Key Transitions In Animal Evolution

Editors

Rob DeSalle

American Museum of Natural History
Division of Invertebrate Zoology
New York
USA

Bernd Schierwater

ITZ, Ecology and Evolution
TiHo Hannover
Germany

CRC Press
Taylor & Francis Group
an **informa** business
www.crcpress.com

6000 Broken Sound Parkway, NW
Suite 300, Boca Raton, FL 33487
270 Madison Avenue
New York, NY 10016
2 Park Square, Milton Park
Abingdon, Oxon OX 14 4RN, UK

Science Publishers
Enfield, New Hampshire

Published by Science Publishers, P.O. Box 699, Enfield, NH 03748, USA
An imprint of Edenbridge Ltd., British Channel Islands

E-mail: *info@scipub.net* **Website:** *www.scipub.net*

Marketed and distributed by:

CRC Press
Taylor & Francis Group
an **informa** business
www.crcpress.com

6000 Broken Sound Parkway, NW
Suite 300, Boca Raton, FL 33487
270 Madison Avenue
New York, NY 10016
2 Park Square, Milton Park
Abingdon, Oxon OX 14 4RN, UK

Copyright reserved © 2011

ISBN 978-1-57808-695-5

Library of Congress Cataloging-in-Publication Data

Key transitions in animal evolution / [edited by] Rob Desalle, Bernd Schierwater.
p. cm.
Includes bibliographical references and index.
ISBN 978-1-57808-695-5 (hardback)
1. Phylogeny. 2. Evolution (Biology) 3. Genetic transcription. I. DeSalle, Rob II. Schierwater, B. (Bernd), 1958-
QH367.5.K49 2010
591.3'8--dc22

2010033462

The views expressed in this book are those of the author(s) and the publisher does not assume responsibility for the authenticity of the findings/conclusions drawn by the author(s). Also no responsibility is assumed by the publishers for any damage to the property or persons as a result of operation or use of this publication and/or the information contained herein.

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying or otherwise, without the prior permission of the publisher, in writing. The exception to this is when a reasonable part of the text is quoted for purpose of book review, abstracting etc.

This book is sold subject to the condition that it shall not, by way of trade or otherwise be lent, re-sold, hired out, or otherwise circulated without the publisher's prior consent in any form of binding or cover other than that in which it is published and without a similar condition including this condition being imposed on the subsequent purchaser.

Printed in the United States of America

Chapter 16

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

John Srouji[1] and *Cassandra Extavour*[2*]

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

[1]Department of Molecular and Cellular Biology, Harvard University, 16 Divinity Avenue, Cambridge MA 02138, USA.
E-mail: jrsrouji@fas.harvard.edu
[2]Department of Organismic and Evolutionary Biology, Harvard University, 16 Divinity, Avenue, Cambridge MA 02138, USA.
E-mail: extavour@oeb.harvard.edu

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 mosacism, 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 acoel 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. Germline 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 (reveiwed 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 acoel flatworms (Hejnol et al. 2009b), also of interest when considering bilaterian germ cell origin because they are a sister group to all other

