

Evolving Pathways Key Themes in Evolutionary Developmental Biology

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Urbisexuality: the evolution of bilaterian germ cell specification and reproductive systems

CASSANDRA G. M. EXTAVOUR

A key focus of evolutionary developmental biology (evo-devo) in recent years has been to elucidate the evolution of developmental mechanisms as a means to 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 (LCA) of the extant Bilateria (Ecdysozoa, Lophotrochozoa and Deuterostomia) (De Robertis and Sasai 1996, Kimmel 1996). 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 region identities (Gilbert and Singer 2006). Perhaps surprisingly, however, no explanations have yet been offered of how it might have achieved the successful reproduction that must have been necessary for it to give rise to still surviving lineages. This chapter will examine the comparative data available on the specification of bilaterian reproductive systems during development, with special emphasis on the cells containing the genetic hereditary material, the germ cells, and speculate on the possible gonad structure and reproductive strategy of Urbilateria.

Before proceeding, we should clarify our expectations as to what the study of extant species can tell us about Urbilateria. In this chapter, I wish to avoid suggesting that extant reproductive systems are simply variations on a defined metazoan reproductive 'Bauplan' theme; the great weakness of the current evo-devo approach stems

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from dilution of explanatory force with inappropriate fixations on strict, confining definitions of this kind (Scholtz 2004, 2005, Hübner 2005). I will review the current and historical literature on germ-cell and somatic gonad anatomy, embryonic specification and development, studies obviously all carried out on extant species, but will not infer from these data that Urbilateria must have had specific, archetypical genetic or developmental characteristics of its reproductive system anatomy or reproductive strategies; rather, I will suggest that these data can tell us what kinds of general features, or basic pattern, its reproductive system was likely to have had, in order for it to have given rise to these systems as manifest in extant bilaterian lineages.

Over the past couple of decades, comparative gene expression patterns, and, to a lesser extent, comparative morphology, have been used as tools in the dig for LCAs. 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, body regionalisation, light-sensing cells, a heart or circulatory system, and a regionalised nervous system. However, no suggestions have been forthcoming as to how this animal might have made gametes, ensured their fertilisation if necessary, and given rise to the first generation of bilaterian LCAs. Several questions about this aspect of Urbilateria come to mind. Was it hermaphroditic or parthenogenetic, or did separate sexes exist? Did it have a dedicated germ-cell population? If so, how was it specified? Did it have a discrete gonad? If so, from which germ layer did it originate? How was fertilisation achieved? To begin to examine some of these questions, we first need to define the components of functional reproductive systems.

COMPONENTS OF BILATERIAN REPRODUCTIVE SYSTEMS

There is a minimum of two aspects to successful sexual reproduction: (1) cells to make gametes, and (2) a fertilisation strategy. Most bilaterian reproductive systems possess a third critical element, which is a dedicated group of somatic cells to enclose, support, and extrude the gametogenic cells.

The germ line

Our starting point is the bilaterian LCA, a multicellular animal with multiple cell types and a division of labour, albeit of unknown extent, among different cell populations. Bilaterian outgroups do show a

distinction between germline and soma: although a dedicated and exclusive gametogenic cell population may not exist (reviewed in Extavour and Akam 2003), most of the cells of these animals are *not* 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. Here, I do not consider this evolutionary innovation in detail; such explanation lies beyond the scope of this paper, and has been dealt with extensively by several researchers. 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 recognise that 'development from more than one cell presents problems, as mutations could occur in some of the cells' (Wolpert *et al.* 2007: 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: 249). Sequestration of a dedicated germ line early in development circumvents this problem, as the organism can thus develop from only one cell, but in its final form be composed of millions. We could reasonably expect that, in order to effectively confer the advantage of protection from somatic mutation, such a lineage might show 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 transposable element mobility (which, although it can be a 'positive' force in adaptive evolution, indisputably leads to increased mutation rates; McDonald 1993, Fedoroff 1999, Deragon and Capi 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. They are relatively transcriptionally quiescent during most of embryonic development, as revealed by diagnostic histone modifications and single-cell transcription analysis (Schaner *et al.* 2003). Finally, RNA-mediated silencing of transposable elements has recently been documented in the germ lines of *Caenorhabditis elegans* and *Drosophila melanogaster* (Aravin *et al.* 2004, Robert *et al.* 2004, Vagin *et al.* 2006).

