., Caskey, C. T. and Swanson, M. S.** (1996). Identification of a (CUG)_n triplet repeat RNA-binding protein and its expression in myotonic dystrophy. *Nucl. Acids Res.* **24**, 4407-4414.
- Tinker, R., Silver, D. and Montell, D. J.** (1998). Requirement for the *vasa* RNA helicase in *gurken* mRNA localization. *Dev. Biol.* **199**, 1-10.
- Tomancak, P., Guichet, A., Zavorsky, P. and Ephrussi, A.** (1998). Oocyte polarity depends on regulation of *gurken* by Vasa. *Development* **125**, 1723-1732.
- Tomancak, P., Piano, F., Riechmann, V., Gunsalus, K. C., Kempthues, K. J. and Ephrussi, A.** (2000). A *Drosophila melanogaster* homologue of *Caenorhabditis elegans* par-1 acts at an early step in embryonic-axis formation. *Nat. Cell Biol.* **2**, 458-460.
- Tsuda, M., Sasaoka, Y., Kiso, M., Abe, K., Haraguchi, S., Kobayashi, S. and Saga, Y.** (2003). Conserved role of nanos proteins in germ cell development. *Science* **301**, 1239-1241.
- Tsunekawa, N., Naito, M., Sakai, Y., Nishida, T. and Noce, T.** (2000). Isolation of chicken *vasa* homolog gene and tracing the origin of primordial germ cells. *Development* **127**, 2741-2750.
- Tsunekawa, N., Nakamura, A., Fukui, A., Asashima, M. and Noce, T.** (2002). Isolation and characterisation of a new *vasa* gene. In *Germ Cells*, pp. 149. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Venables, J. P., Ruggiu, M. and Cooke, H. J.** (2001). The RNA-binding specificity of the mouse Dazl protein. *Nucleic Acids Res.* **29**, 2479-2483.
- Wang, C. and Callard, G. V.** (2001). Molecular cloning and stage-related distribution of a *vasa*-related gene from shark testis. NCBI Database Accession Number AF432868.
- Wang, C., Dickinson, L. K. and Lehmann, R.** (1994). Genetics of *nanos* localization in *Drosophila*. *Dev. Dyn.* **199**, 103-115.
- Wang, C. and Lehmann, R.** (1991). Nanos is the localized posterior determinant in *Drosophila*. *Cell* **66**, 637-647.
- Webster, P. J., Liang, L., Berg, C. A., Lasko, P. and Macdonald, P. M.** (1997). Translational repressor *bruno* plays multiple roles in development and is widely conserved. *Genes Dev.* **11**, 2510-2521.
- Webster, P. J., Suen, J. and Macdonald, P. M.** (1994). *Drosophila virilis oskar* transgenes direct body patterning but not pole cell formation or maintenance of mRNA localization in *D. melanogaster*. *Development* **120**, 2027-2037.
- White, E. K., Moore-Jarrett, T. and Ruley, H. E.** (2001). PUM2, a novel murine puf protein, and its consensus RNA-binding site. *RNA* **7**, 1855-1866.
- Wilson, J. E., Connell, J. E. and Macdonald, P. M.** (1996). aubergine enhances oskar translation in the *Drosophila* ovary. *Development* **122**, 1631-1639.
- Woods, B. G., Ginther, O. J., Wentworth, A., Wentworth, B. and Wiltbank, M.** (2002). Equine VASA homolog. NCBI Database Accession Number AY100475.
- Xu, E. Y., Moore, F. L. and Pera, R. A.** (2001). A gene family required for human germ cell development evolved from an ancient meiotic gene conserved in metazoans. *Proc. Natl. Acad. Sci. USA* **98**, 7414-7419.
- Yoon, C., Kawakami, K. and Hopkins, N.** (1997). 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**, 3157-3166.
- Yoshizaki, G., Sakatani, S., Tominaga, H. and Takeuchi, T.** (2000). Cloning and characterization of a *vasa*-like gene in rainbow trout and its expression in the germ cell lineage. *Mol. Reprod. Dev.* **55**, 364-371.
- Zhao, X. F., Colaizzo-Anas, T., Nowak, N. J., Shows, T. B., Elliott, R. W. and Aplan, P. D.** (1998). The mammalian homologue of mago nashi encodes a serum-inducible protein. *Genomics* **47**, 319-322.

Table S2. Determining the mode of germ cell specification across the Metazoa

PGC origin*		Mode of PGC specification [†]	Experimental evidence [‡]	PGC identification criteria [§]	References
Stage	Location/Derivation				
BASAL LINEAGES					
Porifera	Mesenchyme formation	Mesenchymal cells	E	-	LM, TEM, MM (Gaino et al., 1984; Hyman, 1940-1959; Mochizuki et al., 2001; Pilato, 2000; Tuzet et al., 1970)
Cnidaria					
Anthozoa	Post-embryonic	Gastrodermal cells of mesentery or endocoelic epithelial cells	E	-	TEM, LM (Goffredo et al., 2000; Halvorson and Monroy, 1985; Jennison, 1979; Kumé and Dan, 1968; Ryland, 1997)
Scyphozoa	Post-embryonic	Within ovaries from endodermally derived gastrodermis	E	-	TEM (Eckelbarger and Larson, 1988)
Hydrozoa	Gastrulation	Endodermal core	E	+	LM, TEM, MM (Berrill and Liu, 1948; Brauer, 1891; Carré and Carré, 2000; Hargitt, 1919; Littlefield and Bode, 1986; Martin and Archer, 1997; Martin et al., 1997; Mochizuki et al., 2001; Mochizuki et al., 2000; Noda and Kanai, 1977; Pilato, 2000; Weismann, 1883)
Ctenophora	Early larval stage	Endoderm	E	-	LM (Chun, 1880; Dunlap Pianka, 1974; Dunlap-Pianka, 1966; Garbe, 1901; Komai, 1922; Nernandez-Nicaise, 1991)
BILATERIA (Triploblasts)					
Acoelomorpha	Late embryogenesis	Mesenchymal	E	-	LM, TEM (Falleni and Gremigni, 1990; Gschwentner et al., 2001)
Lophotrochozoa (Protostomes)					
Platyhelminthes					
Turbellaria	Late embryogenesis	Mesenchymal	E	+	LM, TEM, MM (Child, 1906; Falleni et al., 1995; Ladurner et al., 2000; Lucchesi et al., 1995)
Trematoda	First cleavage	First cleavage	P	-	LM (Bednarz, 1962; Bednarz, 1973; Cort, 1944; van der Woude, 1954)
Cestoda	Late embryogenesis	Mesenchymal	E	-	LM, TEM (Gustafsson, 1976)
Rotifera	Before gastrulation	4d cell	P	-	LM (Lechner, 1966; Nachtwey, 1925; Zelinka, 1891)
Entoprocta	nd	nd	nd	-	nd (Mariscal, 1974)
Ectoprocta	Post-embryonic	Mesenchyme: gonadal epithelium	E	-	LM (Brien, 1959; Emschermann, 1982; Ryland, 1970)
Nemertea	Late embryogenesis	Mesodermally derived cells of parenchyma or gonadal epithelium	E	-	LM, TEM (Bierne, 1862; Bürger, 1897-1907; Crandall et al., 1998; Olivier, 1966; Riser, 1974; Wilson, 1900)
Phoronida	Late embryogenesis	Peritoneal epithelium	E	-	LM (Benham, 1889; Ikeda, 1903; Koren and Daniellsen, 1877)
Brachiopoda	Late embryogenesis	Ileo-parietal epithelium	E	-	LM, TEM (James et al., 1991a; James et al., 1991b; Yatsu, 1901)
Gnathostomulida	nd	nd	nd	-	nd (Sterrer, 1974)
Pogonophora	Post-embryonic	Gonadal epithelium	E	-	LM (Ivanov, 1963; Southwood, 1974)
Echiura	Larval stage	Mesoderm	E	-	LM, TEM (Gould-Somero and Holland, 1975; Loosli, 1935; Newby, 1932; Newby, 1940)
Sipunculida	Larval stage	Gonadal epithelium	E	-	LM (Andrews, 1889; Gérould, 1907; Hérubel, 1908; Rice, 1974)
Mollusca					
Aplacophora	Post-larval	Mesodermal?	E	-	LM (Thompson, 1960)
Polyplacophora	Post-embryonic	Gonadal epithelium	E	-	TEM (Selwood, 1968)
Cephalopoda	Blastoderm stage	Blastoderm superficial layer	P	-	LM (Faussek, 1901; Teichmann, 1903)
Gastropoda	Late embryogenesis/early	Mesodermal/early cleavage	E/P	-	LM, TEM (Aubry, 1962; Bounoure and Aubry, 1956; Brisson,

