Key Transitions
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Chapter 16

Redefining Stem Cells and Assembling Germ Plasm: Key Transitions in the Evolution of the Germ Line

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Introduction

A discussion of “key transitions” in the evolution of animals often invokes mental images of large-scale morphological or behavioural changes: the fin-to-limb transition, avian beak shape changes, the transition from simply holding objects to using them purposefully as tools. These types of changes clearly occurred in evolution and had great adaptive value. Other types of changes, however, have also occurred in the morphologies and behaviours of single cells and cell lineages. A complete understanding of many “key transitions” involving new structures and new cell types must therefore incorporate the molecular genetic basis for the novel or modified cell behaviours that can lead to novel structures.

For example, the fin-to-limb transition cannot be considered without first considering the origin of paired fins. The appearance of these appendages clearly predated their adaptive transformation into terrestrial limbs. At the
level of the completed morphological product, the fossil record shows that midline unpaired appendages (fins) were present before paired appendages (Zhang and Hou 2004). At the level of behaviour of the individual cells that participate in the development of these structures, gene expression studies suggest that bilaterally paired groups of cells adopted a developmental program hitherto used by unpaired midline appendage anlagen (Freitas et al. 2006). In other words, cells that had previously held a given developmental capacity were able to expand their biological potential and perform a new function, thus giving rise to a new morphology. In this case, the bilateral anlagen may have co-opted a developmental program that was already in use by other cells in the organism.

A second example illustrates that some features require us to consider the emergence of an apparently new cell type. In this case, rather than cells adopting entire genetic regulatory programs already in use by other cells in the embryo, cells may instead have acquired novel combinations or modifications of genetic regulatory programs, allowing them to perform functions that are new to the embryo. Years of evolutionary analysis of avian beaks have shown that beak shape is an important target of adaptive morphological changes (Darwin 1859, Grant et al. 1976, Grant 1999, Schluter 2000). One important level of analysis has been aimed at the gene regulatory differences that are present in birds with differently shaped upper beaks (Abzhanov et al. 2004, Abzhanov et al. 2006). A second, related level of analysis is possible, however, which focuses on the very existence of these beak regions: the analysis of the neural crest (Douarin and Kalcheim 2009). Neural crest cells give rise to the upper beak pattern itself, and are responsible for most vertebrate craniofacial variation (Noden 1975, Hu et al. 2003). From their origins in the dorsal neural tube, neural crest cells migrate away from the neural tube in an anterior to posterior progression, finally coming to rest at several different places in the embryo. Both during and after this migration, these cells differentiate into an enormous diversity of cell types, including neurons, glia, pigmented cells, cartilage, and bone. The first neural crest cells would thus literally have gone where no cell had gone before.

The neural crest is unique to vertebrates, yet cell lineages with some, but not all, neural crest properties have been identified in non-vertebrate chordates (Jeffery et al. 2004, Ota et al. 2007, Jeffery et al. 2008). The evolution of the “true” neural crest may therefore have involved the acquisition of neural crest-specific characters (including migration in different directions and the potential to give rise to multiple terminal cell fates) that pre-existed in an either fundamentally migratory but not pluripotent, or pluripotent but stationary, cell lineage (Wada 2001, Kee et al. 2007, Sauka-Spengler et al. 2007, Donoghue et al. 2008, Jeffery et al. 2008).
This chapter will examine the evolution of a cell type that, not unlike the neural crest, seems to have arisen at a definable branch point within the Metazoa, and was more likely to have arisen by modification of a pre-existing cell type than to have appeared entirely *de novo*: the dedicated germ line.

**The novelty of the bilaterian germ line**

The cells of the germ line are those uniquely responsible for undergoing gametogenesis during adult reproductive life. The eggs and sperm that germ cells produce ensure both organismal reproduction and species continuity. All sexually reproducing organisms, including plants, specify germ cells at some stage of reproductive life. However, bilaterians are the only clade with a single, embryonically specified cell lineage that is capable only of producing gametes. Many non-bilaterians have pluripotent stem cell-like lineages, which can both undergo gametogenesis and produce a variety of differentiated somatic cell types (discussed further below). In contrast, bilaterians generally have a uniquely specified germ line that is established once during embryogenesis and cannot self-renew. While germ line stem cells (GLSCs), the self-renewing precursors to gametes, exist in some animals, embryonic primordial germ cells (PGCs) are not a naturally self-renewing population before they commit to gametogenesis. Instead, similar to somatic cell types, a limited, species-specific number of germ cells is generated during embryogenesis. These cells generally cannot be replaced if they are lost during embryogenesis (but see Takamura et al. 2002, Modrell et al. submitted), and only they can take on the job of creating gametes in the sexually mature animal.

The emergence of the dedicated germ line during embryogenesis thus represents a key transition in animal evolution: the transition from a state in which any cell could contribute to the next generation, to one where that potential was restricted to a tiny number of cells in the organism. In other words, the issue of the evolution of the germ line is also that of the evolution of the true soma, cells that contribute to the body plan but whose genetic material is barred from being transmitted to future generations.

**The Dedicated Germ Line as an Outcome of Multicellularity**

One of the consequences of the evolution of stable multicellularity was a fundamental shift in reproductive strategy. In unicellular species, each individual is responsible for its own reproduction, and passes on its genes to every one of its descendants. In contrast, in multicellular species, the majority of an organism’s cells will not have their particular gene complement transmitted to the next generation: only a small fraction of the organism’s cells, the germ cells, will have that privilege. Similarly, every
single somatic function that was formerly performed by the unicellular organism (for example, motility, nutrient intake, or waste excretion) then became the responsibility of only a subset of cells in the multicellular organism. Thus, while the physical phenomenon of multicellularity would have required the acquisition of cell adhesion and cell signalling molecules (King and Carroll 2001, Nichols et al. 2006, Abedin and King 2008, Newman and Bhat 2009), the adaptive value of multicellularity is based on the principle of division of labor among cells with theoretically equivalent genetic potential (Willensdorfer 2008). Such equivalent genetic potential, however, is not guaranteed. We must therefore consider what happens in the case that genotypes are not identical, or do not produce identical phenotypes, in cells of a multicellular organism.

**Heterogeneity and cellular competition in the germ line**

Although we usually think of all cells in an organism having identical genomes, in reality somatic mutation at any point after first cleavage can result in a genetically mosaic individual. When cells are not genetically identical, and only some of them can contribute to making gametes, we must consider the impact of cellular competition. Genetic heterogeneity in a cellular population has been shown to lead to competition and natural selection (Keller 1999), in the same way that adult organisms and populations of organisms are subject to selection (Darwin 1859, Wallace 1885). Many scholars have therefore reasoned that because unchecked competition between aggregated cells poses a threat to multicellularity, a dedicated germ line is required for true multicellularity (see for example Buss 1987, Michod and Roze 2001). Higher-order levels of cooperation, such as insect colonies and some mutualisms, have been proposed to represent "organisms" with no need for a germ-soma distinction to ensure organismal cohesion (Queller and Strassmann 2009). However, in a single multicellular organism with a dedicated germ line, reproduction is a "non-exchangeable benefit" (Michod and Roze 2001), because no somatic cell can produce gametes. In this situation, "cooperation" in the form of maintaining multicellularity, is less costly than abandoning the aggregate (Michod 2005, Michod 2007).