It has further been suggested that the invention of a gametogenic lineage 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, 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 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.

The soma

What all somatic reproductive systems have in common is that they comprise a network of non-gametogenic cells whose role is to support, enclose, transport and expel the gametic products of the individual. Beklemishev (1969) defined five components of the somatic reproductive system as follows: (1) gonads (where gametogenesis takes place); (2) genital ducts (for storing, transporting or extruding gametic products); (3) copulatory organs (for transferring gametes between individuals of the opposite sex); (4) adaptations for creating envelopes for ova; (5) adaptations for bearing live young. We shall use these five categories to characterise the reproductive systems of the metazoan phyla, and as will become evident, a successful reproductive strategy may involve all or none of these elements.

Fertilisation strategies

Urbilateria, by definition, must have used some kind of reproductive strategy, but we have no way of knowing what it was. Once gametes have been made, if fertilisation is necessary then this needs to take place. Fertilisation can be wholly external (gametes of both sexes released without copulation), wholly internal (gametes of one sex deposited into the individual of the opposite sex, via copulation) or external-internal (gametes of one sex are released without copulation, then taken up by the opposite sex, so that fertilisation is internal). The type of fertilisation strategy used depends on the anatomy of the somatic reproductive system. For example, genital ducts and copulatory organs are prerequisites for wholly internal fertilisation. For this reason, we will only be able to begin speculation on an urbilaterian reproductive strategy once we have identified some patterns of comparative metazoan somatic gonad structures.

COMPARATIVE DATA ON SOMATIC GONAD SPECIFICATION

Anatomical studies of members of most extant bilaterian phyla provide data on the structure of the somatic reproductive system. More difficult to obtain are data on the developmental origin of the system, and on its functioning during reproduction, as these depend on availability of reliably staged developmental intermediates, and direct observations of copulation and/or fertilisation, respectively. What is immediately apparent even from the data available, however, is that on a bilaterian scale, a strictly phylogenetic consideration of reproductive system anatomy makes no sense without also considering life history and environmental factors.

We will use Beklemishev's (1969) five categories of reproductive system components to characterise the complexity of these systems across the Bilateria. We observe here the full range of complexities of reproductive systems, from free-floating gametes within the body cavity, which are extruded by epidermal rupture to engage in external fertilisation, to gametes confined within elaborate gonads, which can only be exposed to gametes of the opposite sex through copulation, and eventually travel through dedicated ducts to uteri specialised for viviparity (Table 17.1).

Among the protostomes, reproductive system structure can vary not only between phyla, but also within a single phylum. For example, within the Annelida, leeches have true gonads and gonoducts, as do oligochaetes and some polychaetes. However, many polychaete species lack discrete gonads; instead, their gametes mature in coelomic cavities from free-floating gametogonia, are released by body wall rupture and undergo external fertilisation in the water column (Beklemishev 1969). Some onychophorans have not only complex gonad structures but also uteri; fertilisation is internal, embryos develop in uteri, and animals give birth to live young (Manton 1949). As in many other segmented protostomes, somatic gonad components are formed from mesodermal cells of the splanchnic dorsal coelomic wall (Manton 1949, Anderson 1973).

Among the deuterostomes, *Xenoturbella* has the simplest known reproductive system: as in many sponges, cnidarians and flatworms, gametes develop freely in the coelom and are extruded through the mouth upon maturity (Beklemishev 1969). Many marine invertebrate deuterostomes have discrete gonads and gonoducts, but lack copulatory organs, and fertilisation takes place in the water column. Mammals have of course developed specialised copulatory organs, as well as adaptations

Table 17.1 Fertilisation strategies and somatic reproductive systems across the Metazoa.