	cleavage?	blastomere?				1971; Brisson, 1973; Dohmen, 1983; Dohmen and Lok, 1975; Dohmen and Verdonk, 1974; Hogg and Wijdenes, 1979; Lavoillette, 1954; Moor, 1983; Tardy, 1970; Verdonk, 1973)
<i>Bivalvia</i>	Early cleavage	4d cell	P	–	LM	(Woods, 1931; Woods, 1932)
Annelida						
<i>Polychaeta</i>	Early cleavage/post-larval	4d cell/peritoneal vascular epithelium/	E/P	–	LM, TEM	(Dales, 1950; Dehorne, 1933; Dhainaut, 1970; Dorsett, 1961; Eckelbarger, 1984; Fischer, 1975; Fordham, 1925; Garwood, 1981; Iwanoff, 1928; Lieber, 1931; Malaquin, 1924; Malaquin, 1925; Malaquin, 1934; Nusbaum, 1908; Potswald, 1969; Potswald, 1972; Randolph, 1892; Stagni, 1959; Wilson, 1892)
<i>Oligochaeta</i>	Early cleavage/late embryogenesis	4d cell/unknown source before mesoderm formation/unknown source late in development	E/P	+	LM, TEM	(Beddard, 1892; Bergh, 1885a; Bergh, 1885b; Devires, 1971; Goto et al., 1999; Herlant-Meewis, 1946; Iwanoff, 1928; Kutsuna et al., 2001; Lehmann, 1887; Meyer, 1929; Penners, 1929; Penners and Stablein, 1931; Wilson, 1889)
<i>Hirudinea</i>	Early cleavage	D blastomere	P	–	LM, MM	(Bürger, 1902; Kang et al., 2002; Pilon and Weisblat, 1997; Weisblat et al., 1984; Weisblat and Shankland, 1985; Whitman, 1878)
Ecdysozoa (Protostomes)						
Arthropoda						
<i>Collembola</i>	Early cleavage	Early cleavage blastomeres	P	–	LM, TEM	(Claypole, 1898; Garaudy-Tamarelle, 1969; Garaudy-Tamarelle, 1970; Klag, 1977; Klag, 1982; Klag, 1984; Klag and Ostachowska-Gasior, 1997; Klag and Swiatek, 1999; Swiatek et al., 2001; Tamarelle, 1979)
<i>Insecta</i>	Early cleavage/late embryogenesis	Early cleavage blastomere/mesoderm	E/P	+	LM, TEM, SEM, EM, MM, LI	Reviewed elsewhere (C.G.E., unpublished)
<i>Crustacea</i>	Early cleavage/late embryogenesis	Early cleavage blastomere/mesoderm	E/P	–	LM, TEM, MM, LI	Reviewed elsewhere (C.G.E., unpublished)
<i>Chelicerata</i>	Early cleavage/late embryogenesis	Inner blastoderm cells/primary cumulus/secondary cumulus/mesoderm	E/P	–	LM, TEM, SEM, MM	(Aeschlimann, 1958; Balbiani, 1864; Brauer, 1894; Dearden et al., 2003; Dogiel, 1913; Farley, 2001; Faussek, 1891; Heymons, 1904; Iwanoff, 1933; Jubertie, 1964; Morgan, 1891; Moritz, 1957; Mothes-Wagner and Seitz, 1984; Munson, 1898; Packard, 1880; Sánchez, 1959; Schimkewitsch, 1906)
<i>Myriapoda</i>	Late embryogenesis	Mesoderm: coelomic sacs	E	–	LM	(Heymons, 1901; Tiegs, 1940; Tiegs, 1947)
<i>Tardigrada</i>	Late embryogenesis	Mesoderm: coelomic sacs	E	–	LM	(Marcus, 1929; May, 1948; von Erlanger, 1895; von Wenck, 1914)
<i>Onychophora</i>	Gastrulation/late embryogenesis	Blastopore/endoderm/mesoderm	E/P	–	LM	(Evans, 1901; Manton, 1949; Sedgwick, 1887)
<i>Nematoda</i>	First cleavage	First cleavage blastomere	P	+	LM, TEM, SEM, MM, LI	(Bossinger and Schierenberg, 1996; Boveri, 1887; Boveri, 1899; Boveri, 1909; Martini, 1903; Pai, 1928; Spemann, 1895; Ziegler, 1895)
<i>Priapula</i>	nd	nd	nd	–	nd	(van der Land, 1974)
<i>Gastrotricha</i>	Late embryogenesis	Base of proctodeum	E	–	LM	(Beauchamp de, 1929; Sacks, 1955)
<i>Kinorhyncha</i>	nd	Apical cells of gonad	E	–	LM	(Zelinka, 1928)
Deuterostomes						
<i>Chaetognatha</i>	First cleavage	First cleavage blastomere	P	+	LM, TEM, MM, LI	(Buchner, 1910; Bütschli, 1873; Carré et al., 2002; Doncaster, 1902; Elpatievsky, 1909; Ghirardelli, 1954; Hertzwig, 1880; Shimotori and Goto, 2001; Stevens, 1910; Vasiljev, 1925)

Hemichordata	Late embryogenesis	Ectoderm/mesoderm	E	–	LM	(Bateson, 1885; Bateson, 1886; Morgan, 1894; Spengel, 1893; Willey, 1899)
Echinodermata						
<i>Crinoidea</i>	Metamorphosis	Wall of stomatocoel	E	–	LM	(Perrier, 1889)
<i>Asteroidea</i>	Metamorphosis	Wall of stomatocoel	E	–	LM, TEM	(Inoue and Shirai, 1991; MacBride, 1896)
<i>Holothuroidea</i>	Post-larval	Gonadal epithelium	E	+	LM, TEM	(Eckelbarger and Young, 1992; Frick and Ruppert, 1996; Frick et al., 1996; Killie, 1942; Mortensen, 1904)
<i>Echinoidea</i>	Metamorphosis/16-cell stage?	Wall of stomatocoel/small micromeres?	E/P	+	LM, TEM, MM	(Cohen et al., 1975; Davidson et al., 1998; Houk and Hinegardner, 1980; Houk and Hinegardner, 1981; MacBride, 1903; Ogawa et al., 1999; Pehrson and Cohen, 1986; Ransick et al., 1996)
Chordata						
<i>Urochordata</i>	Two-cell stage/post-metamorphosis	B7.6 cells: posterior of embryo	P	+	LM, TEM, MM, LI	(Berrill, 1941; Fujimura and Takamura, 2000; Iseto and Nishida, 1999; Mukai, 1977; Mukai and Watanabe, 1976; Nakamura et al., 2003; Nishida, 1987; Nishikata et al., 1999; Sabbadin and Zaniolo, 1979; Stoner et al., 1999; Stoner and Weissman, 1996; Takamura et al., 2002; Tomioka et al., 2002; Yamamoto and Okada, 1999)
<i>Cephalochordata</i>	Cleavage stages/larval stages	Mesoderm of myocoel/gonadal epithelium/single cleavage stage blastomere?	E/P	–	LM, TEM	(Boveri, 1892; Frick and Ruppert, 1997; Hatschek, 1888; Holland and Holland, 1992)
Agnatha	Gastrulation	Unclear	E	–	LM	(Beard, 1900; Beard, 1902a; Beard, 1902b; Butcher, 1929; Goette, 1890; Hardisty, 1971; Okkelberg, 1921; Walvig, 1963; Wheeler, 1899)
<i>Chondrichthyes</i>	Late cleavage stages/late embryogenesis	Blastoderm/mesoderm	E/P	–	LM	(Balfour, 1878; Beard, 1900; Beard, 1902a; Beard, 1902b; Ruchert, 1888; Wijhe, 1889; Woods, 1902)
<i>Actinopterygii</i>	Cleavage stages/late embryogenesis	Cleavage blastomeres/endoderm	E/P	+	LM, TEM, MM, LI	(Allen, 1911; Braat et al., 1999; De Smet, 1970; Dodds, 1910; Eigenmann, 1891; Goodrich et al., 1934; Hann, 1927; Kobayashi and Iwamatsu, 2000; Maschkowzeff, 1934; Oppenheimer, 1959a; Oppenheimer, 1959b; Richards and Thompson, 1921; Shinomiya et al., 2000; Yoshizaki et al., 2000)
<i>Dipnoi</i>	Late embryogenesis	Unclear	E	–	MM	(Johnson et al., 2002)
<i>Urodela</i>	Late embryogenesis	Lateral plate mesoderm	E	+	LM, TEM, MM	(Humphrey, 1925; Humphrey, 1929; Johnson et al., 2001; Johnson et al., 2002; Johnson et al., 2003; McCosh, 1930; Nieuwkoop, 1947)
<i>Anura</i>	Cleavage stages	Cleavage blastomeres/endoderm	P	+	LM, TEM, MM, LI	(Aubry, 1953; Blackler, 1958; Bounoure, 1927; Gipouloux, 1971; Gipouloux, 1975; Ogiso-Ono and Ikenishi, 1999; Padoa, 1963; Smith, 1966; Swingle, 1921)
<i>Archosauria</i>	Cleavage stages	Cleavage stages	P	+	LM, TEM, EM, MM	(Eyal-Giladi et al., 1981; Karagenc et al., 1996; Matsumoto, 1932; Naito et al., 2001; Swift, 1914; Tsunekawa et al., 2000)
<i>Squamata</i>	Primitive streak formation	Extraembryonic endoderm	E	–	LM, MM	(Hubert, 1969; Jarvis, 1908; Pasteels, 1953; Simkins and Asana, 1930; Tribe and Brambell, 1932)
<i>Testudines</i>	Primitive streak formation	Extraembryonic endoderm	E	–	LM, TEM, MM	(Allen, 1906; Fujimoto et al., 1979; Jordan, 1917; Risley, 1933; Simkins, 1925; Tsunekawa et al., 2000)
<i>Mammalia</i>	Primitive streak formation	Proximal epiblast	E	+	LM, TEM, EM, MM, LI	(Allen, 1904; Eddy et al., 1981; Everett, 1945; Falin, 1969; Heys, 1931; Jiang et al., 1997; Selwood, 2001; Simkins, 1928; Tarkowski, 1959; Ullmann et al., 1997; Vanneman, 1917; Witschi, 1948)

*Since comparing the duration of stages of development in different species is often confusing, we describe relative developmental stages rather than absolute time. nd, no data.

†P, preformation; E, epigenesis

‡+, yes; -, no.

§LM, light microscopic histological analysis, of either whole mounts or sections; TEM, transmission electron microscopy; SEM, scanning electron microscopy; EM, enzymatic markers; MM, molecular markers, usually in situ hybridization or antibody staining; LI, cell lineage studies.