The complexity of the cooperation/conflict problem posed by multicellularity increases, however, even when the gametogenic lineage is restricted, if the germ line itself is genetically mosaic. The most extreme case of such mosaicism occurs in plants, where individual, spatially separated stem cell lineages produce "multiple germ lines." In plants, a stem cell lineage called the shoot apical meristem (SAM) is produced at the tips of the aerial parts of the plant (Sharma et al. 2003). The SAM undergoes self-renewing divisions to give rise to gametogenic cells, and
also produces various somatic cell types during the continuous growth of the plant (Dickinson and Grant-Downton 2009, Stahl and Simon 2009). With the production of every new reproductive organ (flower), germ cells must be established de novo from each SAM. The high levels of somatic mosaicism, combined with the longevity of many plants, means that individual SAMs can differ genetically, resulting in germ lines of multiple genotypes being produced by a single plant (Whitham and Slobodchikoff 1981, Kleckowski 1986, Schultz and Scofield 2009).

The plant mechanism of germ line determination might seem to resemble that seen in some basal bilaterians, where a pluripotent stem cell lineage gives rise to both somatic cells and gametes throughout adult life (discussed below). However, this is only superficially similar to the production of gametes in plants. Whereas basal bilaterian gametogenic stem cells arise from a single founder population during embryogenesis, the individual SAMs of a plant are not all clonally related. Thus, in the plant case, the opportunities for a single plant to produce genetically heterogeneous gametes are greater than those for an individual cnidarian or acocel flatworm. In other words, an important determinant of the possible heterogeneity of the gametic population is whether the germ line arises from a small, early-determined clone, or whether it is polyclonal and derived late in development. Germ line specification mechanisms are therefore highly relevant to the selective pressures and cellular competition that influence the germ line during pre-gametic development.

Once the animal germ line is specified, not all PGCs may have the opportunity to contribute to the gametic population in the next generation. Competition between genetically mosaic somatic cells is a well-documented phenomenon (García-Bellido et al. 1973, Morata and Ripoll 1975, Moreno et al. 2002, Moreno and Basler 2004, Oliver et al. 2004, Oertel et al. 2006). Similarly, when genetic mosaicism is induced in embryonic PGCs, they compete to enter the germ line (Extavour and García-Bellido 2001). Standing genetic variation has also been clearly shown to result in natural selection acting on the germ line in colonial ascidians. In these animals, embryos produce tadpole larvae that join genetically heterogeneous colonies. The larvae mature into individual zooids, which participate in the colony by sharing a common test (outer covering) and by undergoing a degree of internal anatomical fusion to share common nutritive, excretory, and reproductive functions (Milkman 1967). Each zooid has a population of putative stem cells that are established during embryogenesis (Brown et al. 2009) and circulate in the hemolymph of the entire colony. These cells give rise to gametes and thus act as shared, circulating germ line progenitors for the colony (Berrill 1941, Mukai and Watanabe 1976, Sabbadin and Zaniolo 1979). Elegant molecular lineage experiments have shown that the germ line from one zooid can “invade”
a colony, effectively outcompeting the germ cells contributed by other colony members (Stoner and Weissman 1996, Stoner et al. 1999, Laird et al. 2005). These observations support the hypothesis put forward by Buss (1982), who suggested that genetically chimaeric animals should possess a self/non-self recognition system in order to prevent germ line takeover by closely related genomes (de Tomaso et al. 2004, 2005).

Investigation of the developmental origin of the germ line in individual zooids has shown that gametogenic stem cells circulating in the colony can be distinguished from circulating somatic stem cells based on the expression of many of the same genes expressed by germ cells in solitary (non-colonial) animals (Sunanaga et al. 2006, Sunanaga et al. 2007, Sunanaga et al. 2008, Rosner et al. 2009). Recent work on the embryonic origin of these cells has further suggested that the “germ line” contributed by each zooid to the competing gamete precursor pool, is specified as a small, early-derived lineage of cells close to the beginning of embryogenesis. These cells may go on to contribute to gametes years after their initial specification (Brown et al. 2009).

Finally, even in cases where cells are genetically identical, stochastic differences in gene expression and cellular metabolism levels are expected to produce heterogeneous phenotypes, which may be subject to natural selection (discussed in Khare and Shaulsky 2006). Thus germ cells, which can be considered a special subset of stem cells, are themselves fundamental units of selection, and their genomes are critical loci of evolutionary change (Weissman 2000).

Evolutionary Implications of Developmental Timing and Molecular Mechanism of Germ Line Specification

We have established that the developmental origin of the germ line is an important event with implications that are not just reproductive, but also evolutionary. Two key aspects of this developmental origin are the timing of germ line specification and the molecular mechanisms directing it.

Timing of germ cell specification

The timing of germ cell specification during embryogenesis affects the degree of gametic, and subsequent somatic, mosaicism because every round of cell division provides an opportunity for mutation (Drost and Lee 1998). DNA replication errors can result in hereditary mutations, and increased mitotic rates can lead to increased mutation rates (Sweasy et al. 2006) and accelerated aging (Ban and Kai 2009). In most animals, once PGCs have been specified in the embryo, they divide little or not at all until gametogenesis begins. Even after gamete production starts, the fact that more mitoses are required to produce sperm than eggs has been suggested
to cause increased mutation rates, and therefore higher rates of evolution, in genes transmitted by males (reviewed by Ellegren 2007). Haldane’s studies of female carriers of haemophilia led to the first suggestion of male-biased evolution over 70 years ago (Haldane 1935, Haldane 1947), but these ideas were not subjected to further theoretical or empirical studies until decades later (Miyata et al. 1987). In mammals, the mutation rate of male-transmitted genes is higher than that of female-transmitted genes, and is correlated with the higher total number of cell divisions required to produce spermatids as opposed to oocytes (Shimmin et al. 1993, Chang et al. 1994, but see Sandstedt and Tucker 2005). In contrast, some studies in Drosophila have provided evidence for an absence of male-biased mutation (Bauer and Aquadro 1997), while others suggest that some weak male-biased mutation does occur (Bachtrog 2008).

The strength of the bias appears to be greater overall in mammals than in Drosophilids, which may be related to the different timing of germ cell specification in these groups. Most biased mutation studies have calculated the number of cell divisions from “zygote” to gamete, without taking into consideration when during zygotic development the germline is created. Mammalian germ cells are specified after many rounds of embryonic cell division, and they continue to divide extensively between specification and gametogenesis, giving those cells an increased opportunity to collect mutations generated during early mitoses (Chiquoine 1954, Zamboni and Merchant 1973, Clark and Eddy 1975, Tam and Snow 1981, Ginsburg et al. 1990). Drosophilid germ cells, on the other hand, are specified after fewer mitotic divisions, and do not divide between specification and gametogenesis (Sonnenblick 1941, Turner and Mahowald 1976, Foe and Alberts 1983, Campos-Ortega and Hartenstein 1985, Hay et al. 1988). The developmental timing of germ cell specification must thus be addressed not only by developmental biologists, but also in any consideration of metazoan evolution.