	External-external (No copulation: both gametes released externally)		Internal-internal (Copulation; neither gamete released externally)		External-internal (External sperm release & internal fertilisation)		Self-fertile hermaphrodite
Porifera	-	-	-	-	-	-	-
Cnidaria	-	-	-	-	-	-	-
Ctenophora							
Gastrotrichia							
Rotifera							
Annelida	1	2	-	4	-	-	-
Echiura	1	2	-	-	-	-	-
Sipuncula	1	-	-	-	-	-	-
Nemertea	1	2	-	4	-	-	-
Platyhelminthes							
Mollusca	1	-	(3)	4	-	-	-
Gnathostomulida							
Entoprocta							
Nematoda							
Priapulida	1	2	-	4	-	-	-

Kinorhyncha	-	-	-	1	2	(3)	4	-																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																											</
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Note: Numbers 1 through 5 indicate the following components: (1) gonads or genital glands (envelope of somatic cells enclosing gametogenic cells); (2) genital ducts (leading from gonads to gonopores for extrusion of gametes); (3) copulatory organs (may be used either for internal fertilisation or external gamete deposition, e.g. spermatophores); (4) adaptations for creating eggshells for ova (including vitelline membranes and chorions, but excluding extra-embryonic membranes of zygotic origin); (5) adaptations for viviparity (including uteri and placentae). Hyphens indicate phyla using the indicated fertilisation strategy but possessing none of the somatic reproductive system components. Parentheses indicate that some phylum members may not always possess the given feature. Data compiled from references cited in Extavour and Akam (2003) and from Giese and Pearse (1974–1989) and Brusca and Brusca (2003).

for internal fertilisation, embryonic development and viviparity. The mammalian somatic gonad probably derives from the mesonephros and the adjacent coelomic epithelium (McLaren 2000).

For many studied metazoans, it is clear that the somatic and germ-line components of the reproductive system are specified or 'sequestered' separately during development; that is, they share limited or no lineage. The huge diversity in somatic reproductive systems should therefore not be surprising, given that independently sequestered lineages may display a certain modular independence in morphological evolution. West-Eberhard summarises this by saying that 'an increase in modularity . . . sometimes appears to have contributed to increased diversification of *that aspect of the phenotype* during the history of a taxon' (West-Eberhard 2003: 87 [*italics original*]).

Jury still out on urbilaterian gonads

Beyond a mesodermal origin for the somatic structures of the reproductive system, no general pattern emerges from a phylogenetic consideration of these systems across the Bilateria. Convergent evolution of every aspect of the system is apparent not only between phyla, but also within phyla. Most bilaterian outgroups lack true gonads, but while some acoels similarly lack gonads, others display compact, paired, ovaries, and many have male copulatory organs. Data on the molecular mechanisms specifying somatic gonad fate are largely limited to mice (McLaren 2000), nematodes (Hubbard and Greenstein 2000) and fruit flies (Moore *et al.* 1998, DeFalco *et al.* 2004). To date, the evidence for conservation of gene function in somatic gonad cells is limited to the protein product of a single gene (Li *et al.* 2003). We therefore cannot suggest homology of molecular pathways involved, consistent with repeated convergent evolution. In summary, while it is likely that Urbilateria lacked a complex somatic reproductive system, it is at present impossible to speculate on whether it possessed a true gonad, let alone any other somatic adaptations for reproduction.

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. Although germ-cell migration, polarity and differentiation are all fascinating

developmental problems in their own right, 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 that separates germ line from soma.

Over the past two centuries, a battery of tools for germ-cell identification and study has become available to researchers (reviewed in 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, large nuclear:cytoplasmic ratio, rounded nuclei with prominent nucleoli and diffuse chromatin, and granular cytoplasmic inclusions usually localised in the perinuclear cytoplasm associated with nuclear pores; (2) electron-dense cytoplasmic granules (nuage) identifiable by transmission electron microscopy; (3) high levels of alkaline phosphatase activity (this criterion has been useful only in vertebrates); (4) localisation of mRNA or protein products of germ-cell-specific genes, notably the *vasa* and *nanos* gene family products. Some combination of these criteria always holds for germ cells at all stages of development, from their initial embryonic specification as primordial germ cells (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 event giving rise to the germ line. This is because, as Balfour (1885) correctly noted, 'Since it is usually only possible to recognise 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'.