References

- Aeschlimann, A.** (1958). Développement embryonnaire d'*Ornithodoros moubata* (Murray) et transmission transovarienne de *Borrelia duttoni*. *Acta Trop.* **15**, 15-64.
- Allen, B. M.** (1904). The embryonic development of the ovary and testis of the mammals. *Am. J. Anat.* **3**, 8-153.
- Allen, B. M.** (1906). The origin of the sex-cells of *Chrysemys*. *Anat. Anz.* **29**, 217-236.
- Allen, B. M.** (1911). The origin of the sex-cells of *Amia* and *Lepidosteus*. *J. Morphol.* **22**, 1-35.
- Andrews, E. A.** (1889). The reproductive organs of *Phascolosoma gouldii*. *Zool. Anz.* **12**, 140-142.
- Aubry, R.** (1953). Nouveaux essais de stérilisation totale des gonades de *Rana temporaria* par action des rayons ultraviolets sur le pôle inférieur de l'oeuf fécondé. *C. R. Acad. Sci. III* **236**, 1101-1102.
- Aubry, R.** (1962). Étude de l'hermaphroditisme et de l'action pharmacodynamique de hormones de vertébrés chez les Gastéropodes Pulmonés. *Arch. Anat. Microsc. Morphol. Exp.* **50**, 521-602.
- Balbani, M.** (1864). Sur la constitution du germe dans l'oeuf animal avant la fécondation. *C. R. Acad. Sci. III* **58**, 584-588.
- Balfour, F. M.** (1878). A Monograph on the Development of Elasmobranch Fishes. London: MacMillan and Co.
- Bateson, W.** (1885). The later stages in the development of *Balanoglossus kowalevskii*, with a suggestion as to the affinities of the Enteropneusta. *Quart. J. Microscop. Sci.* **25**, 81-122.
- Bateson, W.** (1886). Continued account of the later stages in the development of *Balanoglossus kowalevskii*, and of the morphology of the Enteropneusta. *Quart. J. Microscop. Sci.* **26**.
- Beard, J.** (1900). The morphological continuity of the germ cells in *Raja batis*. *Anat. Anz.* **18**, 465-485.
- Beard, J.** (1902a). The germ cells of *Pristiurus*. *Anat. Anz.* **21**, 50-61.
- Beard, J.** (1902b). The germ cells. I. *Raja batis*. *Zool. Jahrb. Abt. Anat. Ontogenie Tiere* **16**, 615-702.
- Beauchamp de, M. P.** (1929). Le développement des gastrotriches (note préliminaire). *Bul. Soc. Zool. France* **54**, 549-558.
- Beddard, F. E.** (1892). Researches into the embryology of the Oligochaeta. No.1. On certain points in the development of *Acanthrodriilus multiporus*. *Quart. J. Microscop. Sci.* **33**, 497-540.
- Bednarz, S.** (1962). The developmental cycle of germ cells in *Fasciola hepatica* L. 1758 (Trematoda, Digenera). *Zool. Pol.* **12**, 439-466.
- Bednarz, S.** (1973). The developmental cycle of the germ cells in several representatives of Trematoda (Digenera). *Zool. Pol.* **23**, 279-326.
- Benham, W. B.** (1889). The anatomy of *Phoronis australis*. *Quart. J. Microscop. Sci.* **30**, 125-158.
- Bergh, R. S.** (1885a). Die Metamorphose von *Aulostomum gulo*. *Arb. Zool. Zootom. Inst. Wurzburg* **7**, 231-291.
- Bergh, R. S.** (1885b). Über die Metamorphose von *Nephilis*. *Z. Wiss. Zool.* **41**, 284-301.
- Berrill, N. J.** (1941). The development of the bud in *Botryllus*. *Biol. Bull.* **80**, 169-184.
- Berrill, N. J. and Liu, C. K.** (1948). Germplasm, Weismann, and Hydrozoa. *Q. Rev. Biol.* **23**, 124-132.
- Bierne, J.** (1862). La régénération des gonades chez la Némerte *Lineus ruber* Müller. *C. R. Acad. Sci. III* **255**, 185-187.
- Blackler, A. W.** (1958). Contribution to the study of germ cells in the Anura. *J. Embryol. Exp. Morphol.* **6**, 491-503.
- Bossinger, O. and Schierenberg, E.** (1996). Cell-cell communication in nematode embryos: differences between *Cephalobus spec.* and *Caenorhabditis elegans*. *Dev. Genes Evol.* **206**, 25-34.
- Bounoure, L.** (1927). Le chondriome des gonocytes primaires chez *Rana temporaria* et la recherche des éléments aux jeunes stades du développement. *C. R. Acad. Sci. III* **185**, 1304-1305.
- Bounoure, L. and Aubry, R.** (1956). La structure du canal hermaphrodite de *Limnaea stagnalis* et les possibilités de régénération germinale chez les Gastéropodes Pulmonés. *C. R. Acad. Sci. Paris* **243**, 1453-1455.
- Boveri, T.** (1887). Über Differenzierung der Zellkerne während der Furchung des Eies von *Ascaris megalcephala*. *Anat. Anz.* **2**.
- Boveri, T.** (1892). Über die Bildungsstätte der Geschlechtsdrüsen und die Entstehung der Genitalkamern beim *Amphioxus*. *Anat. Anz.* **7**, 170-181.
- Boveri, T.** (1899). Die Entwicklung von *Ascaris megalcephala* mit besonderer Rücksicht auf die Kernverhältnisse. In *Festschr. zum 70ten Geburtstag von Carl von Kupfer*, pp. 383-430. Jena: Gustav Fischer.
- Boveri, T.** (1909). Die Blastomerenkerne von *Ascaris megalcephala* und die theorie der Chromosomenindividualität. *Arch. Zellforsch.* **3**, 181-268.
- Braat, A. K., Speksnijder, J. E. and Zivkovic, D.** (1999). Germ line development in fishes. *Int. J. Dev. Biol.* **43**, 745-760.

