Evolution of the bilaterian germ line: lineage origin and modulation of specification mechanisms

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Synopsis A key focus of evolutionary developmental biology (evo–devo) in recent years has been to elucidate the evolution of developmental mechanisms as a means of reconstructing the hypothetical last common ancestors of various clades. Prominent among such reconstructions have been proposals as to the nature of the mysterious "Urbilateria," originally defined as the last common ancestor of the extant Bilateria (protostomes and deuterostomes). Indeed, drawings of this animal can now be found, as well as detailed information on the genetics and morphological processes that it used to construct its gut, heart, eyes, appendages, segments, and body regions. Perhaps surprisingly, however, no explanations have yet been offered as to how this animal might have achieved the successful reproduction that must have been necessary for it to give rise to those lineages that are ancestral to today's diverse clades. The present article examines the comparative data available to date on the specification of the only cells containing the genetic hereditary material, the germ cells, and speculates on the possible evolutionary and developmental origin of the Urbilaterian germ line.

Introduction

> "*It is perhaps an understatement to say that difficulties confront attempts to infer evolutionary events that occurred during the early evolution of multicellular animals.*" (Blackstone and Ellison 2000, p 102)

Popular conclusions about the morphological and developmental characteristics of Urbilateria have been reached largely through the study of extant species (Balavoine and Adoutte 2003; Carroll et al. 2005; Gilbert and Singer 2006). Comparisons of the patterns of gene expression and, to a lesser extent, comparative morphology, have been used as tools in the dig for last common ancestors (LCAs) (De Robertis and Sasai 1996; Kimmel 1996). The result has been a rather detailed description of the genetic networks, or at least major genetic players, which are proposed to have been active in Urbilateria to give it various features, including axial polarity (Martindale 2005; Marcellini 2006), body regionalization (Pearson et al. 2005), light-sensing cells (Kozmik et al. 2003; Gehring 2005; Kozmik 2005), a heart or circulatory system (Bodmer and Venkatesh 1998) and a regionalized nervous system (Lichtneckert and Reichert 2005). No suggestions have been forthcoming, however, as to how this animal, whether more or less complex in body organization, might have made gametes, ensured their fertilization if necessary, and given rise to the first generation of protostome and deuterostome LCAs. Here, I address two questions about the germ line of Urbilateria: (1) Did it have a dedicated germ cell population? (2) If so, how was it specified?

The germ line

> "*It is the mutual interest of genes in multicellular organisms in decreasing repulsive forces that probably led to the sequestration of a cell lineage set early in development for the production of gametes . . . The separation of the germ line reduced the opportunity for conflict . . . and thus was a first step toward the evolution of individuality.*" (Reeve and Keller 1999, p 12)

Since we are considering the bilaterian LCA, our starting point is a multicellular animal with multiple cell types and a division of labor, albeit of unknown extent, among different cell populations. Bilaterian outgroups do show a germ line/soma distinction: although a dedicated and exclusive gametogenic cell population may not exist (reviewed by Extavour and Akam 2003), most of the cells of these animals are not

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capable of producing gametes. The true innovation in the evolution of the germ line was not, therefore, the generation of a gametogenic lineage, but rather the loss of gametogenic potential from the majority of cells of the organism. I do not consider here this evolutionary innovation in detail; such explanation lies beyond the scope of this article, and has been dealt with extensively by several researchers (see for example Buss 1987; Michod 1996, 1997; Michod and Roze 1997, 2001; West-Eberhard 2003). Nonetheless, it is appropriate to briefly review current ideas as to the evolution of a germ cell lineage.

Even general developmental biology textbooks that do not explicitly include evolutionary biology in their remit, often recognize that ''development from more than one cell presents problems, as mutations could occur in some of the cells.'' (Wolpert et al. 2007, p 521). More explicitly, ''The only way for the genome to be fully tested is to have only one line of germ cells.'' (Gerhart and Kirschner 1997, p 249). Sequestration of a dedicated germ line early in development circumvents this problem, as the organism can thus develop from only one cell, while in its final form be composed of millions. Early segregation of the germ line, however, brings with it the possibility of rapid fixation of mutations, possibly deleterious ones, in the hereditary lineage. Mutations arising during developmment in only a small fraction of embryonic cells may, nonetheless be represented in a majority of the next generation, if the few cells in which the mutation arises happen to be primordial germ cells (PGCs). It has indeed been demonstrated that the developmental biology of PGC formation, regardless of the mechanism of PGC specification, provides an explanation for rapid change in allelic frequencies from one generation to the next (Drost and Lee 1998). Consistent with these calculations are observations that PGCs in a mosaic germline undergo natural selection at the cellular level based on mutational differences between them (Extavour and García-Bellido 2001).

We must therefore reasonably expect that, in order to effectively confer the advantage of protection from somatic mutation, an appropriate hereditary lineage might exhibit reduction of mitotic activity [since more rounds of DNA replication give more opportunity for mutation through copy error (Sweasy et al. 2006)], reduced transcriptional activity [because genes may be more subject to mutation when actively transcribed (Medvedev 1981)], and reduced mobility of transposable elements [which, although it can be a ''positive'' force in adaptive evolution, indisputably leads to increased mutation rates (McDonald 1993; Fedoroff 1999; Deragon and Capy 2000)].

In fact, the germ line displays all of these features. Germ cells are typically mitotically quiescent from the time of their specification during embryogenesis, until the time that gametogenesis begins, usually during larval or adult life (Saffman and Lasko 1999; Gilbert and Singer 2006). They are relatively quiescent transcriptionally during most of embryonic development, as revealed by diagnostic histone modifications and single-cell transcription analysis (see for example Seydoux et al. 1996; Seydoux and Dunn 1997; Saitou et al. 2002; Schaner et al. 2003; Deshpande et al. 2004). Finally, RNA-mediated silencing of transposable elements has been documented in the germlines of *Caenorhabditis elegans* and *Drosophila melanogaster* (Sijen and Plasterk 2003; Aravin et al. 2004; Robert et al. 2004; Vagin et al. 2006).