Molecular mechanisms of germ cell specification

The developmental mechanisms used by metazoans to specify PGCs early in embryogenesis can be grouped into two broad categories. Previous literature has referred to these two types as epigenesis and preformation (Nieuwkoop and Sutasurya 1981, Extavour and Akam 2003). We will avoid using these terms in this chapter, due to the varied and often confusing use of these terms in the biological literature (Haig 2004, Callebaut 2008), and particularly in light of the recent rise of “epigenetics” (in the sense of non-genomically encoded, heritable gene expression phenotype changes (Ko and McLaren 2006, Hayashi and Surani 2009). Instead, we will refer to inherited germ line determinants (formerly “preformation”) as the “inheritance mode,” and to inductive signal-dependent PGC specification(formerly “epigenesis”) as the “inductive mode.”
Under the inductive mode, signals produced from one cell population induce another cell population to adopt germ cell fate (reviewed by Extavour and Akam 2003). The signals are necessary and sufficient for germ cell formation. When PGCs are specified in this way, the specification event takes place relatively late in development. By this time, the cells that become PGCs have already undergone several rounds of cell division, and therefore of putative somatic mutation and selection. Under this scenario, gametic populations are expected to be relatively heterogeneous, as the founder population of PGCs may be relatively large. In animals, the heterogeneity of the gametic founder population never reaches the extremes seen in plants (discussed above), but is still considerable. In mice, for example, the PGC founder population of at least 150 cells has divided mitotically at least 22 times before becoming committed to gametogenesis (summarized by Drost and Lee 1998).

Experimental evidence for the inductive mode in wild-type development, in the form of experimental embryology and genetic knockdown experiments, comes from work on mice (Tam and Zhou 1996, Lawson et al. 1999, Ying et al. 2000, Ying and Zhao 2001, de Sousa Lopes et al. 2004, Ohinata et al. 2009) and salamanders (Humphrey 1929, McCosh 1930, Nieuwkoop 1947). There is also experimental evidence that some animals can employ this mode under abnormal conditions where the endogenous germ line has been removed (Takamura et al. 2002, Modrell et al. submitted). An additional wealth of cytological, histological, embryological, electron microscopy, and lineage tracing data from all major metazoan clades collectively suggests that the inductive mode is the most common mode of germ cell specifications in bilaterians (Extavour and Akam 2003). Outside of the bilaterians, germ line development and gene expression patterns are consistent with the inductive mode of germ cell specification (reviewed by Extavour and Akam 2003, see for example Extavour et al. 2005). In these groups, germ cells are formed either by a dedicated germ line stem cell (GLSC) population, or by a pluripotent stem cell population that can produce both gametes and various somatic cell types. No data currently support the existence of an inheritance mode of PGC specification in bilaterian outgroups.

The inheritance mode is characterized by the maternal provision of germ cell determinants (reviewed by Extavour and Akam 2003). Before the end of oogenesis, or shortly after fertilization, the determinants are asymmetrically localized to a region of the ooplasm (the germ plasm) that will be inherited by the future PGCs. In some cases, the PGCs may incorporate this germ plasm after initial rounds of syncitial cleavage (see classical descriptions of Metschnikoff 1866, Huettner 1923). In other cases, germ plasm is inherited directly through successive rounds of asymmetric, holoblastic cleavage until all of the germ plasm is contained within one or
a few cells, which are the first PGCs (see for example Browne et al. 2005, Extavour 2005). When PGCs are specified by inheritance, their separation from the soma takes place very early in embryogenesis, sometimes as early as second cleavage (Grbic et al. 1998, Donnell et al. 2004, Zhurov et al. 2004) and not later than sixth cleavage (Nishida 1987, Fujimura and Takamura 2000). The germ line in these cases is thus subject to limited somatic selection, and the resulting gametic population is less likely to be genetically heterogeneous. At the same time, fixation of mutations may be more likely than in the inductive mode, since the small founder population of PGCs means that early-occurring mutations will appear in all or most of the gametes (Drost and Lee 1998).

Because this is the mode of germ cell specification displayed by the genetic laboratory model organisms Drosophila melanogaster (fruit fly), Caenorhabditis elegans (nematode), Danio rerio (zebrafish), and Xenopus laevis (frog), it has been examined in great molecular genetic detail (reviewed by Raz 2003, Zhou and King 2004, Hayashi et al. 2007, Strome and Lehmann 2007). Largely due to the enormous amounts of molecular genetic data concerning this mode of PGC specification, it was until recently widely assumed that the inheritance mode represented an ancestral animal developmental mechanism (Wolpert et al. 2002). However, as mentioned above, the examination of PGC specification mode distribution across a broad phylogenetic range suggests that the inheritance mode may have evolved convergently several times during metazoan evolution (Extavour and Akam 2003). This hypothesis now forms part of many synthetic treatments of developmental biology (Gilbert and Singer 2006, Wolpert et al. 2007).

Two Major Transitions in the Evolution of the Germ Line

Of all of the evolutionary steps that must have taken place along the road to the spectrum of mechanisms used by extant animals to specify their germline, two stand out as being of particular interest. The first is the evolution of a lineage-restricted stem cell population (GLSCs) from pluripotent stem cells. The second is the repeated, convergent evolution of an inheritance mode of PGC specification, from an ancestral inductive mode. The following sections deal with each of these two critical transitions, and propose testable hypotheses for further investigation.

From Stem Cell to Germ Cell

Comparative and phylogenetic analyses of PGC specification modes across the metazoans have led to the hypothesis that germ cells have their evolutionary origins in a pluripotent stem cell population that was present in the last common bilaterian ancestor (Extavour 2007a, b).
Similar hypotheses have been proposed by researchers examining the *in vivo* and *in vitro* similarities between germ cells, somatic stem cells, and cultured stem cells of extant animals (Sanchez Alvarado and Kang 2005, Agata et al. 2006). Nonetheless, it is still unclear how the molecular genetic program controlling pluripotent stem cell identity could have been modified to yield a gametogenic stem cell program. To this end, we will first review the relationships, potentials and conversions between these various cell types. A note regarding terminology is in order here: the term “stem cell” is not always consistently defined in developmental biology research, clinical research or the popular media (Shostak 2006, Lander 2009). For the purposes of this chapter, a “stem cell” simply refers to a cell that is self-renewing. Namely, upon mitotic division, one daughter differentiates, and the second daughter does not differentiate, but rather engages in another self-renewing mitosis. The common second criterion of pluripotency will not be applied, so as to be able to include unipotent germ line stem cells (GLSCs). In these cells, mitoses are self renewing, but their differentiated daughters are all of the same cell type, that is, gametes or gametogonia.

**Dedicated Germ Cells from Pluripotent Stem Cells**

Germ cells have sometimes been called the “ultimate” or “mother of all” stem cells because although their immediate differentiated products are of a single cell type, their final products (oocytes, the founder cell of all animal embryogenesis) ultimately give rise to all cell types of an organism (Donovan 1998, Spradling and Zheng 2007, Cinalli et al. 2008, Rangan et al. 2008, Rangan et al. 2009). From such a perspective, germ cells seem “more pluripotent” than some naturally occurring or laboratory-developed (somatic) stem cell populations. However, in order to detect a pattern in the wide variety of observed germ cell/stem cell relationships, it is more useful to think of the production of lineage-restricted germ cells or GLSCs, as a true differentiation event. In other words, deriving either GLSCs or gametes from somatic stem cells will be considered a reduction in pluripotency (Fig. 1).