Specification and origin of extant metazoan PGCs: epigenesis and preformation

In 1979 and 1981, Nieuwkoop and Sutasurya published two excellent volumes summarising all available literature on PGCs across the metazoans, including, but not limited to, their initial specification (Nieuwkoop and Sutasurya 1979, 1981). More focused survey studies dealing specifically with the first embryological sequestration of the germline in both vertebrates and invertebrates are limited to three: two classic monographs of the last century (Bounoure 1939, Wolff 1964), and a modern

review incorporating the last quarter of a century of genetic and experimental data (Extavour and Akam 2003). The results of these studies will be briefly summarised here.

Modern developmental genetic model systems have indicated that two basic types of molecular mechanisms are responsible for germ-cell specification; I will call these two types '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 germ-cell development can be mapped, since at some stage of germ-cell development, both types of mechanism are inevitably used.

Preformation refers to cell-autonomous acquisition of germ-cell fate through localised, inherited cytoplasmic determinants, which 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 showing this mode of PGC specification.

Epigenesis refers to acquisition of germ-cell fate by reception of cell non-autonomous 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, and while in the axolotl the inductive signals have not yet been identified (but see Johnson *et al.* 2003), in mice they are members of the BMP2/4 and 8b families.

Until very recently, it was widely held among most developmental biologists that since preformation was prevalent among 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, 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).

For most ecdysozoans and lophotrochozoans, all studied members of a given phylum appear to use epigenesis to specify PGCs, while a few phyla (Platyhelminthes, Annelida, Mollusca and Arthropoda) contain both members showing epigenesis, and members displaying preformation (Figure 17.1). Only in the Nematoda, Rotifera and Chaetognatha do all studied members exhibit preformation. In other words, across both the Ecdysozoa and the Lophotrochozoa, epigenesis is the most common mechanism of PGC specification.