Several lines of evidence suggest that the granular components of germ plasm that have been a classical cytological marker for germ cells for over 100 years, may represent a molecular link between three important germ cell processes: (1) post-transcriptional silencing; (2) suppression of mobility of transposable elements; and (3) identity of germ cells.

Ribonucleoprotein (RNP) granules called P bodies occur in a variety of eukaryotic cell types. P bodies have been shown to contain proteins involved in translational repression, mRNA surveillance, and RNA-mediated gene silencing, together with the mRNA targets of these proteins (reviewed by Eulalio et al. 2007). The dense granules observed in the germ plasm of all studied metazoans (in the form of nuage, sponge bodies, chromatoid bodies, balbiani bodies, or mitochondrial clouds) can be considered a germ cell-specific variant on the P body, additionally containing gene products conferring germ cell identity (see for example Kotaja and Sassone-Corsi 2007), and thus may be a key molecular hub linking the three processes outlined above.

Firstly, PIWI and ARGONAUTE (AGO) proteins, known to interact with the RISC (RNA-induced silencing complex) to effect RNA-mediated gene silencing, are also P body and germ granule components. The miRNA pathway member Dicer has been shown, in mice and fruit flies, to have an important role in both post-transcriptional silencing of germ line-specific genes and maintenance of pluripotency (Jin and Xie 2007; Murchison et al. 2007; Park et al. 2007). Secondly, PIWI, AGO, miRNAs and rasiRNAs not only regulate germ cell-specific genes (Megosh et al. 2006; Mishima et al. 2006), but also suppress mobility of transposable elements in the germline (Aravin et al. 2004;

Vagin et al. 2006). Thirdly, products of germ line-specific genes such as *vasa* and *nanos* are found in germ granules, where they often engage in positive feedback loops to regulate their own expression and that of other germ line genes (Mahowald 2001; Wilkins and Extavour, manuscript in preparation).

It has further been suggested that the invention of a gametogenic lineage, or at least a pluripotent lineage whose responsibilities included reproduction (see discussion by Sanchez Alvarado and Kang 2005), was not just an added bonus, but in fact a *sine qua non* of the evolution of multicellular organisms that acted, and were acted on by natural selection, as true individuals (Michod 1999). This is because as long as all cells retain the possibility to contribute to future generations, intra-individual competition among cell lineages is predicted to prevent the fitness gains of the group (that is, of the multicellular organism) from exceeding the fitness gains of the component cells. In summary, Urbilateria, as a *bona fide* metazoan, can be assumed to have possessed, if not an exclusively dedicated gametogenic population, at least a majority of truly somatic cells, so that it depended for its reproductive success on the successful specification and protection throughout development of a germ line.

Comparative data on germ cell specification

Germ cells are one of the most extensively studied metazoan cell lineages. They represent a crucial link between developmental biology and evolutionary biology, being responsible for both reproduction of the individual and genetic continuity of the species. I propose that the most crucial aspect of germ cell development for understanding the evolution of the germ line is the first specification event of the lineage, that is, the mechanism separating germ line from soma.

Over the past two centuries, a battery of tools for identification of germ cells and study has become available to researchers (reviewed by Extavour and Akam 2003). Germ cells can almost always be unambiguously distinguished from somatic cells by one or a combination of the following four criteria: (1) characteristic morphology under transmitted white light, including organelle-free cytoplasm, high nuclear:cytoplasmic ratio, rounded nuclei with prominent nucleoli and diffuse chromatin, granular cytoplasmic inclusions usually localized in the perinuclear cytoplasm associated with nuclear pores; (2) electron-dense cytoplasmic granules identifiable by transmission electron microscopy (TEM); (3) high levels of alkaline phosphatase activity (this criterion has been useful only in mammals); (4) localization of mRNA or protein products of germ cell-specific genes, notably the *vasa* and *nanos* gene family products. Some combination of these criteria always hold for germ cells at all stages of development, from their initial embryonic specification as PGCs, until their differentiation as male and female gametes.

Identifying germ cells at some stage of development is therefore feasible for any animal one wishes to study, given access to embryos or adults or both. Much more difficult, however, is discerning the time, place, and mechanism responsible for the initial specification that gave rise to the germ line. This is because, as Francis Maitland Balfour correctly noted, ''Since it is usually only possible to recognize generative elements after they have advanced considerably in development, the mere position of a generative cell, when first observed, can afford . . . no absolute proof of its origin.'' (Balfour 1885).

Specification and origin of extant metazoan PGCs: epigenesis and preformation

In 1979 and 1981, Nieuwkoop and Sutasurya published two excellent volumes summarizing all literature available at that time on PGCs across the metazoans, including, but not limited to, their initial specification (Nieuwkoop and Sutasurya 1979, 1981). More focused surveys dealing specifically with the first embryological sequestration of the germ line in both vertebrates and invertebrates are limited to three: the first classic monographs of the past century by Bounoure and Wolff, (Bounoure 1939; Wolff 1964), and a modern review incorporating the last quarter century of genetic and experimental data that Niuewkoop and Sutasurya were not able to include in their volumes (Extavour and Akam 2003). The results of these studies are briefly summarized here.

Modern developmental genetic model systems have indicated that two basic types of molecular mechanisms are responsible for germ cell specification: ''preformation'' and ''epigenesis'' (Extavour and Akam 2003). It is important to note that the two mechanisms are not necessarily mutually exclusive, but rather are better viewed as two extremes of the continuum along which development of germ cells can be mapped, since at some stage of germ cell development, both types of mechanism are inevitably used.