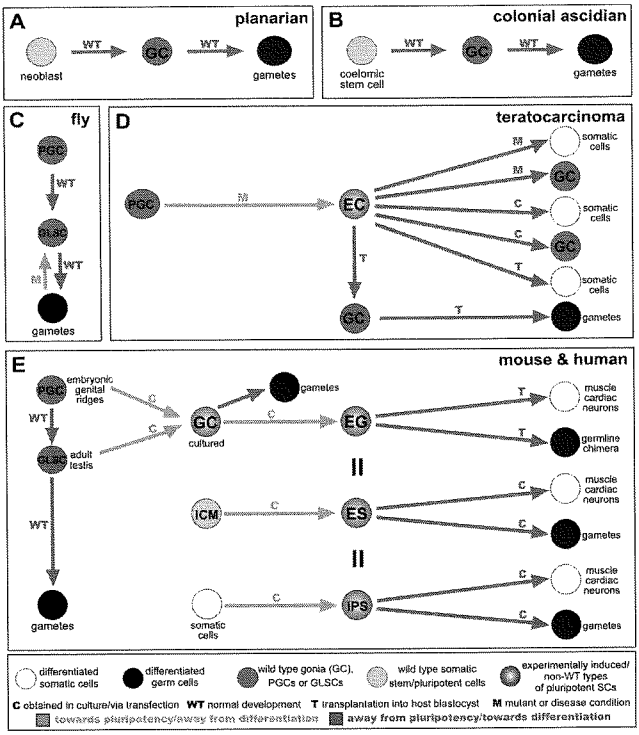

Fig. 1. Stem cell and germ cell relationships and transitions. (A) In platyhelminths, pluripotent stem cells called neoblasts can differentiate into gonia (GC), which generate gametes. **(B)** In colonial ascidians, pluripotent stem cells circulate in the haemolymph, and may differentiate into gonia (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 germ cell 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 gonia (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*.

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 maGSs 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 *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 differentate 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 *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 iPS 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 (Blelloch 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 linage-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

Dixon 1988, Robb et al. 1996). In *C. elegans*, however, an actin-based mechanism controls the cytoplasmic flows that localize germ plasm components (called P granules) (Hird and White 1993, Hird 1996, Hird et al. 1996). In *D. rerio* both actinomyosin function and the mictrotubule cytoskeleton are implicated in germ plasm assembly (Pelegri et al. 1999, Knaut et al. 2000, Urven et al. 2006). Similarly, both microtubules and actin filaments have been shown to play a role in fruit fly germ plasm assembly (Erdelyi et al. 1995, Pokrywka and Stephenson 1995, Lantz et al. 1999, Jankovics et al. 2002, Zimyanin et al. 2008). The mechanisms driving germ plasm assembly in chickens are unknown (Tsunekawa et al. 2000).

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 localisation 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 paralogue, 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*

Oskar (*osk*) was first identified in a screen for maternal effect genes on the third chromosome in *Drosophila melanogaster* (Lehmann and Nüsslein-Volhard 1986). *osk* mRNA accumulation and translation are localized to the posterior cytoplasm during oogenesis (Ephrussi et al. 1991, Kim-Ha et al. 1991, Kim-Ha et al. 1995). Its localization requires microtubules and the plus end-directed motor protein kinesin (Lehmann and Nüsslein-Volhard 1986, Brendza et al. 2000, Zimyanin et al. 2008). Localized translation of Osk protein is achieved both by activating translation of posteriorly localized *osk* transcripts and by inhibiting translation of unlocalized transcripts (Kim-Ha et al. 1995, Micklem et al. 2000, Chekulaeva et al. 2006, Klattenhoff et al. 2007, Klattenhoff and Theurkauf 2008). Osk thus displays the germ plasm localization pattern we would expect of a "germ plasm nucleator".

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 Nusslein-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 Drosophilid genomes and in three mosquito genomes (Fig. 2). Although a "consensus" Drosophilid sequence can be deduced, much of the protein is variable at the amino