**Transitions from stem cell to germ cell in normal development**

This transition occurs naturally during adult reproductive life in extant members of bilaterian outgroups. Cnidarians and sponges have pluripotent stem cells that give rise both to differentiated somatic cells of various types, and to male and female gametes (Fig. 1A) (Müller 2006). Within Bilateria but branching basally to protostomes and deuterostomes are the acelo flatworms (Hejnol et al. 2009b), also of interest when considering bilaterian germ cell origin because they are a sister group to all other
Fig. 1. Stem cell and germ cell relationships and transitions. (A) In platyhelminths, pluripotent stem cells called neoblasts can differentiate into gonias (GC), which generate gametes. (B) In colonial ascidians, pluripotent stem cells circulate in the haemolymph, and may differentiate into gonias (GC), which generate gametes. The existence of lineage-restricted germline stem cells in colonial ascidians remains a possibility under active investigation (Brown et al. 2009, Kawamura and Sunanaga 2010). (C) In Drosophila, embryonically specified primordial germ cells (PGCs) differentiate into unipotent germline stem cells (GLSCs), which generate gametes. However, GLSC daughters that have begun the differentiation process into gametes can be induced to revert to a GLSC fate (Niki and Mahowald 2003). (D) In teratocarcinomas, misguided embryonic PGCs are thought to convert to an “embryonal carcinoma” (EC) state. When transplanted into a host blastocyst, EC descendants can populate both the soma and the germline, and generate functional gametes. In situ, EC cells can differentiate into cells with both somatic and germline characteristics. In in vitro culture, EC cells be induced to differentiate into both somatic cell types and germ cells. (E) In normally developing mouse and human embryos, embryonically specified PGCs colonize the embryonic genital ridges, differentiate either into gonias (in females) or into germline stem cells (in males), and subsequently generate gametises. PGCs from the embryonic genital ridges or GLSCs from the adult male testis, can be cultured to form embryonic germ (EG) cells. EG cells can contribute to both somatic and germline cells when transplanted into host blastocysts. Similarly, embryonic stem (ES) cells derived from culturing cells of the inner cell mass (ICM), and differentiated somatic cells converted into induced pluripotent stem (iPS) cells by treatment with variations on the “Yamanaka factors,” (Takahashi and Yamanaka 2006, Okita et al. 2007, Takahashi et al. 2007) can both be induced to adopt differentiated somatic and germ cell fate characteristics in vitro.

Color image of this figure appears in the color plate section at the end of the book.
bilaterians. Work on acoel gametogenesis suggests that inheritance does not play a role in embryonic germ cell specification (Falleni and Gremigni 1990, Gschwentner et al. 2001, Hejnol and Martindale 2008).

Although the Platyhelminthes, excluding Acoelomorpha, appear to be firmly nested within protosome bilaterians (Dunn et al. 2008, Hejnol et al. 2009a), their embryogenesis is very similar to that described for acoels (Boyer et al. 1998, Henry et al. 2000). Data on platyhelminth germ cell specification may therefore be informative for understanding early bilaterian strategies for PGC and stem cell function. Flatworm germ cells are derived from pluripotent stem cells called neoblasts (Fig. 1A) (Baguñà 1981). These cells can also give rise to several types of somatic cells. Although germ cells and neoblasts have been the subject of much recent work in these animals, there is still no clear evidence supporting the existence of an exclusively gametogenic subpopulation of neoblasts (Reddien et al. 2005, Guo et al. 2006). In contrast, colonial ascidians (discussed above) display multiple stem cell types, one of which is GLSCs (Fig. 1B).

Transitions from stem cell to germ cell in disease conditions

The transition from stem cell to gamete can also occur in vivo as a result of specific genetic or epigenetic alterations. Teratocarcinomas and teratomas are two types of germ cell neoplasms, thus called because they are derived from misplaced or ectopically occurring PGCs (Fig. 1D). Teratomas are generally benign, non-invasive, and do not recur after surgical removal (Stevens and Little 1954, Stevens 1967, Heerema-McKenney et al. 2005). By contrast, teratocarcinomas are malignant, can be invasive and recurring, and are composed of a combination of teratomas and embryonal carcinoma (EC) cells. EC cells, in turn, are thought to be the misregulated products of ectopic or misguided embryonic PGCs (Fig. 1D) (Kleinsmith and Pierce 1964, Martin and Evans 1974, Martin 1975, Graham 1977, Hoei-Hansen et al. 2006). These two types of germ cell tumor can give rise both to germ cells and to several types of somatic cell, resulting in tumors that may contain differentiated hair, bone or teeth (Kleinsmith and Pierce 1964, Kahan and Ephrussi 1970). The demonstrated pluripotency of these tumors is attributed to the EC cells they contain. The higher differentiation potential of EC cells, as compared to cells of non-differentiating tumors, may be attributable to their germ cell origin.

It has been suggested that a “germ cell state” of reprogramming “back to” pluripotency exists, and must be traversed by differentiated cells in order to regain pluripotency (Hayashi and Surani 2009). This hypothesis is consistent with the observation that EC cells of germ cell neoplasms are able to differentiate into many cell types; in other words, they are demonstrably pluripotent. When cultured and subsequently transplanted
into blastocysts, EC cells can contribute both to somatic cells and to the germline of the hosts (Mintz and Illmensee 1975, Stewart and Mintz 1981, Stewart and Mintz 1982). In summary, germ cell neoplasms provide an example of PGCs, or possibly even lineage-restricted GLSCs, that first increase in pluripotency to become EC cells, and then lose pluripotency as they move towards either GLSC, gametic, or somatic cell fate.

**Transitions from stem cell to germ cell in vitro**

The various methods of inducing laboratory-cultured stem cells to differentiate as gametogenic cells again demonstrate the close relationship between these cell types (Fig. 1E). We will consider three main categories of laboratory-derived stem cells. (1) Embryonic Germ (EG) cells are obtained by culturing PGCs from embryonic genital ridges (Matsui et al. 1992, Resnick et al. 1992, Rohwedel et al. 1996, Shamblott et al. 1998, Kanatsu-Shinohara et al. 2004, Kerr et al. 2006). Although cultured spermatogonial cells from adult testes are called SSCs or maGScs in the literature, in this chapter we will consider them within this first category, since they are also stem cells derived from cells that had achieved germline specification through normal development. (2) Embryonic Stem (ES) cells are obtained by culturing cells of the inner cell mass (ICM) of blastocyst stage embryos (Evans and Kaufman 1981, Martin 1981, Thomson et al. 1998). (3) Induced Pluripotent Stem (iPS) cells are obtained by transfecting differentiated somatic cells with an appropriate combination of transcription factors (Takahashi and Yamanaka 2006, Blelloch et al. 2007, Takahashi et al. 2007, reviewed by Amabile and Meissner 2009).