Specification of germ cells via a cell-autonomous mechanism was first formally proposed by Moritz Nussbaum:

> *"The segmented ovum divides accordingly into the cell-material of the individual and into the cells for the preservation of species . . . Both groups of cells and their offspring are propagated quite independently of each other, so that the reproductive cells have no share in the development of the tissues of the individual, and no seminal or ovicular cell arises from the cell-material of the individual."* (from Nussbaum 1880, p 112; translated by Stockberger 1913)

I will use the term preformation to refer to the acquisition of germ cell fate through localized, inherited cytoplasmic determinants, which later are both necessary and sufficient to confer germ cell fate upon the cell containing them. The molecules composing these determinants are both mRNA and protein products of genes that are widely conserved across all metazoans. Dipterans and nematodes are well known, long-standing examples of animals exhibiting this mode of PGC specification (Illmensee and Mahowald 1974, 1976; Strome and Wood 1982; Wolf et al. 1983; Markussen et al. 1995; Mello et al. 1996).

The idea of preformation and immortality of the germ line greatly influenced biologists following August Weismann's treatise on the subject (Weismann 1892). It has become increasingly clear, however, that immortality of the germ line does not hold true for all animal groups, leading many workers to revise the way they think about the germ line:

> *"We must ask ourselves whether the distinction between a separate and in principle continuous, immortal germ line and the mortal somatic tissues of the organism is still valid, or whether it is an artificial distinction which has merely been retained in the literature as a remnant of Weismann's* Keimplasma *theory."* (Nieuwkoop and Sutasurya 1981, p 174)

I will use the term epigenesis to refer to acquisition of germ cell fate by reception of inductive signals from germ layers adjacent to future PGCs. In this case, the signals are themselves necessary and sufficient to induce receiving cells to adopt PGC fate. Mice and axolotls clearly exhibit this mode of PGC specification (Nieuwkoop 1947; Tam and Zhou 1996), and while in the axolotl the inductive signals have not yet been identified (although see Johnson et al. 2003), in mice they are members of the BMP2/4 and 8b families (Lawson et al. 1999; Ying et al. 2000; Ying and Zhao 2001).

Until very recently, it was widely held among most developmental biologists that since preformation was prevalent among most model laboratory organisms, it was probably the most widespread and ancestral mechanism of PGC formation (contrast the second edition of the influential text Wolpert et al. 2002; with the most recent edition, Wolpert et al. 2007). However, if we move beyond the relatively derived species used as genetic model systems, closer examination of the available data demonstrates that this is unlikely to be the case (for details and comprehensive reference lists, see Extavour and Akam 2003).

Within protostomes, all studied members of most phyla appear to use epigenesis to specify PGCs, while a few phyla (Platyhelminthes, Annelida, Mollusca, and Arthropoda) contain members showing epigenesis as well as members showing preformation (Fig. 1). There are only three protostome phyla (Nematoda, Rotifera, and Chaetognatha), all of whose studied members exhibit preformation. In other words, across both the Ecdysozoa and the Lophotrochozoa, epigenesis is the more common mechanism of PGC specification.

Within the deuterostomes, most phyla show the same pattern as the protostomes. For all studied members of the nonchordate phyla, epigenesis is likely used to specify PGCs (Fig. 1). Of the chordates, only Urochordata, Chondrichthyes and Actinopterygii contain some members that use epigenesis et al. that use preformation as a PGC specification mode. Finally, in only two clades (anuran amphibians and archosaurs) do all studied members exhibit evidence for preformation. To summarize, with the exception of some elasmobranchs, the only deuterostome clades containing preformistic members are those containing all model laboratory chordates except for mice. These are (1) the solitary ascidians *Ciona intestinalis* and *Halocynthia roretzi* (Nishikata et al. 1999; Takamura et al. 2002; Shirae-Kurabayashi et al. 2006) [but note that recent data on colonial ascidians (Sunanaga et al. 2006, 2007) is consistent with epigenesis]; (2) the frog *Xenopus laevis* (Heasman et al. 1984; Wylie et al. 1985; Ikenishi et al. 1986); (3) the teleost *Danio rerio* (Olsen et al. 1997; Yoon et al. 1997), and (4) the chicken *Gallus gallus* (Tsunekawa et al. 2000; Naito et al. 2001). All other studied deuterostomes, including the Ambulacraria and Xenoturbellida, show evidence for epigenesis as the mode of PGC specification.