- Brauer, F.** (1891). Über die Entstehung der Geschlechtsprodukte und die Entwicklung von *Tubularia mesembryanthemum*. *Z. Wiss. Zool* **52**, 551-579.
- Brauer, F.** (1894). Beiträge zur Kenntnis der Entwicklungsgeschichte des Skorpions. *Z. Wiss. Zool.* **57**, 402-432.
- Brien, P.** (1959). Classe des Endoproctes ou Kamptozoaires. In *Traité de Zoologie, Anatomie, Systématique, Biologie*, Vol. 1 (ed. P. P. Grassé), pp. 927-1007. Paris: Masson et Cié.
- Brisson, P.** (1971). Castration chirurgicale et régénération gonadique chez quelques planorbides (Gastéropode Pulmoné). *Ann. Embryol. Morphol.* **4**, 180-210.
- Brisson, P.** (1973). Observation ultrastructurale des cellules germinales chez l'embryon d'*Acroloxus lacustris* (L.) (Gastéropode Pulmoné Basommatophore). *C. R. Acad. Sci. III* **277**, 2205-2208.
- Buchner, P.** (1910). Die Schicksale des Keimplasmas der Sagitten in Reifung, Befruchtung, Keimbahn, Ovogenese und Spermatogenese. *Festschr. zum 60ten Geburtst. Hertwigs* **1**.
- Bürger, O.** (1897-1907). Nemertini. In *Bronn's Klassen und Ordnungen des Tierreichs*, Vol. 4, pp. 1-542. Leipzig: Akademische Verlag.
- Bürger, O.** (1902). Weitere Beiträge zur Entwicklungsgeschichte der Hirudineen. Zur Embryologie von *Clepsine*. *Z. Wiss. Zool.* **72**, 525-544.
- Butcher, E. O.** (1929). The origin of the germ cells in the lake lamprey (*Petromyzon marinus unicolor*). *Biol. Bull.* **56**, 87-99.
- Bütschli, O.** (1873). Zur Entwicklungsgeschichte von *Sagitta*. *Z. Wiss. Zool.* **23**, 409-413.
- Carré, D. and Carré, C.** (2000). Origin of germ cells, sex determination, and sex inversion in medusae of the genus *Clytia* (Hydrozoa, Leptomedusae): the influence of temperature. *J. Exp. Zool.* **287**, 233-242.
- Carré, D., Djediat, C. and Sartet, C.** (2002). Formation of a large Vasa-positive granule and its inheritance by germ cells in the enigmatic Chaetognaths. *Development* **129**, 661-670.
- Child, C. M.** (1906). The development of germ cells from differentiated somatic cells in *Moniezia*. *Anat. Anz.* **29**.
- Chun, C.** (1880). Die Ctenophoren des Golfes von Neapel: Fauna Flora Golf. Neapel.
- Claypole, A. M.** (1898). The embryology and oogenesis of *Anurida maritima* (Guer.). *J. Morphol.* **14**, 219-300.
- Cohen, L. H., Newrock, K. M. and Zweidler, A.** (1975). Stage-specific switches in histone synthesis during embryogenesis of the sea urchin. *Science* **190**, 994-997.
- Cort, W. W.** (1944). The germ cell cycle in digenetic trematodes. *Quart. J. Microscop. Sci.* **19**, 275-284.
- Crandall, F. B., Norenburg, J. L. and Gibson, R.** (1998). Gonadogenesis, embryogenesis, and unusual oocyte origin in *Notogaeaneumertes folzae* Riser, 1988 (Nemertea, Hoplonemertea). *Hydrobiol.* **365**, 93-107.
- Dales, R. P.** (1950). Reproduction and larval development of *Nereis diversicolor*. *J. Mar. Biol. Assoc. UK* **29**, 321-360.
- Davidson, E. H., Cameron, R. A. and Ransick, A.** (1998). Specification of cell fate in the sea urchin embryo: summary and some proposed mechanisms. *Development* **125**, 3269-3290.
- De Smet, W. M. A.** (1970). The germ cells of *Polypterus* (Brachiopterygii, Pisces). *Acta Morphol. Neerl.-Scand.* **8**, 133-141.
- Dearden, P., Grbic, M. and Donly, C.** (2003). Vasa expression and germ-cell specification in the spider mite *Tetranychus urticae*. *Dev. Genes Evol.* **212**, 599-603.
- Dehorne, A.** (1933). La schizometamerie et les segments tetragemmes du *Dodecaceria caulleryi* sp. n. *Bull. Biol. Fr. Belg.* **67**, 298-326.
- Devires, J.** (1971). Origine de la lignée germinale chez le Lombricien *Eisenia faetida*. *Ann. Embryol. Morphol.* **4**, 37-43.
- Dhainaut, A.** (1970). Étude en microscopie électronique et par autoradiographie à haute résolution des extrusions nucléaires au cours de l'ovogenèse de *Nereis pelagica* (Annelide Polychete). *J. Microsc. (Paris)* **9**, 99-118.
- Dodds, G. S.** (1910). Segregation of the germ cells of the teleost, *Lophius*. *J. Morphol.* **21**, 563-612.
- Dogiel, V.** (1913). Embryologische studien an Pantopoden. *Z. Wiss. Zool.* **107**, 4.
- Dohmen, M. R.** (1983). Gametogenesis. In *The Mollusca: Development*, Vol. 3 (ed. N. H. Verdonk, J. A. M. van den Biggelaar and A. S. Tompa), pp. 1-49. New York: Academic Press.
- Dohmen, M. R. and Lok, D.** (1975). The ultrastructure of the polar lobe of *Crepidula fornicata*. *J. Embryol. Exp. Morphol.* **34**, 419-428.
- Dohmen, M. R. and Verdonk, N. H.** (1974). The structure of a morphogenetic cytoplasm, present in the polar lobe of *Bithynia tentaculata* (Gastropoda, Prosobranchia). *J. Embryol. Exp. Morphol.* **31**, 423-433.
- Doncaster, L.** (1902). On the development of *Sagitta*, with notes on the anatomy of the adult. *Quart. J. Microscop. Sci.* **46**.
- Dorsett, D. A.** (1961). The reproduction and maintenance of *Polydora ciliata* (Johnst.) at Whitstable. *J. Mar. Biol. Assoc. UK* **41**, 383-396.
- Dunlap Pianka, H.** (1974). Ctenophora. In *Reproduction of Marine Invertebrates: Acoelomate*, Vol. 1 (ed. A. C. Giese and J. S. Pearse), pp. 201-265. New York: Academic Press.
- Dunlap-Pianka, H.** (1966). *Oogenesis in the Ctenophora*. Seattle: University of Washington.
- Eckelbarger, K. J.** (1984). Comparative Aspects of Oogenesis in Polychaetes. *Fortschr. Zool.* **29**, 123-148.
- Eckelbarger, K. J. and Larson, R. L.** (1988). Ovarian morphology and oogenesis in *Aurelia-Aurita* (Scyphozoa, Semaestomae) - ultrastructural evidence of heterosynthetic yolk formation in a primitive Metazoan. *Mar. Biol.* **100**, 103-115.
- Eckelbarger, K. J. and Young, C. M.** (1992). Ovarian ultrastructure and vitellogenesis in 10 species of shallow-water and bathyal sea-cucumbers (Echinodermata, Holothuroidea). *J. Mar. Biol. Assoc. UK* **72**, 759-781.
- Eddy, E. M., Clark, J. M., Gong, D. and Fenderson, B. A.** (1981). Origin and migration of primordial germ-cells in mammals. *Gamete Res.* **4**, 333-362.

- Eigenmann, C. H.** (1891). On the precocious segregation of the sex-cells in *Micrometrus aggregatus*. *J. Morphol.* **5**, 481-493.
- Elpatievsky, W.** (1909). Die Urgeschlechtszellenbildung bei *Sagitta*. *Anat. Anz.* **35**, 226-239.
- Emschermann, P.** (1982). The present state of our knowledge of the anatomy, the development and biology and the phylogeny of the Entoprocta (Kamptozoa). *Bull. Soc. Zool. Fr. Evol. Zool.* **107**, 317.
- Evans, R.** (1901). On the Malayan species of Onychophora. *Quart. J. Microscop. Sci.* **45**, 41-88.
- Everett, N. B.** (1945). The present status of the germ-cell problem in vertebrates. *Biol. Rev.* **20**, 45-55.
- Eyal-Giladi, H., Ginsburg, M. and Farbarov, A.** (1981). Avian primordial germ cells are of epiblastic origin. *J. Embryol. Exp. Morphol.* **65**, 139-147.
- Falin, L. I.** (1969). The development of genital glands and the origin of germ cells in human embryogenesis. *Acta Anat (Basel)* **72**, 195-232.
- Falleni, A. and Gremigni, V.** (1990). Ultrastructural study of oogenesis in the acoel turbellarian *Convoluta*. *Tissue Cell* **22**, 301-310.
- Falleni, A., Lucchesi, P. and Gremigni, V.** (1995). Ultrastructural and cytochemical studies of the female gonad of *Prorhynchus sp.* (Platyhelminthes, Lecithoepitheliata). *Hydrobiol.* **305**, 199.
- Farley, R. D.** (2001). Development of segments and appendages in embryos of the desert scorpion *Paruroctonus mesaensis* (Scorpiones: Vaejovidae). *J. Morphol.* **250**, 70-88.
- Faussek, V.** (1891). Zur Anatomie und Embryologie der Phalangiden. *Trav. Soc. nat. St. Petersburg, Zool. & Physiol.* **22**.
- Faussek, V.** (1901). Untersuchungen über die Entwicklung der Cephalopoden. *Mith. Zool. Stat. Neapel* **14**, 83.
- Fischer, A.** (1975). The structure of symplasmic early oocytes and their enveloping sheath cells in the polychaete, *Platynereis dumerilii*. *Cell Tissue Res.* **160**, 327-343.
- Fordham, M.** (1925). *Aphrodite aculeata*. *Mem. Lpool. mar. biol. comm.* **27**.
- Frick, J. E. and Ruppert, E. E.** (1996). Primordial germ cells of *Synaptula hydriformis* (Holothuroidea; Echinodermata) are epithelial flagellated-collar cells: their apical-basal polarity becomes primary egg polarity. *Biol. Bull.* **191**, 168-177.
- Frick, J. E. and Ruppert, E. E.** (1997). Primordial germ cells and oocytes of *Branchiostoma virginiae* (Cephalochordata, Acrania) are flagellated epithelial cells: relationship between epithelial and primary egg polarity. *Zygote* **5**, 139-151.
- Frick, J. E., Ruppert, E. E. and Wourms, J. P.** (1996). Morphology of the ovotestis of *Synaptula hydriformis* (Holothuroidea, Apoda): an evolutionary model of oogenesis and the origin of egg polarity in echinoderms. *Invert. Biol.* **115**, 46-66.
- Fujimoto, T., Ukeshima, A., Miyayama, Y., Horio, F. and Ninomiya, E.** (1979). Observations of primordial germ cells in the turtle embryo (*Caretta caretta*): light and electron microscopic studies. *Dev. Growth Diff.* **21**, 3-10.
- Fujimura, M. and Takamura, K.** (2000). Characterization of an ascidian DEAD-box gene, *Ci-DEAD1*: specific expression in the germ cells and its mRNA localization in the posterior-most blastomeres in early embryos. *Dev. Genes Evol.* **210**, 64-72.
- Gaino, E., Burlando, B., Zunino, L., Pansini, M. and Buffa, P.** (1984). Origin of Male Gametes from Choanocytes in *Spongia officinalis* (Porifera, Demospongiae). *Int. J. Invert. Repr. Dev.* **7**, 83-93.
- Garaudy-Tamarelle, M.** (1969). Quelques observations sur le développement embryonnaire de l'ébauche génitale chez le Collembole *Anurida maritima* Guérin. *C. R. Acad. Sci. III* **268**, 945-947.
- Garaudy-Tamarelle, M.** (1970). Observations sur la ségrégation de la lignée germinale chez le Collembole *Anurida maritima* Guérin. Explication de son caractère intravitellin. *C. R. Acad. Sci. III* **270**, 1149-1152.
- Garbe, A.** (1901). Untersuchungen über die Entstehung der Geschlechtsorgane bei der Ctenophoren. *Z. Wiss. Zool.* **69**, 472-491.
- Garwood, P. R.** (1981). Observations on the cytology of the developing female germ-cell in the polychaete *Harmothoe imbricata* (L). *Int. J. Invert. Repr.* **3**, 333-345.
- Gérould, B.** (1907). Studies on the embryology of the spiunculidae. II. The development of *Phascolosoma*. *Zool. Jarhb. Anat.* **23**, 77-162.
- Ghirardelli, E.** (1954). Studi sul determinante germinale (d.g.) nei Chetognati: Ricerca sperimentali su *Spadella cephaloptera* Busch. *Pubbls. Staz. zool. Napoli* **25**, 444-453.
- Gipouloux, J. D.** (1971). Effets de l'extrusion totale ou partielle du cytoplasma germinal au cours des premiers stades de la ségmentation sur la fertilité des larves d'Amphibiens Anoures. *C. R. Acad. Sci. III* **273**.
- Gipouloux, J. D.** (1975). Cytoplasme germinale et détermination germinale chez les Amphibiens Anoures. *Ann. Biol.* **14**, 475-487.
- Goette, A.** (1890). Entwicklungsgeschichte des Flussneunauges (*Petromyzon fluviatilis*). *Abh. zur Ent. der Thiere* **5**, 95.
- Goffredo, S., Telo, T. and Scanabissi, F.** (2000). Ultrastructural observations of the spermatogenesis of the hermaphroditic solitary coral *Balanophyllia europaea* (Anthozoa, Scleractinia). *Zoomorphology* **119**, 231-240.
- Goodrich, H. B., Dee, J. E., Flynn, C. M. and Mercer, R. N.** (1934). Germ cells and sex differentiation in *Lebistes reticulatus*. *Biol. Bull.* **67**, 83-96.
- Goto, A., Kitamura, K., Arai, A. and Shimizu, T.** (1999). Cell fate analysis of teloblasts in the *Tubifex embryo* by intracellular injection of HRP. *Dev. Growth Diff.* **41**, 703-713.
- Gould-Somero, M. and Holland, L.** (1975). Oocyte differentiation in *Urechis caupo* (Echiura): a fine structural study. *J. Morphol.* **147**, 475-505.
- Gschwentner, R., Ladurner, P., Nimeth, K. and Rieger, R.** (2001). Stem cells in a basal bilaterian. S-phase and mitotic cells in *Convolutriloba longifissura* (Acoela, Platyhelminthes). *Cell Tissue Res.* **304**, 401-408.
- Gustafsson, M. K. S.** (1976). Studies on cytodifferentiation in the neck region of *Diphyllobothrium dendriticum* Nitze 1824 (Cestoda, Pseudophyllidea). *Parasitenk* **50**, 323-329.