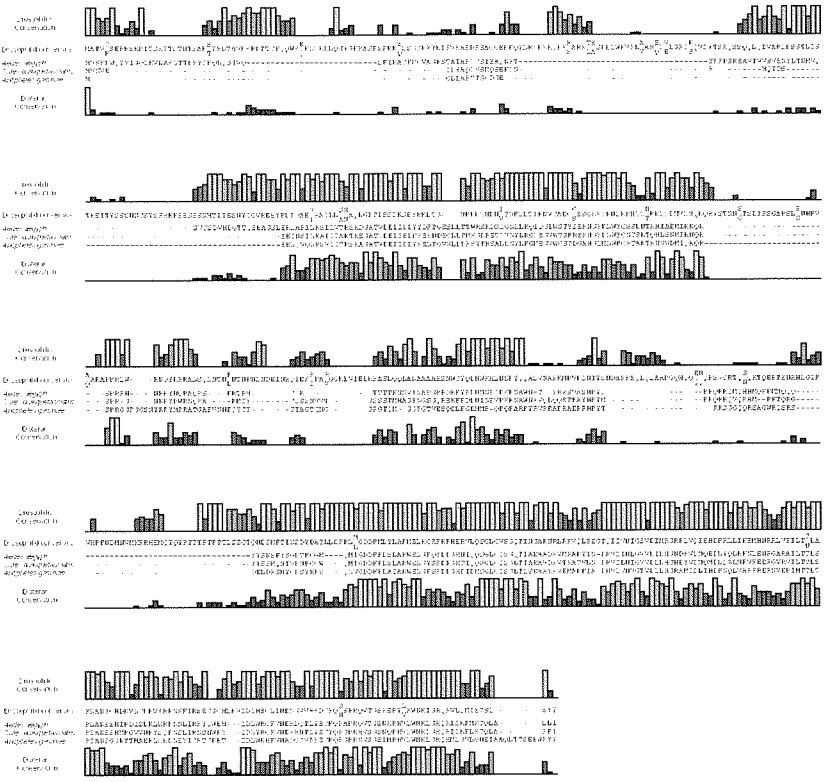

Fig. 2. Alignment of dipteran Osk homologs. An amino acid MAFFT (Katoh and Toh 2008) alignment of twelve Drosophilid and three probable mosquito *osk* homologs (identified via BLAST) has been condensed to the modal consensus Drosophilid *osk* sequence (top line) and the three mosquito proteins (bottom lines). Where two residues in a column are found to be most and equally frequent in the Drosophilid 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 Drosophilid *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.

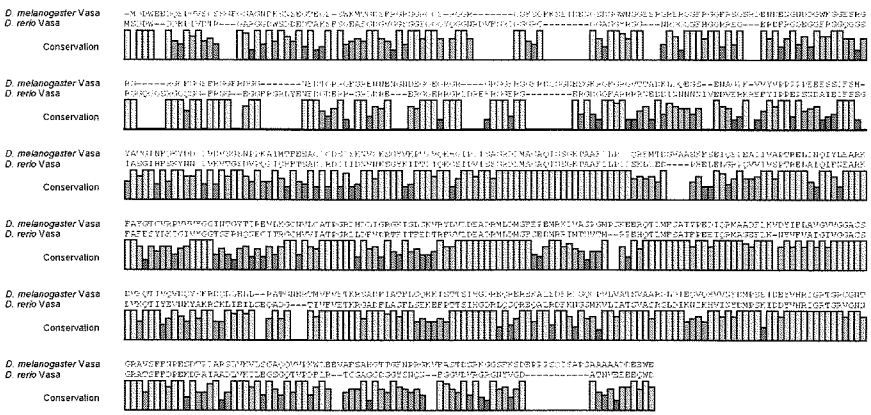

Fig. 3. Vasa proteins show clear homology between *D. melanogaster* and *D. rerio*. Needleman-Wunsch global pairwise alignment of *Drosophila melanogaster* and *Danio rerio* Vasa proteins is presented with physico-chemical conservation (generated in Jalview v2.4 (Waterhouse et al. 2009)) at each residue 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. virilis* (Remsen and O'Grady 2002, Tamura et al. 2004). Similar to *immosk*, *D. virilis 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. virilis* 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. virilis* 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. melanogster 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).