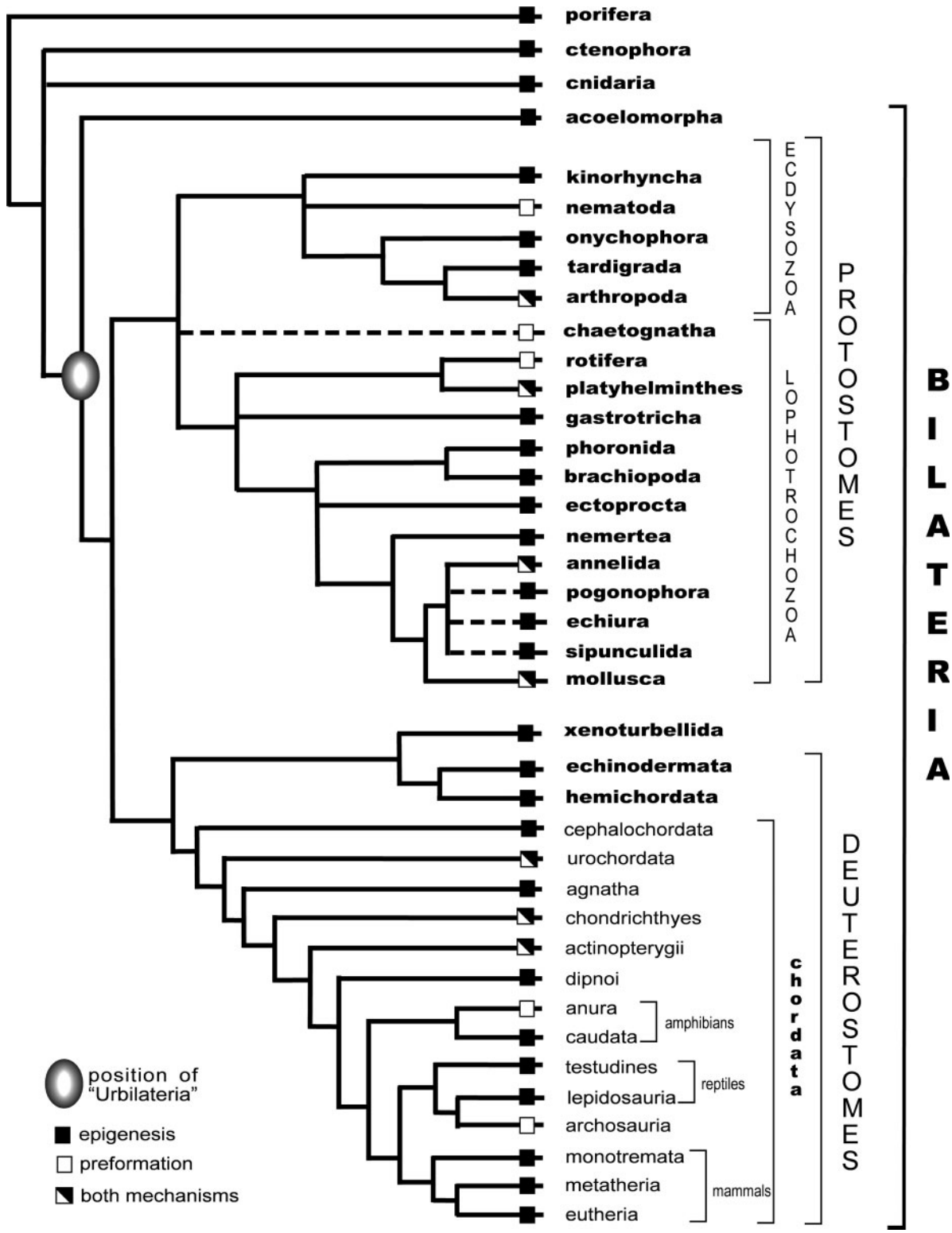

Fig. 1 Distribution of PGC specification mechanisms across the Metazoa. Position of "Urbilateria" indicated by shaded oval. Epigenesis (black boxes), preformation (white boxes), or both mechanisms (black and white boxes) are indicated only for phyla for which at least two independent primary data sources provide morphological, cell lineage, experimental, or molecular evidence. Details of source data are as described by Extavour and Akam (2003). Adapted from Extavour and Akam (2003) with modifications as follows: assignation of *Xenoturbella* to its own phylum within the deuterostomes (Bourlat et al. 2003, 2006); evidence for epigenetic PGC specification in a colonial ascidian (Sunanaga et al. 2006, 2007); changed phylogenetic relationship of Urochordata and Cephalochordata within the Chordata (Bourlat et al. 2006; Delsuc et al. 2006; Vienne and Pontarotti 2006) and affiliation of Chaetognatha with the protostomes (Marletaz et al. 2006; Matus et al. 2006).

A stem cell origin of Urbilaterian PGCs

The data summarized above, taken together with the observation that there are no data supporting preformation of the germ line in any of the bilaterian outgroups (Extavour and Akam 2003) (Fig. 1), strongly suggest that epigenetic establishment of the germ line was present in Urbilateria. However, even if it is likely that inductive signals were used to establish urbilaterian germ cells, we are still left with the problem of understanding the evolutionary origin of the germ line. Just as the evolution of mesoderm needs to be considered, in order to understand the transition from diploblasty to triploblasty (Technau and Scholz 2003; Martindale et al. 2004), the evolution of the germ line as a separate cell type needs to be considered, in order to understand the evolution of a "true soma," devoid of reproductive capability, and the division of labor that accompanied the evolution of multicellularity. It is therefore useful to consider how bilaterian outgroups generate a germ line.

Sponges, cnidarians, and acoel flatworms use very similar strategies to obtain gametogenic cells. They all contain a population of endodermally derived pluripotent stem cells (sponge archaeocytes, cnidarian interstitial cells, and acoel neoblasts) that acquire their fate in early to mid-embryogenesis, and can give rise to both somatic cell types and gametes (reviewed by Agata et al. 2006). These cells are scattered throughout the body cavity and/or intercalated between other somatic cells. Urbilateria was unlikely to have had all of its gametogenic cells clustered together in one region, but rather might have had them scattered throughout the body (Extavour 2007). These potential PGCs would have been pluripotent stem cells: some of them would have been capable of creating or regenerating adult somatic tissue as well, throughout the lifetime of the animal. The closest extant cell population to the urbilaterian germ line may be similar to the archaeocytes of sponges, which share both the characteristics listed above and some gene expression with the germ cells of triploblasts (Perovic-Ottstadt et al. 2004; Muller 2006).