- Halvorson, H. O. and Monroy, A.** (1985). *The Origin and Evolution of Sex*. New York: Alan R. Liss.
- Hann, H. W.** (1927). The history of the germ cells of *Cottus bairdii* Girard. *J. Morphol.* **63**, 427-498.
- Hardisty, M. W.** (1971). Gonadogenesis, Sex Differentiation and Gametogenesis. In *The Biology of Lampreys*, Vol. 1 (ed. M. W. Hardisty and I. C. Potter), pp. 295-359. London: Academic Press.
- Hargitt, G. T.** (1919). Germ cells of Coelenterates. VI. General considerations, discussion, conclusions. *J. Morphol.* **33**, 1-60.
- Hatschek, B.** (1888). Über die Schintzenbau von *Amphioxus*. *Arb. a. d. Zool. Inst. Wein* **4**.
- Herlant-Meewis, H.** (1946). Contribution à l'étude de la régénération chez les Oligochètes. II. Reconstitution du germe chez *Lumbricillus lineatus* (Enchytraeidae). *Arch. Biol.* **57**, 197-306.
- Hertzwig, O.** (1880). Über die Entwicklungsgeschichte der Sagitten. *Jenaische Zeitschr. f. Naturwiss.* **14**, 196-303.
- Hérubel, M.** (1908). Recherches sur les sipunculides. *Mém. Soc. Zool. France* **20**, 107-419.
- Heymons, R.** (1901). Entwicklungsgeschichte der Scolopender. *Zoologica* **33**, 1-244.
- Heymons, R.** (1904). Entwicklung und Morphologie der Solifugen. In *Congr. Intern. Zool.: Die flügelartige Anhang (Lateralorganen) der Solifugen*, Vol. 8, pp. 429-436. Berlin: Sitz. Ber. Akad. Wiss. Berlin, Math. Phys. Kl.
- Heys, F.** (1931). The problem of the origin of germ cells. *Q. Rev. Biol.* **6**, 1-45.
- Hogg, N. A. and Wijdenes, J.** (1979). A study of gonadal organogenesis, and the factors influencing regeneration following surgical castration in *Deroceras reticulatum* (Pulmonata: Limacidae). *Cell Tissue Res.* **198**, 295-307.
- Holland, L. Z. and Holland, N. D.** (1992). Early development in the lancelet (=Amphioxus) *Branchiostoma floridae* from sperm entry through pronuclear fusion: presence of vegetal pole plasm and lack of conspicuous ooplasmic segregation. *Biol. Bull.* **182**, 77-96.
- Houk, M. S. and Hinegardner, R. T.** (1980). The formation and early differentiation of sea urchin gonads. *Biol. Bull.* **159**, 280-294.
- Houk, M. S. and Hinegardner, R. T.** (1981). Cytoplasmic inclusions specific to the sea urchin germ line. *Dev. Biol.* **86**, 94-99.
- Hubert, J.** (1969). Localisation précoce et mode de migration des gonocytes primordiaux chez quelques reptiles. *Ann. Embryol. Morphol.* **2**, 479-494.
- Humphrey, R. R.** (1925). The primordial germ cells of *Hemidactylum* and other Amphibia. *J. Morphol. Physiol.* **41**, 1-43.
- Humphrey, R. R.** (1929). The early position of the primordial germ cells in Urodeles: evidence from experimental studies. *Anat. Rec.* **42**, 301-314.
- Hyman, L.** (1940-1959). *The Invertebrates*. New York: McGraw-Hill.
- Ikeda, I.** (1903). On the development of the sexual organs and of their products in *Phoronis*. *Annot. Zool. Jap.* **4**, 141-153.
- Inoue, C. and Shirai, H.** (1991). Origin of germ-cells and early differentiation of gonads in the starfish, *Asterina pectinifera*. *Dev. Growth Diff.* **33**, 217-226.
- Iseto, T. and Nishida, H.** (1999). Ultrastructural studies on the centrosome-attracting body: electron-dense matrix and its role in unequal cleavages in ascidian embryos. *Dev. Growth Diff.* **41**, 601-609.
- Ivanov, A. V.** (1963). Pogonophora. London: Academic Press.
- Iwanoff, P. P.** (1928). Die Entwicklung der Larvalsegmente bei den Anneliden. *Zeit. Morph. U. Okol.* **10**, 62-161.
- Iwanoff, P. P.** (1933). Die Embryonalentwicklung von *Limulus moluccanus*. *Zool. Jahrb. Abt. Anat. Ontogenie Tiere* **56**, 163-348.
- James, M. A., Ansell, A. D. and Curry, G. B.** (1991a). Functional Morphology of the Gonads of the Articulate Brachiopod *Terebratulina retusa*. *Mar. Biol.* **111**, 401-410.
- James, M. A., Ansell, A. D. and Curry, G. B.** (1991b). Oogenesis in the Articulate Brachiopod *Terebratulina retusa*. *Mar. Biol.* **111**, 411-423.
- Jarvis, M. M.** (1908). The segregation of the germ cells of *Phrynosoma cornutum*: preliminary note. *Biol. Bull.* **15**, 119-126.
- Jennison, B. L.** (1979). Gametogenesis and reproductive cycles in the sea anemone *Anthopleura elegantissima* (Brandt, 1835). *Can. J. Zool.* **57**, 403-411.
- Jiang, F. X., Clark, J. and Renfree, M. B.** (1997). Ultrastructural characteristics of primordial germ cells and their amoeboid movement to the gonadal ridges in the tammar wallaby. *Anat. Embryol.* **195**, 473-481.
- Johnson, A. D., Bachvarova, R. F., Drum, M. and Masi, T.** (2001). Expression of axolotl *DAZZL* RNA, a marker of germ plasm: widespread maternal RNA and onset of expression in germ cells approaching the gonad. *Dev. Biol.* **234**, 402-415.
- Johnson, A. D., Drum, M. and Bachvarova, R.** (2002). A common mechanism of germ cell determination in Axolotls and mice demonstrates that germ plasm is not conserved. *Conference on Germ Cells, Cold Spring Harbor Laboratory*, 55.
- Johnson, A. D., Drum, M., Bachvarova, R. F., Masi, T., White, M. E. and Crother, B. I.** (2003). Evolution of Predetermined Germ Cells in Vertebrate Embryos: Implications for Macro-evolution. *Evol. Dev.* (in press).
- Jordan, H. E.** (1917). Embryonic history of the germ cells of the Loggerhead turtle (*Caretta caretta*). *Carnegie Institute of Washington Publications* **11**, 313-344.
- Juberthie, C.** (1964). Recherches sur la biologie des Opilions. *Ann. Spél.* **19**, 1-237.
- Kang, D., Pilon, M. and Weisblat, D. A.** (2002). Maternal and zygotic expression of a *nanos*-class gene in the leech *Helobdella robusta*: primordial germ cells arise from segmental mesoderm. *Dev. Biol.* **245**, 28-41.