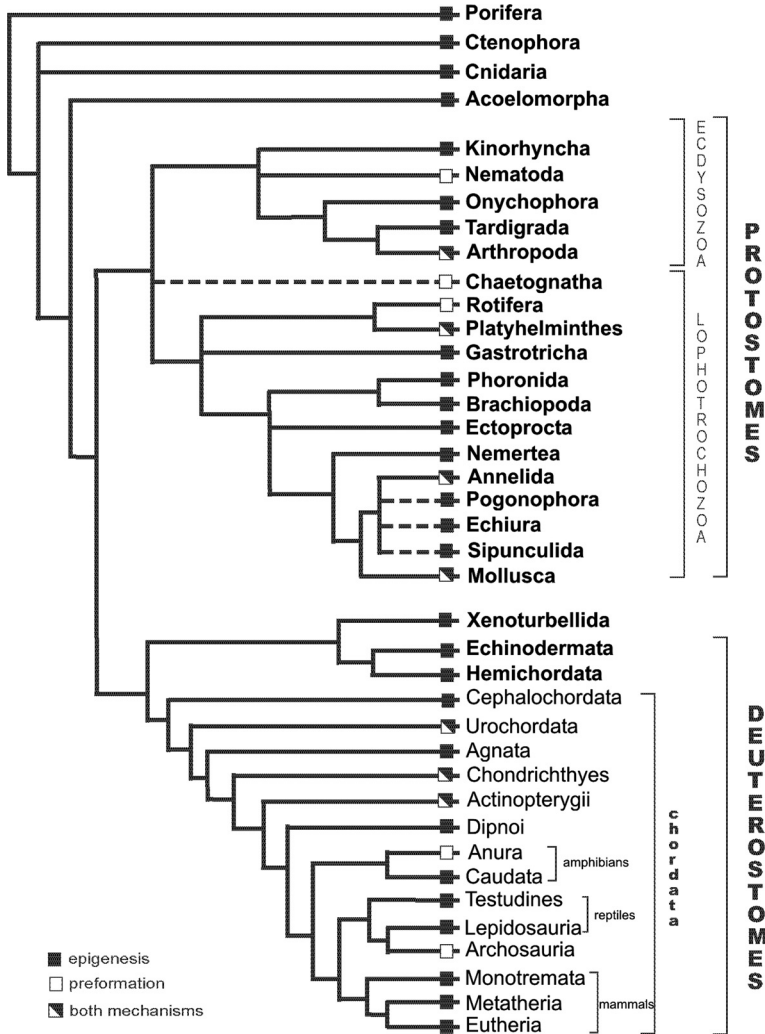


Figure 17.1 Distribution of PGC specification mechanisms across the Metazoa. Epigenesis (black boxes), preformation (white boxes), or both mechanisms (black and white boxes) are indicated only in phyla for which at least two independent primary data sources provide morphological, cell lineage, experimental or molecular evidence; phyla for which the data on germ-cell specification mechanisms are insufficient have been omitted. Dashed lines indicate phyla for which phylogenetic relationships are still unclear. Details of source data are as described in Extavour and Akam (2003). Adapted from Extavour and Akam (2003) with modifications as follows: assignment of *Xenoturbella* to its own phylum within the deuterostomes (Bourlat *et al.* 2003, 2006); evidence for epigenetic PGC

Within the deuterostomes, all studied members of these phyla, including all non-chordates, probably use epigenesis to specify PGCs (Figure 17.1). Of the chordates, only Urochordata, Chondrichthyes and Actinopterygii contain some members that use epigenesis and others that use preformation as a PGC specification mode. Finally, in only two clades (anuran amphibians and archosaurs) do all studied members exhibit preformation. To summarise, with the exception of some elasmobranchs, the only deuterostome clades containing preformistic members are those containing chordate model laboratory organisms other than mice: the solitary ascidians *Ciona intestinalis* and *Halocynthia roretzi* (but note that recent data on colonial ascidians are consistent with epigenesis; Sunanaga *et al.* 2006a,b); the frog *Xenopus laevis*; the teleost *Danio rerio*; and the chicken *Gallus gallus*. All other studied deuterostomes, including the Ambulacraria and *Xenoturbella*, show evidence for epigenesis as the mode of PGC specification.

A stem-cell origin of urbilaterian PGCs

The acoelomorph, protostome and deuterostome data summarised 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, Figure 17.1), strongly suggest that epigenetic establishment of the germ line was present in Urbilateria. 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. These cells are scattered throughout the gastral cavity and/or intercalated between other somatic cells. As we will see below from the basic patterns of somatic gonad structure, 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. 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.

Fig. 17.1 (cont.) 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).

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 nuage material invariably found in germ cells using transmission electron microscopy has also been found in stem-cell lineages (Eddy 1975). Pluripotent cells often display all of 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 have 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 localised to both germ cells and other pluripotent cell types. If *PL10* homologues are isolated and incorrectly assigned *vasa* homology because of insufficient analysis, using them to identify germ cells can give rise to ambiguous or inaccurate lineage assignment (see for example Shibata *et al.* 1999). On the morphological and gene expression levels, then, germ cells and stem cells are very similar.

Another level of similarity between germ cells and stem cells has been revealed by functional analysis in both vertebrate and invertebrate systems. Mammalian germ cells grown in culture and treated with fibroblast growth factor (FGF) can be induced to become pluripotent stem cells, called embryonic germ (EG) cells, that are very similar in differentiation potential to embryonic stem (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). *Drosophila* germ cells already en route towards oogenic differentiation can be induced to revert back to a germline stem-cell state (Kai and Spradling 2004). 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. 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 teratocarcinoma cells can contribute not only to many somatic tissues, but also to the germ line, of the resulting progeny (Stewart and Mintz 1981).

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. However, Zwaka and Thomson (2005) have hypothesised 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 is sufficient to 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 non-bilateria and basal bilaterians, and that 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, with the obvious caveat that somatic support structures are almost always required for successful gamete production, even in preformistic species. To demonstrate how this proposal represents a modification of previous models of germline continuity, I will compare it with the three major previous models of pangenesis, continuity, and modified continuity with somatic selection.