Once in culture, all of these stem cell types can be induced to "reduce pluripotency" by differentiating either as somatic cells, or as germ cells (reviewed by Marques-Mari et al. 2009). EG cells show germline transmission when injected into donor blastocysts, indicating that they can give rise to functional gametogenic cells (Kanatsu-Shinohara et al. 2003, Guan et al. 2006). ES cells are capable of adopting many germ cell characteristics (Kee et al. 2006, Clark et al. 2004). They can produce "oocytes" that are competent to enter meiosis, form follicle-like structures, and parthenogenetically produce blastocyst-like masses (Hübner et al. 2003). They can upregulate germ cell-specific genes and undergo spermatogenesis (Toyooka et al. 2003). Finally, iPS cells can produce cells that upregulate germ cell-specific genes and contribute to the germline of a host blastocyst (Okita et al. 2007, Park et al. 2009). For all of these laboratory-cultured stem cells, however, fully functional germ cells have never been achieved without passage through an embryonic system (host blastocyst), suggesting that additional factors are required to drive true germ cell differentiation in vitro (Niwa 2007).
In summary, pluripotent stem cells have the capacity to move away from pluripotency towards a special, restricted stem cell identity as a GLSC or germ cell. They can do this as a part of normal development, as in the case of flatworms or colonial ascidians, in disease conditions such as teratocarcinomas, and under culture conditions. Both in the latter case and in wild type development, upregulation of germ cell-specific genes such as \textit{vasa} accompanies the transition.

**Pluripotent Stem Cells from Dedicated Germ Cells**

A number of lines of evidence suggest that not only can pluripotent stem cells reduce pluripotency to acquire germ cell or GLSC fate, but also that the reverse is true: somatic cells, GLSCs, PGCs, or even germ cells that have already begun to enter gametogenesis, can “backtrack” to acquire or increase pluripotency. Fruit fly germ cells that have already begun to differentiate into oocytes can be induced to dedifferentiate and revert to a germ line stem cell identity (Fig. 1C) (Niki and Mahowald 2003). The most notable \textit{in vitro} examples of this are cultured PGCs or GLSCs that give rise to embryonic germ (EG) cells, cultured ICM cells that give rise to ES cells, and iP\$S cells.

PGCs from the embryonic genital ridges, or GLSCs from adult testes, can acquire pluripotency if cultured with leukemia inhibitory factor (LIF), fibroblast growth factor (bFGF), and the mitogen or survival factor kit ligand (KL) (Dolci et al. 1991, Godin et al. 1991, Matsui et al. 1991). For ICM cells to make the transition to ES cells, they must be cultured with a feeder layer providing at least LIF and bone morphogenetic proteins (BMPs 2/4). However, the specific family and levels of BMP are critical, since addition of appropriate concentrations of BMP4 and/or BMP8b can cause failure to acquire pluripotency, and adoption of the more restricted fate of gametes (reviewed by Zhang and Li 2005). Finally, differentiated somatic cells of many kinds can be induced to acquire pluripotency by transfection or other delivery (Bleloch et al. 2007, Okita et al. 2008, Stadtfeld et al. 2008) of the so-called “Yamanaka factors,” comprising a cocktail of transcription factors that promote pluripotency (Yamanaka et al. 2006, Takahashi et al. 2007, Yamanaka 2007).

Factors that are not directly dependent on transcriptional regulation have also been shown to influence the stem cell/germ cell decision. Extended mitosis of cultured cells inhibits germ cell differentiation, and is associated with a tendency towards continued self-renewal (Kimura et al. 2003). From an evolutionary point of view, this is consistent with positive selection acting on cells that have undergone fewer mutagenic events (mitoses) as founders of the germ line.
Increased pluripotency by vertebrate stem cells is often accompanied or regulated by the transcription factors Oct4 and Nanog (Chambers et al. 2003, Mitsui et al. 2003, Hatano et al. 2005, Lavial et al. 2007). These genes clearly play an important role in vertebrate stem cell biology. However, they are lineage-restricted genes (unique to deuterostomes) (Booth and Holland 2004, Odintsova 2009). As a result, they cannot play a part in any evolutionary scenario concerning the transition, in a bilaterian ancestor, from pluripotent stem cell to dedicated PGC or GLSC.

Modifying Stem Cell Programs: From Generic to Germ Line-Specific

Many experiments aimed at achieving a molecular definition of "stemness" have suggested that the transcriptional regulatory landscape is largely similar between vertebrate stem cells and germ cells (Burns and Zon 2002, Ivanova et al. 2002, Evsikov and Solter 2003, Fortunel et al. 2003, Ramalho-Santos and Willenbring 2007, Sun et al. 2007). However, other studies have succeeded in identifying clear transcriptional and proteomic profile differences between GLSCs and ES cells (Sperger et al. 2003, Fujino et al. 2006, Kurosaki et al. 2007). Moreover, the upregulation of germline-specific genes in more "generic" stem cell types is correlated with a transition from somatic stem cell to GLSC in vivo (Sunanaga et al. 2006, Sunanaga et al. 2007), and has been shown to induce GC characteristics in somatic stem cells in vitro (Lavial et al. 2009). At the level of specific genes, several conserved molecules are expressed in both germ cells and all types of stem cells. These include Piwi family proteins, Tudor family proteins, and PL10 gene products. Genes specifically upregulated in dedicated germlines, but downregulated in or absent from pluripotent stem cell types include the vasa family members, and possibly nanos (reviewed by Ewen-Campen et al. 2010).

In summary, the extensive molecular signatures and functional potential of germ cells and stem cells suggest a shared evolutionary origin for these cell types. A dedicated germ line is likely to have appeared before the divergence of Bilateria, but after the advent of animal multicellularity. This in turn suggests that germ cell-specific components would have been added to a pre-existing pluripotency network. An ancestral pluripotency network would have been responsive to BMP signalling. It would also have included members of Piwi and Tudor class proteins, which are conserved components of both germ plasm and stem cell cytoplasm across the metazoans (reviewed by Ewen-Campen et al. 2010). PL10, the ancestor of the vasa gene family, is expressed in both germ line and stem cell populations in extant metazoans (Mochizuki et al. 2001). It is therefore likely that PL10, rather than a true vasa family member, participated in an ancestral "germ cell regulatory module".
From Inductive Signalling to Germ Plasm Inheritance

The transition from "generic" stem cell to germ line stem cell would, by parsimony, have happened once in the lineage leading to the Bilateria. The ancestral mechanism of germ cell fate acquisition was likely to have been inductive signalling, possibly via BMP response as outlined above. Subsequently, however, an inheritance mechanism would have evolved independently in multiple branches of both protostomes and deuterostomes. Given that it has arisen in at least ten metazoan phyla (Extavour and Akam 2003, Extavour 2007a), some researchers have addressed the possible selective advantages of evolving inheritance mechanisms (Johnson et al. 2003, Crother et al. 2007); these issues will not be discussed here. Instead, the following section will consider molecular developmental mechanisms that could explain such convergence, given what we know about germ cell specification and development in extant groups.