- Karagenc, L., Cinnamon, Y., Ginsburg, M. and Petite, J. N.** (1996). Origin of primordial germ cells in the prestreak chick embryo. *Dev. Genet.* **19**, 290-301.
- Killie, F. R.** (1942). Regeneration of the reproductive system following binary fission in the sea-cucumber, *Holothuria parvula* (Selenka). *Biol. Bull.* **83**, 55-87.
- Klag, J.** (1977). Differentiation of primordial germ cells in the embryonic development of *Thermobia domestica*, Pack. (Thysanura): an ultrastructural study. *J. Embryol. Exp. Morphol.* **38**, 93-114.
- Klag, J.** (1982). Germ line of *Tetradontophora bielaniensis* (Insecta, Collembola). Ultrastructural study on the origin of primordial germ cells. *J. Embryol. Exp. Morphol.* **72**, 183-195.
- Klag, J.** (1984). Germ line of *Tetradontophora bielaniensis* (Insecta, Collembola) 4. Nucleolus-like bodies extruded in toto from the nuclei of primordial germ cells become part of the 'nuage'. *Cytobios* **40**, 7-20.
- Klag, J. and Ostachowska-Gasior, A.** (1997). A cytochemical study on 'nuage' accumulations in primordial germ cells of *Tetradontophora bielaniensis* (Insecta, Collembola). *Folia Biol. (Krakow)* **45**, 15-20.
- Klag, J. and Swiatek, P.** (1999). Differentiation of primordial germ cells during embryogenesis of *Allacma fusca* (L.) (Collembola: Symphyleona). *Int. J. Insect Morphol. Embryol.* **28**, 161-168.
- Kobayashi, H. and Iwamatsu, T.** (2000). Development and fine structure of the yolk nucleus of previtellogenic oocytes in the medaka *Oryzias latipes*. *Dev. Growth Diff.* **42**, 623-632.
- Komai, T.** (1922). Studies on two aberrant ctenophores, *Coeloplana* and *Gastrodes*. Kyoto: T. Komai.
- Koren and Danielsen.** (1877). *Fauna littoralis norvegiae*, pp. 120-123. Bergen.
- Kumé, M. and Dan, K.** (1968). *Invertebrate Embryology*. Belgrade: Prosveta.
- Kutsuna, J., Yoshida-Noro, C., Shibata, N., Agata, K. and Tochinal, S.** (2001). Origin of germ cells in a regenerating oligochaete, *Enchytraeus japonensis*. In *14th International Congress of Developmental Biology*, pp. S12-P55. Kyoto, Japan.
- Ladurner, P., Rieger, R. and Bagnù, J.** (2000). Spatial distribution and differentiation potential of stem cells in hatchlings and adults in the marine platyhelminth *Macrostomum sp.*: a bromodeoxyuridine analysis. *Dev. Biol.* **226**, 231-241.
- Lavoillette, P.** (1954). Étude cytologique et expérimentale de la régénération germinale après castration chez *Arion rufus*. *Ann. Sci. Nat.* **2**, 427-535.
- Lechner, M.** (1966). Untersuchungen zur Embryonalentwicklung des Rädertieren *Asplanchna girodi* de Guerne. *Roux's Arch. Dev. Biol.* **157**, 117-173.
- Lehmann, O.** (1887). Beiträge zur Frage von der Homologie der Segmental-organe und Ausführgänge der Geschlechtsprodukte bei den Oligochaeten. *Jenaische Zeitschr. f. Naturwiss.* 322-360.
- Lieber, A.** (1931). Zur Oogenese einiger Diopatra-arten. *Z. Wiss. Zool.* **138**, 580-649.
- Littlefield, C. L. and Bode, H. R.** (1986). Germ cells in *Hydra oligactis* males. II. Evidence for a subpopulation of interstitial stem cells whose differentiation is limited to sperm production. *Dev. Biol.* **116**, 381-386.
- Loosli, M.** (1935). Über die Entwicklung und den Bau der indifferenten und männlichen Larven von *Bonellia viridis* Rol. *Pubbl. Staz. zool. Napoli* **15**, 16-59.
- Lucchesi, P., Falleni, A. and Gremigni, V.** (1995). The ultrastructure of the germarium in some Rhabdocoela. *Hydrobiol.* **305**, 207.
- MacBride, E. W.** (1896). The development of *Asterina gibbosa*. *Quart. J. Microscop. Sci.* **38**, 339-411.
- MacBride, E. W.** (1903). The development of *Echinus esculentus*, together with some points in the development of *E. miliaris* and *E. acutus*. *Phil. Trans. R. Soc. Lond. Ser. B. Biol. Sci.* **195**, 285-327.
- Malaquin, M. A.** (1924). Les glandes genitales et les cellules sexuelles primordiales chez l'Annelide *Salmacina dysteri* (Huxley). *C. R. Acad. Sci. III* **179**, 1348-1351.
- Malaquin, M. A.** (1925). La ségrégation, au cours de l'ontogénèse, de deux cellules sexuelles primordiales, souches de la lignée germinale, chez *Salmacina dysteri* (Huxley). *C. R. Acad. Sci. III* **180**, 324-327.
- Malaquin, M. A.** (1934). Nouvelles observations sur la lignée germinale de l'Annelide *Salmacina dysteri*, Huxley. *C. R. Acad. Sci. III* **198**, 1804-1806.
- Manton, S. M.** (1949). Studies on the Onychophora. VII. The Early Embryonic Stages of Peripatopsis, and Some General Considerations Concerning the Morphology and Phylogeny of the Arthropoda. *Phil. Trans. R. Soc. Lond. B.* **233**, 483-580.
- Marcus, E.** (1929). Zur Embryologie der Tardigraden. *Zool. Jahrb. Abt. Anat. Ontogenie Tiere* **50**, 333-384.
- Mariscal, R. N.** (1974). Entoprocta. In *Reproduction of Marine Invertebrates: Entoprocts and Lesser Coelomates*, Vol. 2 (ed. A. C. Giese and J. S. Pearse), pp. 1-42. New York and London: Academic Press.
- Martin, V. J. and Archer, W. E.** (1997). Stages of larval development and stem cell population changes during metamorphosis of a hydrozoan planula. *Biol. Bull.* **192**, 41-52.
- Martin, V. J., Littlefield, C. L., Archer, W. E. and Bode, H. R.** (1997). Embryogenesis in hydra. *Biol. Bull.* **192**, 345-363.
- Martini, E.** (1903). Über Furchung und Gastrulation bei *Cucullanus elegans*. *Z. Wiss. Zool.* **74**, 501-556.
- Maschkowzeff, A.** (1934). Zur Phylogenie der Geschlechtsdrüsen und der Geschlechtsausführgänge bei den Vertebrata auf Grund von Forschungen betreffend die Entwicklung des Mesonephros und der Geschlechtsorgane bei den Acipenseridae, Salmoniden und Amphibien. I. Die Entwicklung des Mesonephros und der Genitaldrüse bei den Acipenseridae und Salmonidae. *Zool. Jahrb. Abt. Anat. Ontogenie Tiere* **59**, 1-68.
- Matsumoto, T.** (1932). On the early localization and history of the so-called primordial germ-cells in the chick embryo (Preliminary report). *Sci. Rep. Tohoku Imp. Univ.* **4** **45**, 89-127.
- May, R.-M.** (1948). *La vie des Tardigrades*. Paris: Gallimard.
- McCosh, G. K.** (1930). The origin of the germ cells in *Amblystoma maculatum*. *J. Morphol.* **50**, 569-611.