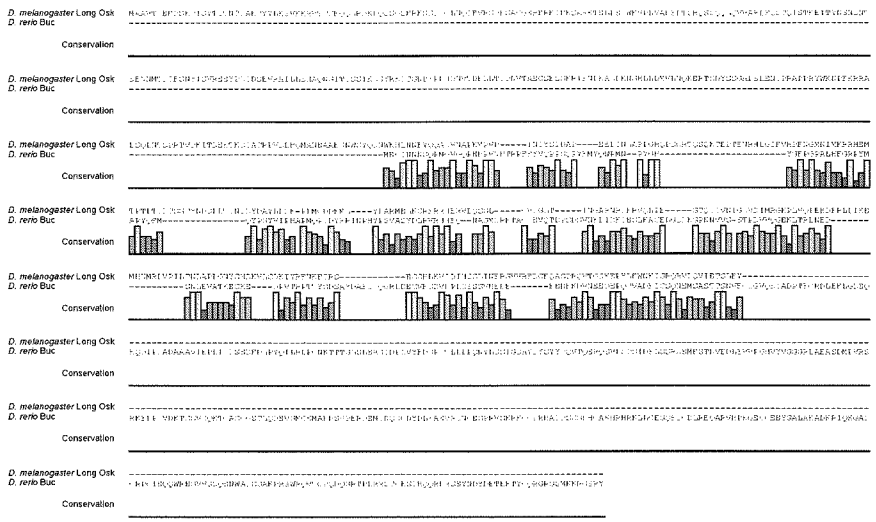

Fig. 4. *D. melanogaster* Long Osk and *Danio rerio* bucky ball are not homologues. Needleman-Wunsch global pairwise alignment of *Drosophila melanogaster* Long Osk and *Danio rerio* Buc proteins is presented with physico-chemical conservation (generated in Jalview v2.4 (Waterhouse et al. 2009)) at each 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 of 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.

Acknowledgements

This work was supported by funding from the National Science Foundation (NSF IOS-0817678).

References

Abedin, M. and N. King. 2008. The premetazoan ancestry of cadherins. Science 319: 946–948.

Abzhanov, A. and M. Protas, B.R. Grant, P.R. Grant, and C.J. Tabin. 2004. Bmp4 and morphological variation of beaks in Darwin's finches. Science 305: 1462–1465.

Abzhanov, A. and W.P. Kuo, C. Hartmann, B.R. Grant, P.R. Grant, and C.J. Tabin. 2006. The calmodulin pathway and evolution of elongated beak morphology in Darwin's finches. Nature 442: 563–567.

Agata, K. and E. Nakajima, N. Funayama, N. Shibata, Y. Saito, and Y. Umesono. 2006. Two different evolutionary origins of stem cell systems and their molecular basis. Sem. Cell Dev. Biol. 17: 503–509.

Amabile, G. and A. Meissner. 2009. Induced pluripotent stem cells: current progress and potential for regenerative medicine. Trends Mol. Med. 15: 59–68.

Bachtrog, D. 2008. Evidence for male-driven evolution in *Drosophila*. Mol. Biol. Evol. 25: 617–619.

Baguñà, J. 1981. Planarian neoblasts. Nature 290: 14–15.

Balbiani, M. 1864. Sur la constitution du germe dans l'oeuf animal avant la fecondation. Comptes Rendus de l Academie des Sciences. Serie III, Sciences de la Vie 58: 584–588.

Ban, N. and M. Kai. 2009. Implication of replicative stress-related stem cell ageing in radiation-induced murine leukaemia. Br. J. Cancer 101: 363–371.

Bauer, V.L. and C.F. Aquadro. 1997. Rates of DNA sequence evolution are not sex-biased in *Drosophila melanogaster* and *D. simulans*. Mol. Biol. Evol. 14: 1252–1257.

Berrill, N.J. 1941. The development of the bud in *Botryllus*. Biol. Bull. 80: 169–184.

Blelloch, R. and M. Venere, J. Yen, and M. Ramalho-Santos. 2007. Generation of induced pluripotent stem cells in the absence of drug selection. Cell Stem Cell 1: 245–247.

Bontems, F. and A. Stein, F. Marlow, J. Lyautey, T. Gupta, M.C. Mullins, and R. Dosch. 2009. Bucky Ball Organizes Germ Plasm Assembly in Zebrafish. Curr. Biol. 19: 414–422.

Booth, H.A.F. and P.W.H. Holland. 2004. Eleven daughters of NANOG. Genomics 84: 229–238.

Boyer, B.C. and J.J. Henry, and M.Q. Martindale. 1998. The cell lineage of a polyclad turbellarian embryo reveals close similarity to coelomate spiralians. Dev. Biol. 204: 111–123.

Breitwieser, W. and F.-H. Markussen, H. Horstmann, and A. Ephrussi. 1996. Oskar protein interaction with Vasa represents an essential step in polar granule assembly. Genes & Development 10: 2179–2188.

Brendza, R.P. and L.R. Serbus, J.B. Duffy, and W.M. Saxton. 2000. A function for kinesin I in the posterior transport of *oskar* mRNA and Staufen protein. Science 289: 2120–2122.