As well as using the general pattern of metazoan germ cell specification modes to infer that Urbilateria's germ cells were a subpopulation of stem cells, we can also obtain evidence from modern molecular and functional comparisons between stem cells and germ cells. The electron dense granules inevitably found in germ cells using transmission electron microscopy (TEM), have also been found in stem cell lineages (Eddy 1975). Pluripotent cells often display all the morphological features commonly used to identify germ cells, such as a large round nucleus with diffuse chromatin and a prominent nucleolus. This can lead to an inability to distinguish between germ cells and other types of stem cells (see for example Potswald 1969, 1972). Similarly, when using molecular markers to identify germ cells, unless careful phylogenetic analysis of the gene homologues is carried out, researchers run the risk of isolating genes that will not distinguish between germ cells and other pluripotent cells. For example, the products of *vasa* gene family members are nearly always exclusive to the germ cell lineage (Raz 2000; Extavour and Akam 2003). The *vasa* gene family is thought to have evolved from the *PL10* family of helicases, which share significant structural similarity with *vasa* genes (Mochizuki et al. 2001). *PL10* products are usually localized both in germ cells and in other pluripotent cell types. If *PL10* homologues are isolated and incorrectly assigned *vasa* homology due to insufficient analysis, using them to identify germ cells can give rise to ambiguous or inaccurate lineage assignation (see for example Shibata et al. 1999). The *vasa* expression in combination with the expression of other germ line genes, however, can allow distinction between germ line cells and somatic cells, even in animals with large populations of pluripotent stem cells (see Sato et al. 2006 and references therein).

Genes used by both germ cells and other stem cell types are not limited to *vasa* family members. Much recent work has been dedicated to elucidating both shared elements and distinguishing features of the specific gene regulatory networks of the germ line and other types of stem cells (see Table 1 for a guide to the nomenclature of stem cell types). Several nongermline stem cell types display large groups of highly expressed genes, which may underlie their individual identities (Ivanova et al. 2002; Ramalho-Santos et al. 2002; Fortunel et al. 2003; Sun et al. 2007). The appealing idea of molecular genetic "stemness," or a common genetic regulatory logic shared by all stem cell types, is consistent with the observed plasticity of stem cells (Filip et al. 2004) and potentially useful as a systems-property concept (Robert et al. 2006). However, it is largely unsupported by comparison of gene-expression profiles of different stem cells, both within and between species (Burns and Zon 2002; Evsikov and Solter 2003; Fortunel et al. 2003; Sun et al. 2007). Nonetheless, clear transcriptional profile differences are apparent between germ line stem cells (GLSCs) and embryonic (ES) cells (Fujino et al. 2006). Moreover, at both the transcriptome and proteome

Table 1 Commonly used nomenclature relevant to metazoan stem cell types

Acronym	Full name	Animal group found	Naturally occurring	Derivation	Differentiation Potential	References
–	Neoblast	Planarians	Yes	Embryo	Soma/Gametes	(Shibata et al. 1999; Sanchez Alvarado and Kang 2005; Sato et al. 2006)
–	Archaeocyte	Sponges	Yes	Embryo	Soma/Gametes	(Pilato 2000; Muller 2006)
–	Interstitial cell	Cnidarians	Yes	Embryo	Soma/Gametes	(Littlefield 1985, 1991; Littlefield and Bode 1986; Bode 1996; Pilato 2000; Muller et al. 2004)
–	Coelomic stem cells	Colonial ascidians	Yes	Embryo	Soma/Gametes	(Sunanaga et al. 2006, 2007)
(similar to GS)	Oocyte	*D. melanogaster*	Yes	GLSC	GLSC/Gametes	(Kai and Spradling 2004)
AE	Amniotic epithelial cells	Mammals	Yes	Embryonic epiblast	Soma	(Miki et al. 2005; Miki and Strom 2006)
AFS/AFMSC	Amniotic fluid derived stem cells	Mammals	Yes	Adult amniotic fluid	Soma	(Tsai et al. 2006; De Coppi et al. 2007)
EC	Embryonal carcinoma cell	Mammals	Yes	Teratocarcinoma	Soma/Gametes	(Kleinsmith and Pierce 1964; Stevens 1967; Kahan and Ephrussi 1970; Stewart and Mintz 1981)
EG	Embryonic germ cell	Mammals	No	Embryonic PGCs	Soma/Gametes	(Matsui et al. 1992; Resnick et al. 1992; Rohwedel et al. 1996; Shamblott et al. 1998)
ES	Embryonic Stem Cell	Mammals	No	Embryo: ICM	Soma/Gametes	(Hubner et al. 2003; Aflatoonian and Moore 2006; Niwa 2007)
GLSC/GSC	Germ line stem cell	Mammals, *D. melanogaster, C. elegans*	Yes	Embryo: PGCs	Gametes	(Kimble and White 1981; McLaren 2000; Johnson et al. 2004; Wong et al. 2005; Kirilly and Xie 2007)
GS	Germ line stem cell	Mammals	No	Neonatal male testis	Soma/GLSC/Gametes	(Kanatsu-Shinohara et al. 2003, 2004)
HSC	Hematopoietic stem cell	Mammals	Yes	Embryonic mesoderm	Soma	(Cumano and Godin 2007)
HUBCSC	Umbilical cord derived stem cells	Mammals	Yes	Umbilical cord/amnion/placenta	Soma	(Nakahata et al. 1985; Weiss and Troyer 2006)
ICM	Inner cell mass	Mammals	Yes	Mammalian blastocyst	Soma/Gametes	(Gardner 1985; Yamanaka et al. 2006)
MaGS	Multipotent adult germ line stem cell	Mammals	No	Adult male testis	Soma/GLSC/Gametes	(Guan et al. 2006)

(continued)

Table 1 Continued

Acronym	Full name	Animal group found	Naturally occurring	Derivation	Differentiation Potential	References
MSC/BM-MSC	Mesenchymal stem cells	Mammals	Yes	Adult bone marrow	Soma	(Caplan 1991; Bobis et al. 2006)
NSC	Neuronal stem cell	Mammals	Yes	Brain	Soma	(Price et al. 1987; Price and Thurlow 1988; Taupin 2006)
PGC	Primordial germ cell	Mammals	Yes	Embryo	GLSC/Gametes	(Nieuwkoop and Sutasurya 1979; 1981; Saffman and Lasko 1999; Extavour and Akam 2003)