The key to achieving the inheritance mode of germ line specification is the germ plasm. This is a special cytoplasm whose components are deposited during oogenesis. Thus, the developing progeny are dependent upon the fidelity of the mother’s genome for proper germ cell formation. The components are then assembled (asymmetrically localized) into a special region of cytoplasm either before the end of oogenesis or immediately following fertilization. This specialized cytoplasm may have a diffuse appearance of loosely localized molecular components, often called nuage or simply germ plasm (coined by Weismann 1892, see for example Noda and Kanai 1977). Germ plasm can also display a compact appearance in the form of a discrete (though not membrane-bounded) organelle(s), which may be called germinal granules, Balbiani bodies, or oosomes (first described by Balbiani 1864, see for example Strome and Wood 1982, Gutzeit 1985). In the inheritance mode, germ plasm components are retained at the end of oogenesis and inherited by the embryo. Animals with an inductive mode of germ cell specification show similar cytoplasmic inclusions in germ cells once they acquire their fate, and during gametogenesis. However, in this mode, germ plasm components are cleared from the ooplasm before the end of oogenesis, leaving PGCs in the developing embryo to assemble their germ plasm de novo.

Heterochrony of Germ Plasm Component Expression or Localization

A comparison of asymmetrical germ plasm localization in different groups with the inheritance mode reveals that the molecular mechanisms necessary to assemble germ plasm are variable. Microtubule-driven localisation is critical for germ plasm assembly in X. laevis (Ressom and

The timing of localization also differs between groups. In fruit flies and zebrafish, germ plasm assembly begins before the end of oogenesis (Illmensee et al. 1976, Olsen et al. 1997), but in C. elegans asymmetric localization of P granules takes place following fertilization (Strome and Wood 1982). X. laevis employs a two-step assembly mechanism, where some components are localized during oogenesis, and others following fertilization (reviewed by King et al. 2005).

In contrast to these differing mechanisms of assembly, the molecules that make up the germ plasm are remarkably conserved. The vasa, tudor, and piwi gene families discussed above, as well as nanos and staufen homologues, have been identified in all metazoans studied, and their gene products are germ plasm components. Strikingly, many of these gene products are not only localized to embryonic germ plasm and PGCs, but are also expressed during and required for gametogenesis. In the inheritance mode, PGCs therefore acquire these gene products directly from the ooplasm. In animals that use the inductive mode, however, these genes must be upregulated de novo in PGCs. An evolutionary switch from induction to inheritance could therefore be explained if a mechanism for stabilization, retention, and localization of germ plasm molecules during oogenesis were to evolve in some lineages. In other words, a heterochronic shift in the timing of regulation and/or localization of germ plasm genes could explain the repeated convergent evolution of the inheritance mode in metazoans (Extavour 2007a, Extavour 2007b).

Evidence for such a heterochronic regulatory change is provided by analysis of the evolution and expression of vasa and PL10 homologues from an anthozoan cnidarian (Extavour et al. 2005). In the sea anemone Nematostella vectensis, the vasa locus has undergone a gene duplication event after diverging from the PL10 ancestor. During embryogenesis, PL10 and one of the vasa duplicates are not expressed during late oogenesis, and show zygotic expression in presumptive germ cells. The later-diverging parologue, however, displays both maternal and zygotic expression. This suggests that following duplication the second vasa locus
evolved regulatory mechanisms that allowed its prolonged expression during oogenesis, such that the transcript was available for cytoplasmic inheritance by cells in the early embryo.

Germ Plasm Nucleators

Conceptually, the easiest way to achieve germ plasm assembly at a given time would be to have a single molecule that was itself necessary and sufficient to nucleate germ plasm components. Under the inheritance model, cytoplasmic germ cell determinants are necessary and sufficient for specifying PGCs. Indeed, when germ plasm is removed or damaged by physical means, germ cell formation is either disrupted or eliminated (see for example Hegner 1908, Geigy 1931, Buehr and Blackler 1970). Conversely, when transplanted wholesale to ectopic locations, germ plasm can be sufficient to autonomously specify ectopic germ cells (Illmensee and Mahowald 1974, Okada et al. 1974, Illmensee and Mahowald 1976). The prediction for a necessary and sufficient “germ plasm nucleator” molecule would therefore be that it, too, would be able to drive PGC formation ectopically, and impede PGC formation when removed. Two metazoan genes are known whose products possess these properties.

Drosophila oskar provides a solution to the localization problem


Functional studies have also shown that the genetic and biochemical properties of osk satisfy the necessity and sufficiency requirements for a germ plasm nucleator. Loss-of-function oskar mutants do not form germ cells (Lehmann and Nüsslein-Volhard 1986). Conversely, osk gene products can autonomously recruit germ plasm components, resulting in ectopic germ cells that are capable of functional gametogenesis (Ephrussi et al. 1991, Ephrussi and Lehmann 1992, Smith et al. 1992). However, osk’s ability to ectopically assemble germ plasm depends on the presence of other germ
plasm factors: ectopic osk cannot produce ectopic PGCs in vas or tud mutants (Ephrussi and Lehmann 1992). Accordingly, Osk protein has been shown to directly interact with Vasa protein, and with Staufen protein, another germ plasm component (Breitwieser et al. 1996). Osk also recruits nanos mRNA (Ephrussi et al. 1991, Smith et al. 1992, Kim-Ha et al. 1995), a third component of germ plasm that is also needed for posterior and abdominal patterning (Nüsslein-Volhard et al. 1987, Lehmann and Nüsslein-Volhard 1991). These data are consistent with a model where the role of Osk is to recruit germ plasm components rather than to induce PGC fate directly.

**Zebrafish bucky ball as a germ plasm nucleator**

Until recently, osk was the only gene known to be both necessary and sufficient for germ plasm nucleation, and therefore for germ cell formation. However, a recent zebrafish screen for mutants affecting anterior-posterior polarity (Dosch et al. 2004) uncovered the gene bucky ball (buc), whose phenotype is strikingly similar to that of oskar’s. Like oskar transcripts during fly oogenesis, during zebrafish oogenesis buc transcripts are localised to the vegetal pole, where germ plasm begins to accumulate. Following fertilization, germ plasm becomes localised to the early cleavage furrows of the zebrafish embryos (Olsen et al. 1997, Yoon et al. 1997), as does Buc protein (Marlow and Mullins 2008). buc loss-of-function mutants do not form germ cells (Marlow and Mullins 2008), and germ plasm components, including transcripts of the vas, dazl, and nos genes, are not localized correctly (Bontems et al. 2009). buc appears to be not just sufficient, but also necessary for germ cell formation, as ectopic expression of buc in early embryonic cells that would normally not give rise to germ cells results in supernumerary PGCs. These ectopic cells are derived from the cells containing ectopic buc, and they localize germ plasm components and migrate to the gonad along with wild type PGCs (Bontems et al. 2009).