- Meyer, A.** (1929). Die Entwicklung der Nephridien und Gonoblasten bei *Tubifex rivulorum* Lam. nebst Bemerkungen zur natürlich System der Oligochaten. *Z. Wiss. Zool.* **133**, 517-562.
- Mochizuki, K., Nishimiya-Fujisawa, C. and Fujisawa, T.** (2001). Universal occurrence of the *vasa*-related genes among metazoans and their germline expression in *Hydra*. *Dev. Genes Evol.* **211**, 299-308.
- Mochizuki, K., Sano, H., Kobayashi, S., Nishimiya-Fujisawa, C. and Fujisawa, T.** (2000). Expression and evolutionary conservation of *nanos*-related genes in *Hydra*. *Dev. Genes Evol.* **210**, 591-602.
- Moor, B.** (1983). Organogenesis. In *The Mollusca: Development*, Vol. 3 (ed. N. H. Verdonk J. A. M. van den Biggelaar and A. S. Tompa), pp. 123-178. New York: Academic Press.
- Morgan, T. H.** (1891). A contribution to the embryology and phylogeny of the pycnogonids, p. 76. Baltimore: Johns Hopkins University.
- Morgan, T. H.** (1894). The development of *Balanoglossus*. *J. Morphol.* **9**, 1-86.
- Moritz, M.** (1957). Zur Embryonalentwicklung der Phalangiiden (Opiliones, Palpatores) unter besonderer Berücksichtigung der ausseren Morphologie, der Bildung der Mitteldarmes und der Genitalanlage. *Zool. Jarhb. Anat.* **76**, 331-370.
- Mortensen, T.** (1904). Zur Anatomie und Entwicklung von *Cucumaria glacialis*. *Z. Wiss. Zool.* **57**.
- Mothes-Wagner, U. and Seitz, K.-A.** (1984). Ultrahistology of oogenesis and vitellogenesis in the spider mite *Tetranychus urticae*. *Tissue Cell* **2**, 179-194.
- Mukai, H.** (1977). Comparative studies on the structure of reproductive organs of four botryllid ascidians. *J. Morphol.* **152**, 363-380.
- Mukai, H. and Watanabe, H.** (1976). Studies on the formation of germ cells in a compound ascidian, *Botryllus primigenus* Oka. *J. Morphol.* **148**, 337-362.
- Munson, J. P.** (1898). The ovarian egg of *Limulus*. A contribution to the problem of the centrosome and yolk-nucleus. *J. Morphol.* **15**, 113-221.
- Nachtwey, R.** (1925). Untersuchungen über die Keimbahn, Organogenese und Anatomie von *Asplanchna priodonta* Gosse. *Z. Wiss. Zool.* **126**, 239-492.
- Naito, M., Sano, A., Matsubara, Y., Harumi, T., Tagami, T., Sakurai, M. and Kuwana, T.** (2001). Localization of primordial germ cells or their precursors in stage X blastoderm of chickens and their ability to differentiate into functional gametes in opposite-sex recipient gonads. *Reproduction* **121**, 547-552.
- Nakamura, Y., Makabe, K. W. and Nishida, H.** (2003). Localization and expression pattern of type I postplasmic mRNAs in embryos of the ascidian *Halocynthia roretzi*. *Gene Expr. Patterns* **3**, 71-75.
- Nernandez-Nicaise, M.-L.** (1991). Ctenophora. In *Microscopic Anatomy of Invertebrates: Placozoa, Porifera, Cnidaria, and Ctenophora*, Vol. 2 (ed. F. W. Harrison), pp. 359-418. Wiley-Liss.
- Newby, W. W.** (1932). The early embryology of the Echiuroid, *Urechis*. *Biol. Bull.* **63**, 387-399.
- Newby, W. W.** (1940). *The embryology of the echiuroid worm Urechis caupo*. Philadelphia: Memoirs of the American Philosophical Society.
- Nieuwkoop, P. D.** (1947). Experimental observations on the origin and determination of the germ cells, and on the development of the lateral plates and germ ridges in the urodeles. *Arch. Neerl. Zool.* **8**, 1-205.
- Nishida, H.** (1987). Cell lineage analysis in ascidian embryos by intracellular injection of a tracer enzyme. III. Up to the tissue restricted stage. *Dev. Biol.* **121**, 526-541.
- Nishikata, T., Hibino, T. and Nishida, H.** (1999). The centrosome-attracting body, microtubule system, and posterior egg cytoplasm are involved in positioning of cleavage planes in the ascidian embryo. *Dev. Biol.* **209**, 72-85.
- Noda, K. and Kanai, C.** (1977). An ultrastructural observation of *Pelmatohydra robusta* at sexual and asexual stages, with a special reference to 'germinal plasm'. *J. Ultrastruct. Res.* **61**, 284-294.
- Nusbaum, J.** (1908). Weitere Regenerationsstudien an Polychaeten. Über Regeneration von *Nereis diversicolor*. *Z. Wiss. Zool.* **89**, 109-163.
- Ogawa, M., Amikura, R., Akasaka, K., Kinoshita, T., Kobayashi, S. and Shimada, H.** (1999). Asymmetrical distribution of mitochondrial rRNA into small micromeres of sea urchin embryos. *Zool. Sci.* **16**, 445-451.
- Ogiso-Ono, Y. and Ikenishi, K.** (1999). Cause of the decreased number of PGC in albino *Xenopus*: Analysis of the number and position of pPGC in albino embryos during and after cleavage. *Dev. Growth Diff.* **41**, 745-750.
- Okkelberg, P.** (1921). The early history of the germ cells in the Brook Lamprey, *Entosphenus wilderi* (Gage). *J. Morphol.* **35**, 1-152.
- Olivier, J.** (1966). Cytochimie de l'Ovocyte au cours de la vitellogenèse chez *Lineus ruber* (Nemerte). *Ann. Univ. Reims L'Arers* **4**, 158-165.
- Oppenheimer, J. M.** (1959a). Extraembryonic transplantation of fragmented shield grafts in *Fundulus*. *J. Exp. Zool.* **142**, 441-460.
- Oppenheimer, J. M.** (1959b). Extraembryonic transplantation of sections of the *Fundulus* embryonic shield. *J. Exp. Zool.* **140**, 247-268.
- Packard, A. S.** (1880). The anatomy, histology, and embryology of *Limulus polyphemus*. *Ann. Mem. Boston Soc. Nat. Hist.* 1-45.
- Padoa, E.** (1963). Qualche precisazione sulla possibilita di distruggere con l'ultravioletto il plasma germinale della uova di *Rana esculenta*. *Boll. Soc. Ital Biol. Sper.* **40**, 272-275.
- Pai, S.** (1928). Die Pasen des Lebenscyclus der *Anguillula aceti* Ehrbg. *Z. Wiss. Zool.* **131**, 293-244.
- Pasteels, J.** (1953). Contribution a l'étude du développement des reptiles. I. Origine et migration des gonocytes chez deux Lacertiliens (*Mabuia megalura* et *Chamaeleo bitaeniatus*). *Arch. Biol.* **64**, 227-245.
- Pehrson, J. R. and Cohen, L. H.** (1986). The fate of the small micromeres in sea urchin development. *Dev. Biol.* **113**, 522-526.
- Penners, A.** (1929). Entwicklungsgeschichte Untersuchungen an maninen Oligochaten. I. Furchung, Keimstreif, Vorderdarm und Urkeimzellen von *Pelosclex benedini* Udekem. *Z. Wiss. Zool.* **134**, 307-344.
- Penners, A. and Stablein, A.** (1931). Über die Urkeimzellen bei Tubificiden (*Tubifex rivulorum* Lam. und *Limnodrilus udekemianus* Claparède). *Z. Wiss. Zool.* **137**, 606-626.
- Perrier, E.** (1889). Mémoire sur l'organisation et le développement de la Comatule de la Méditerranée (*Antedon Rosacea*, Linck). *Nouv. Arch. Mus. Hist. Nat. Paris* **9**, 54-348.
- Pilato, G.** (2000). The ontogenetic origin of germ cells in Porifera and Cnidaria and the 'theory of the endoderm as secondary layer'. *Zool. Anz.* **239**, 289-295.

- Pilon, M. and Weisblat, D. A.** (1997). A *nanos* homolog in leech. *Development* **124**, 1771-1780.
- Potswald, H. E.** (1969). Cytological observations on the so-called neoblasts in the Serpulid *Spirorbis*. *J. Morphol.* **128**, 241-260.
- Potswald, H. E.** (1972). The relationship of early oocytes to putative neoblasts in the Serpulid *Spirorbis borealis*. *J. Morphol.* **137**, 215-228.
- Randolph, H.** (1892). The regeneration of the tail in Lumbriculus. *Journal of Morphology* **7**, 317-344.
- Ransick, A., Cameron, R. A. and Davidson, E. H.** (1996). Postembryonic segregation of the germ line in sea urchins in relation to indirect development. *Proc. Natl. Acad. Sci. USA* **93**, 6759-6763.
- Rice, M. E.** (1974). Sipuncula. In *Reproduction of Marine Invertebrates: Entoprocts and Lesser Coelomates*, Vol. 2 (ed. A. C. Giese and J. S. Pearse), pp. 67-128. New York and London: Academic Press.
- Richards, A. and Thompson, J. T.** (1921). The migration of the primordial sex-cells of *Fundulus heteroclitus*. *Biol. Bull.* **40**, 325-348.
- Riser, N. W.** (1974). Nemertinea. In *Reproduction of Marine Invertebrates: Acoelomate and Pseudocoelomate Metazoans*, Vol. 1 (ed. A. C. Giese and J. S. Pearse), pp. 359-390. New York and London: Academic Press.
- Risley, P. L.** (1933). Contributions on the development of the reproductive system in *Sternotherus odoratus* (Latreille). I. The embryonic origin and migration of the primordial germ cells. *Zeit. Zellforsch. mikrosk. Anat.* **18**, 459-492.
- Ruchert, J.** (1888). Über die Entstehung der Excretionsorgane bei Selachiern. *Arch. Anat. u. Phys., Anat. Abt.* **2**, 205-278.
- Ryland, J. S.** (1970). Bryozoans. London: Hutchinson and Co.
- Ryland, J. S.** (1997). Reproduction in Zoanthidea (Anthozoa: Hexacorallia). *Invert. Repr. Dev.* **31**, 177-188.
- Sabbadin, A. and Zaniolo, G.** (1979). Sexual differentiation and germ cell transfer in the colonial ascidian *Botryllus schlosseri*. *J. Exp. Zool.* **207**, 289-304.
- Sacks, M.** (1955). Observations on the embryology of an aquatic Gastrotrich, *Lepidodermella squammata* (Dujardin, 1841). *J. Morphol.* **96**, 473-498.
- Sánchez, S.** (1959). Le développement des Pycnogonides et leurs affinités avec les Arachnides. *Arch. Zool. Exp. Gen.* **98**, 1-101.
- Schimkewitsch, W.** (1906). Entwicklung von *Theluphonus caudatus*. *Z. Wiss. Zool.* **81**.
- Sedgwick, A.** (1887). The development of the Cape species of *Peripatus*. Part 3. *Quart. J. Microscop. Sci.* **27**, 467-550.
- Selwood, L.** (1968). Interrelationships between developing oocytes and ovarian tissues in the chiton *Sypharochiton septentriones* (Ashby) (Mollusca, Polyplacophora). *J. Morphol.* **125**, 71-103.
- Selwood, L.** (2001). Mechanisms for pattern formation leading to axis formation and lineage allocation in mammals: a marsupial perspective. *Reproduction* **121**, 677-683.
- Shimotori, T. and Goto, T.** (2001). Developmental fates of the first four blastomeres of the chaetognath *Paraspadella gotoi*: relationship to protostomes. *Dev. Growth Diff.* **43**, 371-382.
- Shinomiya, A., Tanaka, M., Kobayashi, T., Nagahama, Y. and Hamaguchi, S.** (2000). The *vasa*-like gene, *ovas*, identifies the migration path of primordial germ cells during embryonic body formation stage in the medaka, *Oryzias latipes*. *Dev. Growth Diff.* **42**, 317-326.
- Simkins, C. S.** (1925). Origin of the germ cells in *Trionyx*. *Am. J. Anat.* **36**, 185-213.
- Simkins, C. S.** (1928). Origin of the germ cells in Man. *Am. J. Anat.* **41**, 249-293.
- Simkins, C. S. and Asana, J. J.** (1930). Development of the sex-glands of *Calotes*. I. Cytology and growth of the gonads prior to hatching. *Quart. J. Microscop. Sci.* **74**, 133-151.
- Smith, L. D.** (1966). The role of a 'germinal plasm' in the formation of primordial germ cells in *Rana pipiens*. *Dev. Biol.* **14**, 330-347.
- Southwood, E. C.** (1974). Pogonophora. In *Reproduction of Marine Invertebrates: Entoprocts and Lesser Coelomates*, Vol. 2 (ed. A. C. Giese and J. S. Pearse), pp. 129-156. New York and London: Academic Press.
- Spemann, H.** (1895). Zur Entwicklung des *Strongylus paradoxus*. *Zool. Jarhb. Morph.* **3**, 301-317.
- Spengel, J. W.** (1893). *Die Enteropneusten des Golfes von Neapel*. Napoli: Fauna Flora Golfes con Neapel Monogr.
- Stagni, A.** (1959). Fenomeni rigenerativi e origine degli elementi germinali in *Spirorbis pagentecheri*. *Boll. lab. zool. gen. agr. Portici* **26**, 397-403.
- Sterrer, W.** (1974). Gnathostomulida. In *Reproduction of Marine Invertebrates: Acoelomate and Pseudocoelomate Metazoans*, Vol. 1 (ed. A. C. Giese and J. S. Pearse), pp. 345-358. New York and London: Academic Press.
- Stevens, N. M.** (1910). Further studies on reproduction in *Sagitta*. *J. Morphol.* **21**, 279-319.
- Stoner, D. S., Rinkevich, B. and Weissman, I. L.** (1999). Heritable germ and somatic cell lineage competitions in chimeric colonial protochordates. *Proc Natl Acad Sci USA* **96**, 9148-9153.
- Stoner, D. S. and Weissman, I. L.** (1996). Somatic and germ cell parasitism in a colonial ascidian: possible role for a highly polymorphic allorecognition system. *Proc. Natl. Acad. Sci. USA* **93**, 15254-15259.
- Swiatek, P., Klag, J. and Romek, M.** (2001). Do germ-line cells in *Allacma fusca* (Insecta, Collembola, Symphypleona) have a higher metabolic rate than somatic cells? *Folia Biol. (Krakow)* **49**, 85-90.
- Swift, C. H.** (1914). Origin and early history of the primordial germ-cells of the chick. *Am. J. Anat.* **15**, 483-516.
- Swingle, W. W.** (1921). The germ cells of anurans. I. The male sexual cycle of *Rana catesbeiana* larvae. *J. Exp. Zool.* **32**, 235-331.
- Takamura, K., Fujimura, M. and Yamaguchi, Y.** (2002). Primordial germ cells originate from the endodermal strand cells in the ascidian *Ciona intestinalis*. *Dev. Genes Evol.* **212**, 11-18.
- Tamarelle, M.** (1979). Recherches ultrastructurales sur la ségrégation et le développement de la lignée germinale chez les embryons de quatre collemboles (Insecta: Apterygota). *Int. J. Insect Morphol. Embryol.* **8**, 95-111.