In the "Naturally Occurring" column, "Yes" refers to cell types that arise during development of wild-type animals or in wild-type adult organs; "No" refers to cell types that are induced from in vitro cultures. In the "Differentiation potential" Column, "Soma" indicates multiple somatic cell types; "Gametes" indicates either oocytes or spermatocytes; "GLSC" indicates germ line stem cell as defined in this table. The literature on stem cells is too voluminous for the references given here to be comprehensive; this reference list is therefore meant only as a guide to direct the reader to key primary publications and/or useful reviews.

levels, all studied nongerm line stem cell types are more similar to each other, than they are to GLSCs or to PGC-derived stem cells (Sperger et al. 2003; Kurosaki et al. 2007). In summary, based on morphological data and on gene expression, germ cells and somatic stem cells are similar enough to suggest a shared evolutionary origin, but different enough to argue that germ cells arose as a lineage-restricted population of somatic stem cells, as a result of changes in gene regulation specific to the germ line at transcriptional, and possibly also post-transcriptional, levels (see also Agata et al. 2006).

A further level of similarity between germ cells and stem cells has been revealed by functional analysis in both vertebrate and invertebrate systems. Mammalian embryonic germ cells or male GLSCs grown in culture can be induced to become pluripotent stem cells, called embryonic germ (EG) cells or germ line stem (GS) cells respectively, that are very similar in differentiation potential to ES cells derived from the inner cell mass (ICM) of the blastocyst (Matsui et al. 1992; Resnick et al. 1992; Rohwedel et al. 1996; Shamblott et al. 1998; Kanatsu-Shinohara et al. 2003, 2004; Guan et al. 2006). *Drosophila* germ cells already en route towards oogenic differentiation can be induced to revert to a germ line stem cell state (Kai and Spradling 2004). Finally, in what is, in a sense, the wild-type converse of the *Drosophila* experimental result, recent evidence suggests that germ line cells can be derived from preexisting somatic stem cell populations, through *de novo* gene expression of a germ line-specific gene, in planarians (Sato et al. 2006) and colonial ascidians (Sunanaga et al. 2007).

Similar dedifferentiation and redifferentiation is seen in cells from teratocarcinomas. These are malignant tumours probably formed from ectopic or aberrant primordial germ cells, which contain multiple differentiated tissues as well as undifferentiated stem cells called embryonal carcinoma (EC) cells (Stevens 1967). Cultures of EC cells, used as *in vitro* models of mammalian differentiation and development, have demonstrated that PGCs may be able, after "dedifferentiation" into EC cells, to "redifferentiate" as multiple somatic cell types (Kleinsmith and Pierce 1964; Kahan and Ephrussi 1970). Even more strikingly, when transplanted into blastocysts, which are then implanted into host female uteri, mouse teratocarinoma cells can contribute (albeit at low frequencies) not only to many somatic tissues, but also to the germ line, of the resulting progeny (Mintz and Illmensee 1975; Stewart and Mintz 1981, 1982).

Because ES cells are usually derived from blastocyst ICM cells, they are generally assumed to be equivalent to ICM cells. Observed differences between ES cells and ICM cells might simply be the result of ES culture conditions. Zwaka and Thomson (2005), however, have hypothesized that EG, ES, and EC cells may all have their closest *in vivo* equivalent not in ICM cells, but rather in germ cells. This hypothesis may explain the developmental origins of ES cells, but to explain the evolutionary origins of germ cells, we need to invert the hypothesis. I propose that PGCs may have their closest evolutionary equivalent in the pluripotent stem cells that are found in extant nonBilateria and basal bilaterians, which almost certainly existed in Urbilateria.

Convergent evolution of preformation

If epigenesis was used by Urbilateria to specify the germ line, then preformation must have evolved convergently several times during the bilaterian radiation. We therefore require a feasible framework for conceiving the following: Urbilaterian germ cells were a subpopulation of somatic cells, and repeatedly, in several descendant lineages of Urbilateria, germ cells acquired a cell-autonomous specification mechanism, and became a lineage independent of somatic cells. To demonstrate how this proposal represents a modification of previous models of germ line continuity, I compare it with the three major previous models: (1) pangenesis; (2) continuity; and (3) modified continuity with somatic selection.

Darwin's (1859) pangenesis theory provided a biological explanation for Lamarck's (1809) ideas about inheritance of acquired characteristics: all somatic cells produced particles, called gemmules, which traveled through the body and lodged in the germ cells. Since germ cells did not initially contain all of the information necessary to reproduce the adult form in successive generations, including acquired characteristics, they needed to receive this information from the gemmules. The germ line was neither immortal nor continuous, as it produced only the soma of the next generation, and that soma would produce the next germ line (Fig. 2A). Weismann, on the other hand, was sure that germ cells were autonomously totipotent from the moment of their formation, and that their nuclear information was both impervious to somatic influence and sufficient for reproduction of the adult form (Weismann 1892). In other words, the germ line was both immortal and continuous, and the source of both soma and germ line of subsequent generations (Fig. 2B). Since at least