Darwin's (1859) pangenesis theory provided a biological explanation for Lamarck's ideas about inheritance of acquired characteristics (Lamarck 1809): all somatic cells produce invisible particles called gemmules, which travel through the body and lodge in the germ cells. Since germ cells do not initially contain all of the information necessary to reproduce the adult form in successive generations, including acquired characteristics, they need to receive this information from the

gemmules. The germ line is neither immortal nor continuous, as it produces only the soma of the next generation, and that soma would produce the next germ line (Figure 17.2A). Weismann, on the other hand, was sure that germ cells are autonomously totipotent from the moment of their formation, and that their nuclear information is both impervious to somatic influence and sufficient for reproduction of the adult form (Weismann 1892). In other words, the germ line is both immortal and continuous, and the source of both soma and germ line of subsequent generations (Figure 17.2B). Since at least 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). Buss (1983) has proposed an elegant revision to Weismann's hypothesis that takes into account both epigenetic germline origin and intra-individual cellular selection. In this model, while germline continuity may exist in some species (Figure 17.2C, bottom series), somatic

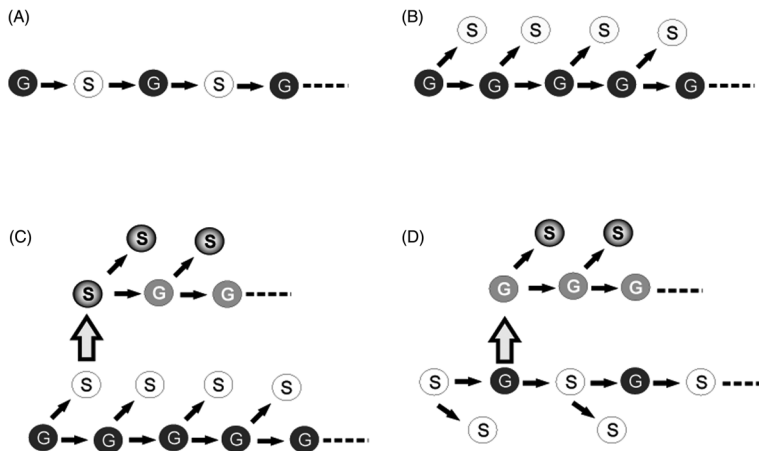


Figure 17.2 Models for the evolution of the relationship between germ line and soma. A, Pangenesis: the soma (white) informs and specifies the germ line (black), which in turn gives rise only to the soma. B, Immortality/continuity: the germ line is the sole progenitor of both germ line and soma, receiving 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 pre-formation from epigenesis: germline mutation (grey) may confer continuity on the germ line (top series), representing a deviation (large arrow) from its usual somatic stem-cell origin (bottom series).

mutation may sometimes allow a subpopulation of the soma to produce gametes (Figure 17.2C, top series).

To explain repeated evolution of preformation from epigenesis, it suffices to invert Buss's model (Figure 17.2D). Urbilateria would have segregated germ cells epigenetically, as a subpopulation of somatic cells: soma therefore gave rise to both germ line and soma (Figure 17.2D, bottom series). Where Buss's model suggests that mutations affecting the soma could allow somatic cells to produce gametes, I suggest that mutations affecting the germ line could allow cell-autonomous segregation of germ cells in a subsequent generation (Figure 17.2D, top series). This mechanism of preformation would then be inherited in subsequent generations. In order to understand what kind of germline mutation could have had this effect, in the next section we will consider known examples of germ cells that segregate by preformation.

Evolving preformation from epigenesis: a transitional model

All known molecular mechanisms of preformation rely on localisation of germ-cell-specific molecules (germ plasm components) to a particular place in the oocyte, either before or after fertilisation (see for example Illmensee *et al.* 1976, Ressom and Dixon 1988, Carré *et al.* 2002). In several cases, notably the *vasa* and *nanos* gene families, the genes encoding these molecules, and their germline 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 expression timing and gene product localisation 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 fertilised egg and cannot therefore be inherited cell-autonomously by PGCs; instead the genes must be zygotically activated in PGCs through epigenetic signalling (Figure 17.3A). In preformation, germ-cell-specific gene products persist through completion of oogenesis in the zygotic cytoplasm, and are therefore available for inclusion into PGCs before the initiation of zygotic transcription (Figure 17.3B). In this context, we can now see that in order to make the transition from epigenesis to preformation, only two things are necessary: (1) persistence (and