**Commonalities Between Germ Plasm Nucleators**

Given the data available for fruit fly osk and zebrafish buc, one might predict that in other groups using the inheritance mode of germ plasm specification, osk and buc homologues would provide the key to early germ plasm assembly. However, both of these genes appear to be recent evolutionary novelties within Diptera and Vertebrata, respectively. Moreover, both of these genes encode novel proteins with neither identifiable functional domains nor predictable secondary structure, making it difficult to understand how they are able to perform analogous biological functions. In the case of oskar, sequence and functional comparisons even within closely related species suggest that this gene is not only a relatively recent dipteran novelty, but is also evolving rapidly within the Diptera.
Functional comparison of dipteran oskar homologues

oskar homologues are identifiable in all 12 Drosophila genomes and in three mosquito genomes (Fig. 2). Although a "consensus" Drosophila sequence can be deduced, much of the protein is variable at the amino

Fig. 2. Alignment of dipteran Osk homologs. An amino acid MAFFT (Katoh and Toh 2008) alignment of twelve Drosophila and three probable mosquito osk homologs (identified via BLAST) has been condensed to the modal consensus Drosophila osk sequence (top line) and the three mosquito proteins (bottom lines). Where two residues in a column are found to be most and equally frequent in the Drosophila consensus sequence, both are presented; a "+" denotes when more than two residues are found to be equally frequent. Physico-chemical conservation (generated in Jalview v2.4 (Waterhouse et al. 2009)) among the twelve Drosophila osk proteins is shown in the histogram above the alignment, and among all fifteen Dipteran proteins in the histogram below the alignment. Highly conserved positions are lighter and taller in the histogram, whereas poorly conserved positions are darker and shorter. Sequences (and accession numbers in parentheses) aligned are from Drosophila melanogaster (NP_731295), D. sechellia (XP_002031969), D. simulans (XP_002104196), D. yakuba (XP_002096875), D. erecta (XP_001980894), D. ananassae (XP_001953297), D. persimilis (XP_002017385), D. pseudoobscura pseudoobscura (XP_001359508), D. virilis (Q24741), D. mojavensis (XP_002000116), D. grimshawi (XP_001994345), D. immigrans (ABH12272), Aedes aegypti (XP_001656415), Culex quinquefasciatus (XP_001848641), and Anopheles gambiae (XP_313289).
acid level even when comparing species that are thought to have diverged as little as 12 million years ago (Tamura et al. 2004). Two large regions, an ~100 amino acid N-terminal domain, and a larger ~210 amino acid C-terminal domain, show a relatively high level of physico-chemical conservation within Drosophilids. Comparison with three probable mosquito homologues shows that these two domains are the areas of highest physico-chemical conservation across all compared dipterans. However, the extent of similarity decreases when the mosquito sequences are added to the analysis. Furthermore, the mosquito sequences are highly diverged from the Drosophilid sequences even in these regions. Finally, outside of these regions, the mosquito sequences are essentially not alignable with the Drosophilid sequences, due to significant insertions in the Drosophilid lineages.

Only two oskar homologues besides D. melanogaster osk have been assessed functionally. Cytoplasm from the posterior (pole plasm) of the early D. immigrans embryo can induce ectopic, functional germ cells when introduced into D. melanogaster embryos (Mahowald et al. 1976). This D. immigrans pole plasm potential appears to be exclusively due to the D. immigrans oskar homologue (immosk), which can rescue both the posterior patterning and germ cell formation defects of D. melanogaster oskar loss of function mutants (Jones and Macdonald 2007). However, the morphology of D. immigrans germ plasm is very distinct from that of D. melanogaster (Mahowald 1962, Mahowald 1968), suggesting that immosk interacts with its germ plasm binding partners differently in the two species. However, the known binding partners of Osk, including Vasa, are very highly conserved across the metazoans at the amino acid level (Fig. 3) (Mochizuki et al. 2001, Extavour et al. 2005), much more so than the Osk proteins. This provides an interpretation for the observation that when immosk replaces endogenous osk in D. melanogaster embryos, the morphology of the resulting germ plasm matches that of D. immigrans, rather than that of D. melanogaster (Jones and Macdonald 2007). We hypothesize that despite high conservation of most germ plasm components, germ plasm morphology differs between these two species because the amino acid changes between the two Osk proteins affect specific molecular interactions with their conserved binding partners. Since diverging from their last common ancestor 30-40 million years ago (Spicer 1988, Russo et al. 1995, Remsen and O’Grady 2002), the evolutionary changes in D. melanogaster and D. immigrans osk have been sufficient to change Osk protein’s specific physical interactions with germ plasm components. However, the changes have not been sufficient to disrupt these interactions altogether, since both Osk proteins can provide posterior patterning (presumably via nanos mRNA localization) and germ plasm assembly.
Fig. 3. Vasa proteins show clear homology between *D. melanogaster* and *D. rerio*. Needleman-Wunsch global pairwise alignment of *Drosophila melanogaster* and *Danio rerio* Vasa proteins is presented with physico-chemical conservation (generated in Jalview v2.4 (Waterhouse et al. 2009)) at each residue in the histogram below the alignment. Histogram interpretation is as in Figure 2. Sequences (and accession numbers in parentheses) aligned are from *Drosophila melanogaster* (P25158) and *Danio rerio* (A1L1Z2).

A similar divergence time separates *D. melanogaster* from *D. viridis* (Remsen and O'Grady 2002, Tamura et al. 2004). Similar to immosk, *D. viridis oskar (virosk)* is able to rescue the posterior and abdominal patterning defects exhibited by *D. melanogaster osk* loss of function mutants (Webster et al. 1994). However, *virosk* cannot maintain *osk* mRNA levels in the germ plasm, and cannot rescue the germ cell formation phenotype of *D. melanogaster osk* mutants. Furthermore, when introduced into wild type *D. melanogaster* (with two functioning copies of endogenous *osk*), *virosk* disrupts endogenous *osk* localization, and induces dominant maternal-effect lethality. These observations indicate that significant changes in *osk* sequence and function occurred in the 30-60 million years separating *D. viridis* and *D. melanogaster* from their last common ancestor (Remsen and O'Grady 2002, Tamura et al. 2004). Specifically, an Osk protein ancestral to those in *D. viridis* and *D. melanogaster* may have had the ability to localize *nanos* mRNA, thus ensuring posterior and abdominal patterning, but not the ability to bind and nucleate other germ plasm components. This hypothesis is consistent with the absence of pole cells (PGCs arising early in embryogenesis) reported for some lower dipterans (Rohr et al. 1999), which may either lack an *osk* homologue altogether, or lack one with the ability to nucleate germ plasm.
Functional similarity between buc and osk cannot be explained by homology

More data on the function of other dipteran osk homologues are clearly needed to broaden our understanding of a gene that has played a key role in the evolution of the inheritance mode of germ cell specification. bucky ball, the zebrafish “solution” to the inheritance mode problem seems to be a functional analogue of D. melanogaster oskar. However, there is no evidence that buc and osk are homologues in the classical sense of common descent from an shared ancestral genomic sequence (Remane 1952). Although comparing the two sequences may appear to show some physico-chemical similarity in a central domain (Fig. 4), these predictions are in fact artefacts of a pairwise comparison between two sequences that cannot confidently be aligned.

The conundrum of these two non-homologous proteins that interact with homologous binding partners to achieve an analogous biological function may be explained by similarities at a level higher than amino acid sequence. The observed molecular interactions of Osk with Vasa and Staufen proteins, and the hypothesized interactions of Buc with the zebrafish homologues of Osk and Vasa, will be governed by the specific biochemical properties of Osk and Buc, including tertiary structure. If three-dimensional structure is similar for both Osk and Buc, conserved molecular interactions with other (homologous) germ plasm components could explain their analogous roles in germ cell specification. Such a finding would mean that the convergent evolution driving acquisition of the inheritance mode in flies and fish was due to selection acting on the level of protein structure, rather than at the nucleotide or amino acid sequence level.