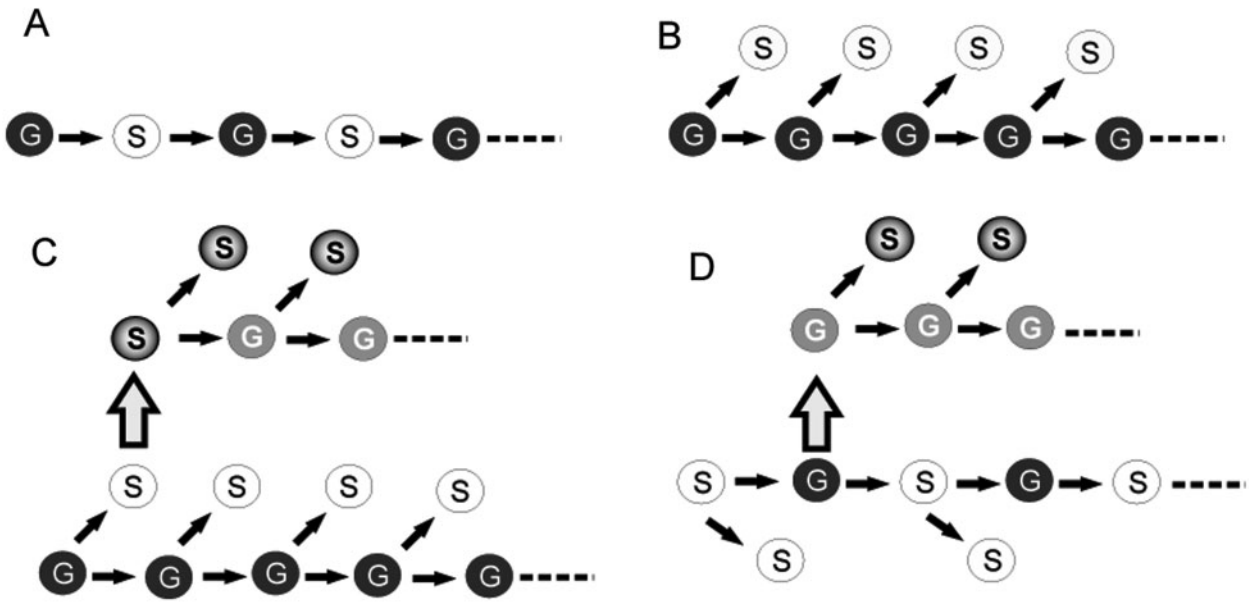

Fig. 2 Models for the evolution of the relationship between germ line and soma. (**A**) Pangenesis: the soma (white) informs and specifies the germ line (black), which in turn gives rise only to the soma. (**B**) Immortality/Continuity: the germ line is the sole progenitor of both germ line and soma, receiving no somatic input. (**C**) Continuity allowing for somatic selection: somatic mutation (gradient) may allow specification of germ line (grey) from somatic cells (top series), representing a deviation (large arrow) from the usual continuity of the germ line (bottom series). (**D**) Evolution of preformation from epigenesis: germ line mutation (grey) may confer continuity on the germ line (top series), representing a deviation (large arrow) from the usual somatic origin for stem cells (bottom series).

the 1920s, however, it has become increasingly clear that Weismann's hypothesis is in need of serious revision, given the existence of epigenesis in germ line specification in many species (Hargitt 1919; Heys 1931; Berrill and Liu 1948). Leo Buss (1983) has proposed an elegant revision of Weismann's hypothesis that takes into account both epigenetic germ line origin and intra-individual cellular selection. In this model, while germ line continuity may exist in some species (Fig. 2C, bottom series), somatic mutation may sometimes allow a subpopulation of the soma to produce gametes (Fig. 2C, top series).

To explain repeated evolution of preformation from epigenesis, it suffices to invert Buss' model (Fig. 2D). Urbilateria would have segregated germ cells epigenetically, as a subpopulation of somatic cells; soma therefore gave rise to both germ line and soma (Fig. 2D, bottom series). Where Buss' model suggests that mutations affecting the soma could allow somatic cells to produce gametes, I suggest that mutations affecting the germ line could allow autonomous segregation of germ cells in a subsequent generation (Fig. 2D, top series). This mechanism of preformation would then be inherited in subsequent generations. In order to understand what kind of germ line mutation could have had this effect, in the next section we consider known examples of germ cells that are specified by preformation.

Evolving preformation from epigenesis: a transitional model

All known molecular mechanisms of preformation rely on localization of germ cell-specific molecules (germ plasm components) to a particular place in the oocyte, either before or after fertilization (see for example Illmensee et al. 1976; Ressom and Dixon 1988; Carré et al. 2002). In several cases, the genes encoding these molecules, and their germ line expression, are conserved across all bilaterian species for which data are available (Extavour and Akam 2003). Many germ plasm components are expressed and required not only in primordial germ cells, but also during gametogenesis (see for example Styhler et al. 1998; Tanaka et al. 2000; Extavour et al. 2005). The major difference between epigenesis and preformation is thus the relative time of gene expression and gene product localization of germ cell-specific genes: in epigenesis, these genes are downregulated and/or their products are eliminated from the oocyte, after gametogenesis. Their products are not present in the cytoplasm of the fertilized egg and cannot therefore be autonomously inherited by PGCs; instead the genes must be zygotically activated in PGCs through epigenetic signaling (Fig. 3A). In preformation, germ cell-specific gene products persist through the completion of oogenesis in the zygotic cytoplasm, and are therefore available for inclusion into PGCs before the initiation of zygotic transcription (Fig. 3B). The molecular genetic