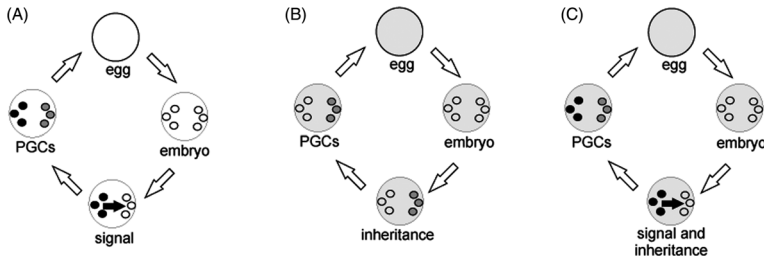


Figure 17.3 A transitional model for the evolution of preformation from epigenesis. A, Epigenesis: germ-cell-specific molecules expressed during gametogenesis are not present in oocytes at the time of fertilisation. During embryogenesis, inductive signals (black) specify PGCs, which begin zygotic expression of germ-cell-specific molecules (dark grey). Germ cells produce gametes to complete the cycle. B, Preformation: maternal germ-cell determinants (light grey) are localised to oocyte cytoplasm and inherited cell-autonomously by PGCs forming in early embryogenesis. Germ cells produce gametes to complete the cycle. C, Transition from epigenesis to preformation: germ-cell-specific molecules expressed during gametogenesis are retained in oocytes through to the time of fertilisation. They are inherited cell-autonomously by PGCs forming in early embryogenesis. Inductive signals (black) produced during embryogenesis are now redundant with respect to PGC formation. Germ cells 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.

possibly, through further refinement of the mechanism, cytoplasmic localisation within the oocyte) of germ-cell-specific gene products through the end of gametogenesis; and (2) inheritance of these products, which would now constitute germ plasm components, by future PGCs (Figure 17.3C).

Mutations arising in the germ line that affected oocyte cytoskeletal dynamics or mRNA or protein localisation of germ cell molecules could allow persistence and/or localisation of these molecules in mature oocytes. Once preformation had arisen in a heritable way through such mutation(s), signals from somatic tissues to induce germline fate would no longer be necessary to ensure species survival. We would therefore expect gradual loss of these signalling mechanisms, since ‘unnecessary but costly structures or activities should be lost in evolution’ (Michod 1999: 55). This model can explain why we see the repeated evolution of autonomous germline determinants in several groups (Figure 17.1), but never observe examples of epigenesis in phyla where preformation is plesiomorphic (e.g. Rotifera, Chaetognatha, Nematoda).

One prediction of the model is the existence at some time of species in which both preformation and epigenesis were operative, or at least operable. In most preformistic model organisms, however, when PGCs or their precursors are eliminated through physical ablation or genetic manipulation, the resulting animals are sterile, presumably unable to replace the ablated germ line through epigenetic mechanisms (reviewed in Saffman and Lasko 1999). These animals may belong to lineages in which preformation evolved so long ago that epigenetic signalling mechanisms have become unusable through lack of positive selection. Given that all currently used developmental genetic model organisms are derived with respect to many other aspects of embryogenesis, this explanation is not unreasonable. Alternatively, our failure thus far to observe widespread coexistence of both PGC specification mechanisms may simply be reflective of poor taxon sampling. Intriguingly, in the solitary ascidian *C. intestinalis*, although convincing embryological and molecular genetic data indicate that preformation specifies PGCs, when the PGCs are ablated in larval stages the resulting adults are still fertile (Takamura *et al.* 2002). The mechanism responsible for this germ line replacement 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

Urbilateria was unlikely to have had a complex somatic reproductive system, but whatever somatic support it did have for gametogenic cells was almost certainly of mesodermal origin. The changes in life histories undergone by urbilaterian descendant lineages, as they occupied different ecological niches, led to morphogenetic modification of these mesodermal derivatives, resulting in convergent evolution of different elements of somatic reproductive systems, including gonads, gonoducts and gonopores, copulatory organs and adaptations for viviparity. Urbilaterian germ cells were likely probably specified as a subpopulation of pre-existing somatic pluripotent stem cells, through inductive signals of unknown molecular identity. Its germ cells expressed *vasa* gene family members and possibly also *nanos* gene(s). Changes in the expression timing (heterochrony) and ooplasmic localisation (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 signalling later in embryonic development, resulting in convergent evolution of preformation. In descendant lineages that had evolved preformation, epigenetic germ-cell specification mechanisms would have gradually deteriorated owing to lack of positive selection.

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