There is evidence from other systems that selective pressures acting on tertiary structure can result in highly similar biological functions in the absence of demonstrable genetic homology. The relationship between the bacterial protein MreB and eukaryotic Actin proteins provides one such example. These genes are domain-specific: actin genes are specific to eukaryotes, while mreB homologues are found in several bacterial species (Doi et al. 1988, Jones et al. 2001). Primary sequence alignment between Actin and MreB homologues (Jones et al. 2001) does not reveal greater amino acid identities, or longer regions of amino acid similarity, than an Osk and Buc alignment (Fig. 4). At the amino acid level, there is little identity, and there are very limited regions of physico-chemical similarity (Jones et al. 2001). However, structural analysis of MreB and Actin has shown that there is extensive tertiary similarity between these two proteins, such that their crystal structure models are nearly identical (van den Ent et al. 2001). MreB has also been shown to polymerize into filaments close to the cell cortex, similar to Actin (van den Ent et al. 2001).
Fig. 4. *D. melanogaster* Long Osk and *Danio rerio* bucky ball are not homologues. Needleman-Wunsch global pairwise alignment of *Drosophila melanogaster* Long Osk and *Danio rerio* Buc proteins is presented with physico-chemical conservation (generated in Jalview v2.4 (Waterhouse et al. 2009)) at each residue in the histogram below the alignment. Histogram interpretation is as in Figure 2. Sequences (and accession numbers in parentheses) aligned are from *Drosophila melanogaster* (NP_731295) and *Danio rerio* (XP_688879).

**Evolving Early Germ Plasm Assembly: Selective Pressure on Tertiary Structure**

While phylogenetic analysis suggests that *mreB* and *actin* genes are not true genetic homologues, many have argued that their striking structural similarities argue for cryptic homology (Kabsch and Holmes 1995, Egelman 2001, van den Ent et al. 2001, Doolittle and York 2002). Under this scenario, selective pressures would have maintained tertiary structure, and therefore biochemical function, by permitting extensive amino acid changes as long as 3D structure was sufficiently unchanged. The accumulated amino acid changes are predicted to be so great that the true homology relationships of the descendant proteins in extant groups are obscured. New approaches to understanding how evolution acts on protein structure and function may be needed to shed light on this problem (Halabi et al. 2009).

While we cannot definitively rule out the possibility that *bucky ball* and *oskar* are cryptic evolutionary homologues, their primary structures provide no evidence in favor of such a hypothesis. This leads us to suggest an intriguing scenario to explain the convergent evolution of maternal or early embryonic germ plasm assembly, and therefore the inheritance mode of germ cell specification. We hypothesize that the driving force behind this convergence was selective pressure on the tertiary structure
of germ plasm nucleators, such that they were able to bind pre-existing, conserved germ plasm factors. The genes encoding these factors, such as *vasa*, *nanos*, and *staufen*, are clearly ancient in metazoans and predate the evolution of the inheritance mode.

**Summary**

The germ cell lineage of extant metazoans is hypothesized to share ancestry with other pluripotent stem cell populations. An ancestral stem cell lineage with the capacity to give rise to both somatic and gametogenic cells, would have undergone modifications to its genetic regulatory program such that it acquired the specialization characteristic of the germ line. These modifications included changes to previously generic pluripotency regulators, such as *PL10* family genes and *piwi* class genes. *PL10* family modifications resulted in the *vasa* family of genes. To test this hypothesis, assessment of the physico-chemical properties and functional abilities with respect to gametogenesis and PGC development should be carried out on bilaterian *vasa* homologues and *PL10* homologues from bilaterian outgroups.

In the evolution of multicellularity, all divisions of labor in the form of specialization of cell types, must have been the result of cell-cell signalling (Pires-daSilva and Sommer 2003). This is consistent with the hypothesis that the inductive signalling mode of germ cell specification is ancestral to the metazoans. Early germ line genetic regulatory networks may have been responsive to signalling from BMP family members. Indeed, germ cell and stem cell function in extant animals can be regulated by BMP family members even in animals using an inheritance mode to specify germ cells (Chen and McKearin 2003, Kawase et al. 2004, Pan et al. 2007, Guo and Wang 2009, Rhiner et al. 2009, Wilkinson et al. 2009), or in cultured cells undergoing an *in vitro* switch from pluripotency to immortality or from pluripotency to gamete production (reviewed by Zhang and Li 2005). Further studies of the possible involvement of BMPs in specifying germ cells in animals with no evidence for early germ plasm will help provide support for or against this hypothesis. Similarly, testing the responsiveness of germ cells to BMP signalling in animals that use the inheritance mode will inform our understanding of the evolution of signals that induce germ cell formation.

A transition from an ancestral inductive mode to a comparatively derived inheritance mode occurred in multiple lineages of the bilaterian radiation. Few similarities are observed in the molecular mechanisms used by different animals to achieve early germ cell specification. *Drosophila oskar* and zebrafish *bucky ball* gene products are the only examples to date of molecules that are both necessary and sufficient for germ cell formation.
The lack of homology between these two genes stands in contrast to the high conservation of the germ plasm factors with which they interact. We therefore hypothesize that selective pressure at the level of tertiary structure of germ plasm nucleator molecules was the driving force behind the convergent evolution of the inheritance mode in these two clades. Comparative structural, biochemical, and functional studies of these two proteins will be needed to evaluate this hypothesis.

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References


Redefining Stem Cells and Assembling Germ Plasm


Chapter 16

Fig. 1. Stem cell and germ cell relationships and transitions. (A) In platyhelminths, pluripotent stem cells called neoblasts can differentiate into gonial (GC), which generate gametes. (B) In colonial ascidians, pluripotent stem cells circulate in the haemolymph, and may differentiate into gonial (GC), which generate gametes. The existence of lineage-restricted germline stem cells in colonial ascidians remains a possibility under active investigation (Brown et al. 2009, Kawamura and Sunanaga 2010). (C) In Drosophila, embryonically specified primordial germ cells (PGCs) differentiate into unipotent germline stem cells (GLSCs), which generate gametes. However, GLSC daughters that have begun the differentiation process into gametes can be induced to revert to a GLSC fate (Niki and Mahowald 2003). (D) In teratocarcinomas, misguided embryonic PGCs are thought to convert to an “embryonal carcinoma” (EC) state. When transplanted into a host blastocyst, EC descendants can populate both the soma and the germline, and generate functional gametes. In situ, EC cells can differentiate into cells with both somatic and germline characteristics. In in vitro culture, EC cells can be induced to differentiate into both somatic cell types and germ cells. (E) In normally developing mouse and human embryos, embryonically specified PGCs colonize the embryonic genital ridges, differentiate either into gonial (in females) or into germline stem cells (in males), and subsequently generate gametes. PGCs from the embryonic genital ridges or GLSCs from the adult male testis, can be cultured to form embryonic germ (EG) cells. EG cells can contribute to both somatic and germline cells when transplanted into host blastocysts. Similarly, embryonic stem (ES) cells derived from culturing cells of the inner cell mass (ICM), and differentiated somatic cells converted into induced pluripotent stem (iPS) cells by treatment with variations on the “Yamanaka factors,” (Takahashi and Yamanaka 2006, Okita et al. 2007, Takahashi et al. 2007) can both be induced to adopt differentiated somatic and germ cell fate characteristics in vitro.