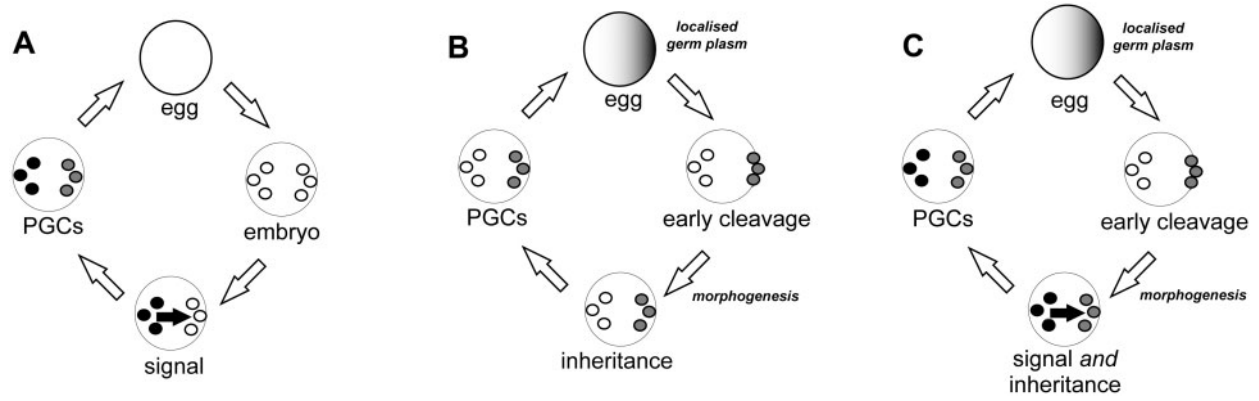

Fig. 3 A transitional model for the evolution of preformation from epigensis. (**A**) Epigenesis: germ cell-specific molecules expressed during gametogenesis are not present in oocytes at the time of fertilization. During embryogenesis, inductive signals (black) specify PGCs, which begin zygotic expression of germ cell-specific molecules (dark grey). Germ cells produce gametes to complete the cycle. (**B**) Preformation: maternal germ cell determinants (light grey) are localized to oocyte cytoplasm and inherited cell-autonomously by PGCs forming in early cleavage stages. Germ cells are localized to gonads during morphogenesis and produce gametes to complete the cycle. (**C**) Transition from epigenesis to preformation: germ cell-specific molecules expressed during gametogenesis are retained in the oocytes until fertilization. They are localized in the oocyte cytoplasm, and inherited cell-autonomously by PGCs forming in early cleavage stages. Inductive signals (black) produced during embryogenesis are now redundant with respect to PGC formation, although they may still be operative. Germ cells are localized to gonads during morphogenesis and produce gametes to complete the cycle. Loss of inductive signals is predicted over evolutionary time, so that this system comes to be like that shown in (B).

interactions that may account for the evolution of persistent germ plasm will be discussed elsewhere (Wilkins and Extavour, manuscript in preparation). Even without invoking the involvement of specific gene products, however, it is clear that in order to make the transition from epigenesis to preformation, only three things are necessary: (1) persistence of germ cell-specific gene products through the end of gametogenesis; (2) cytoplasmic localization of these germ cell-specific gene products within the oocyte; and (3) inheritance of these products, which would now constitute germ plasm components, by future PGCs (Fig. 3C).

Mutations arising in the germ line that affected the cytokskeletal dynamics of oocytes, translational mRNA regulation, or protein localization of germ cell molecules could allow persistence and localization of these molecules in mature oocytes. Once preformation had arisen in a heritable way through such mutation(s), signals from somatic tissues that induce germ line fate would no longer be necessary to ensure survival of a species. We would therefore expect gradual loss of these signaling mechanisms, since ''unnecessary but costly structures or activities should be lost in evolution.'' (Michod 1999, p 55). This model is consistent with our observation of repeated evolution of autonomous germ line determinants in several groups (Fig. 1), and with the complete absence of examples of epigenesis in phyla in which preformation is plesiomorphic (e.g., Rotifera, Chaetognatha, Nematoda).

This model predicts the existence at some time of species in which both preformation and epigenesis were operative, or at least operable. In all preformistic model organisms, however, when PGCs or their precursors are eliminated through physical ablation or genetic manipulation, the resulting animals are sterile, presumably because they are unable to replace the ablated germ line through epigenetic mechanisms (reviewed by Saffman and Lasko 1999). These animals may belong to lineages in which preformation evolved so long ago that their epigenetic signaling mechanisms have become unusable through lack of positive selection. This explanation is not unreasonable, given that all currently used genetic model organisms are derived with respect to many other aspects of embryogenesis. Alternatively, our failure thus far to observe widespread coexistence of both PGC specification mechanisms may simply be reflective of inadequate sampling of taxa. Intriguingly, in the solitary ascidian *C. intestinalis*, although convincing embryological and molecular genetic data suggest that preformation specifies PGCs (Iseto and Nishida 1999; Takamura et al. 2002; Nakamura et al. 2003; Shirae-Kurabayashi et al. 2006), when the PGCs are ablated in larval stages, the resulting adults are still fertile (Takamura et al. 2002). Similarly, although mitotic dynamics and patterns of gene expression suggest an early germ line segregation event in sea urchins (Pehrson and Cohen 1986; Juliano et al. 2006), regulative replacement of germ cells has been shown to occur if the early lineage is experimentally ablated (Ransick et al. 1996). The mechanism(s) responsible for such replacement of the germ line is currently unknown. I suggest that as more species from the diversity of the Bilateria become amenable to molecular analysis of embryogenesis and development, further examples of species able to use both epigenetic and preformation to specify germ cells will emerge.

Conclusions

Urbilaterian germ cells were likely specified as a subpopulation of preexisting somatic pluripotent stem cells, through inductive signals of unknown molecular identity. Changes in the timing of expression (heterochrony) and of ooplasmic localization (heterotopy/heterotypy) of germ cell differentiation genes led to early embryonic cytoplasmic inheritance of germ cell determinants that was both heritable and independent of somatic epigenetic signaling later in embryonic development, resulting in convergent evolution of preformation. In descendant lineages that had evolved preformation, epigenetic mechanisms of germ cell specification would have gradually deteriorated due to lack of positive selection.

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