- Tardy, M. J.** (1970). Organogenèse de l'appareil génital chez les Mollusques. *Bul. Soc. Zool. France* **95**, 407.
- Tarkowski, A. K.** (1959). Experiments on the development of isolated blastomeres of mouse eggs. *Nature* **184**, 1286-1287.
- Teichmann, E.** (1903). Die frühe Entwicklung der Cephalopoden. *Verh. D. Zool. Ges.* **13**, 42.
- Thompson, T. E.** (1960). The development of *Neomenia carinata* Tullberg (Mollusca, Aplacophora). *Proc. R. Soc. Lond. B. Biol. Sci.* **153**, 263-278.
- Tiegs, O. W.** (1940). The embryology and affinities of the Symphyla, based on a study of *Hanseniella agilis*. *Quart. J. Microscop. Sci.* **82**, 1-225.
- Tiegs, O. W.** (1947). The development and affinities of the Paupoda, based on a study of *Paupopus silvaticus*. *Quart. J. Microscop. Sci.* **88**, 165-336.
- Tomioaka, M., Miya, T. and Nishida, H.** (2002). Repression of zygotic gene expression in the putative germline cells in ascidian embryos. *Zool. Sci.* **19**, 49-55.
- Tribe, M. and Brambell, F. W. R.** (1932). The origin and migration of the primordial germ cells of *Sphenodon punctatus*. *Quart. J. Microscop. Sci.* **75**, 251-282.
- Tsunekawa, N., Naito, M., Sakai, Y., Nishida, T. and Noce, T.** (2000). Isolation of chicken *vasa* homolog gene and tracing the origin of primordial germ cells. *Development* **127**, 2741-2750.
- Tuzet, O., Garrone, R. and Pavans de Caccatty, M.** (1970). Origine choanocytaire de la ligné germinale male chez la Démonosponge *Aplysilla rosea* Schulze (Dendroceratides). *C. R. Acad. Sci. III* **270**, 955-957.
- Ullmann, S. L., Shaw, G., Alcorn, G. T. and Renfree, M. B.** (1997). Migration of primordial germ cells to the developing gonadal ridges in the tammar wallaby *Macropus eugenii*. *J. Reprod. Fertil.* **110**, 135-143.
- van der Land, J.** (1974). Priapulida. In *Reproduction of Marine Invertebrates: Entoprocts and Lesser Coelomates*, Vol. 2 (ed. A. C. Giese and J. S. Pearse), pp. 55-66. New York and London: Academic Press.
- van der Woude, A.** (1954). The germ cell cycle of *Megalodiscus temperatus* (Stafford, 1905) Harwood 1932 (Paramphistomidae: Trematoda). *Amer. Midl. Nat.* **51**, 172-202.
- Vanneman, A. S.** (1917). The early history of the germ cells in the armadillo, *Tatusia novemcincta*. *Am. J. Anat.* **22**, 341-363.
- Vasiljev, A.** (1925). La fécondation chez *Spadella cephaloptera* LGHRS. et l'origine du corps déterminant la voie germinative. *Biol. Gen.* **1**, 249-278.
- Verdonk, N. H.** (1973). Cytoplasmic localization in *Bithynia tentaculata* and its influence on development. *Malacol. Rev.* **6**, 57.
- von Erlanger, R.** (1895). Beitrage zur Morphologie der Tardigraden. I. Zur Embryologie eines Tardigraden: *Macrobotus macronyx* Dujardin. *Morph. Jb.* **22**, 491-513.
- von Wenck, W.** (1914). Entwicklungsgeschichte untersuchungen an tardigraden (*Macrobotus lacustris* Duj.). *Zool. Jahrb. Abt. Anat. Ontogenie Tiere* **37**, 465-514.
- Walvig, F.** (1963). The Gonads and the Formation of the Sexual Cells. In *The Biology of Myxine* (ed. A. Brodal and R. Fänge), pp. 530-580. Oslo: Universitetsforlaget.
- Weisblat, D. A., Kim, S. Y. and Stent, G. S.** (1984). Embryonic origins of cells in the leech *Helobdella triserialis*. *Dev. Biol.* **104**, 65-85.
- Weisblat, D. A. and Shankland, M.** (1985). Cell lineage and segmentation in the leech. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **312**, 39-56.
- Weismann, A.** (1883). *Die Entstehung der Sexualzellen bei den Hydromedusen*. Jena: Gustav Fischer.
- Wheeler, W. M.** (1899). The development of the urogenital organs of the lamprey. *Zool. Jahrb. Abt. Anat. Ontogenie Tiere* **13**, 1-88.
- Whitman, C. O.** (1878). A contribution to the history of the germ-layers in *Clepsine*. *J. Morphol.* **1**, 105-182.
- Wijhe, J. W.** (1889). Über die Mesodermsegments des Rumpfes und die Entwicklung des Excretionssystems bei Selachiern. *Arch. mikr. Anat. Ent.* **33**, 461-516.
- Willey, A.** (1899). *Enteropneusta from the South Pacific, with notes on the West Indian species*. London: Cambridge University Press.
- Wilson, E. B.** (1889). The embryology of the Earthworm. *J. Morphol.* **3**, 387-462.
- Wilson, E. B.** (1892). The cell-lineage of *Nereis*: a contribution to the cytogeny of the Annelid body. *J. Morphol.* **6**, 361-480.
- Wilson, E. B.** (1900). The habit and early development of *Cerebratulus lacteus*. *Quart. J. Microscop. Sci.* **43**, 97-198.
- Witschi, E.** (1948). Migration of the germ cells of human embryos from the yolk sac to the primitive gonadal folds. *Contrib. Embryol.* **209**, 67-80.
- Woods, F. A.** (1902). Origin and migration of the germ cells in *Acanthias*. *Am. J. Anat.* **1**, 307-320.
- Woods, F. H.** (1931). History of the germ cells in *Sphaerium striatinum* (Lam.). *J. Morphol. Physiol.* **51**, 545-595.
- Woods, F. H.** (1932). Keimbahn determinants and continuity of the germ cells in *Sphaerium striatinum* (Lam.). *J. Morphol.* **53**, 345-365.
- Yamamoto, M. and Okada, T.** (1999). Origin of the gonad in the juvenile of a solitary ascidian, *Ciona intestinalis*. *Dev. Growth Diff.* **41**, 73-79.
- Yatsu, N.** (1901). On the development of *Lingula anatina*. *J. Coll. Sci. Imp. Univ. Tokyo* **17**, 1-112.
- Yoshizaki, G., Sakatani, S., Tominaga, H. and Takeuchi, T.** (2000). Cloning and characterization of a *vasa*-like gene in rainbow trout and its expression in the germ cell lineage. *Mol. Reprod. Dev.* **55**, 364-371.
- Zelinka, C.** (1891). Studien über Rädertiere III. *Z. Wiss. Zool.* **53**, 1-159.
- Zelinka, C.** (1928). Monographie Der Echinodera. Leipzig: Wilhelm Engelmann.
- Ziegler, H. E.** (1895). Untersuchungen über die ersten Entwicklungsvorgänge der Nematoden. *Z. Wiss. Zool.* **47**, 218-260.