Brown, F.D. and S. Tiozzo, M.M. Roux, K. Ishizuka, B.J. Swalla, and A.W. De Tomaso. 2009. Early lineage specification of long-lived germline precursors in the colonial ascidian *Botryllus schlosseri*. Development 136: 3485–3494.

Browne, W.E. and A.L. Price, M. Gerberding, and N.H. Patel. 2005. Stages of embryonic development in the amphipod crustacean, *Parhyale hawaiensis*. Genesis 42: 124–149.

Buehr, M. and A.W. Blackler. 1970. Sterility and partial sterility in the South African clawed toad following the pricking of the egg. J. Embryol. Exp. Morphol. 23: 375–384.

Burns, C.E. and L.I. Zon. 2002. Portrait of a stem cell. Dev. Cell. 3: 612–613.

Buss, L.W. 1982. Somatic cell parasitism and the evolution of somatic tissue compatibility. Proc. Natl. Acad. Sci. USA 79: 5337–5341.

Buss, L.W. 1987. The Evolution of Individuality. Princeton University Press, Princeton.

Callebaut, M. 2008. Historical evolution of preformistic versus neoformistic (epigenetic) thinking in embryology. Belg. J. Zool. 138: 20–35.

Campos-Ortega, J.A. and V. Hartenstein 1985. The Embryonic Development of *Drosophila melanogaster*. Springer-Verlag, Jena.

Chambers, I. and D. Colby, M. Robertson, J. Nichols, S. Lee, S. Tweedie, and A. Smith. 2003. Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. Cell 113: 643–655.

Chang, B.H. and L.C. Shimmin, S.K. Shyue, D. Hewett-Emmett, and W.H. Li. 1994. Weak male-driven molecular evolution in rodents. Proc. Natl. Acad. Sci. USA 91: 827–831.

Chekulaeva, M. and M.W. Hentze, and A. Ephrussi. 2006. Bruno acts as a dual repressor of *oskar* translation, promoting mRNA oligomerization and formation of silencing particles. Cell 124: 521–533.

Chen, D. and D. McKearin. 2003. Dpp signaling silences *bam* transcription directly to establish asymmetric divisions of germline stem cells. Curr. Biol. 13: 1786–1791.

Chiquoine, A.D. 1954. The identification, origin, and migration of the primordial germ cells in the mouse embryo. Anat. Rec. 2: 135–146.

Cinalli, R.M. and P. Rangan, and R. Lehmann. 2008. Germ cells are forever. Cell 132: 559–562.

Clark, J.M. and E.M. Eddy. 1975. Fine structural observations on the origin and associations of primordial germ cells of the mouse. Dev. Biol. 47: 136–155.

Crother, B.I. and M.E. White, and A.D. Johnson. 2007. Inferring developmental constraint and constraint release: Primordial germ cell determination mechanisms as examples. J. Theor. Biol. 248: 322–330.

Darwin, C. 1859. On the Origin of Species by Means of Natural Selection. Grant Richards, London.

de Sousa Lopes, S.M. and B.A. Roelen, R.M. Monteiro, R. Emmens, H.Y. Lin, E. Li, K.A. Lawson, and C.L. Mummery. 2004. BMP signaling mediated by ALK2 in the visceral endoderm is necessary for the generation of primordial germ cells in the mouse embryo. Genes Dev. 18: 1838–1849.

Dickinson, H.G. and R. Grant-Downton. 2009. Bridging the generation gap: flowering plant gametophytes and animal germlines reveal unexpected similarities. Biol. Rev. Camb. Philos. Soc. 84: 589–615.

Doi, M. and M. Wachi, F. Ishino, S. Tomioka, M. Ito, Y. Sakagami, A. Suzuki, and M. Matsuhashi. 1988. Determinations of the DNA sequence of the mreB gene and of the gene products of the mre region that function in formation of the rod shape of *Escherichia coli* cells. J. Bacteriol. 170: 4619–4624.

Dolci, S. and D.E. Williams, M.K. Ernst, J.L. Resnick, C.I. Brannan, L.F. Lock, S.D. Lyman, H.S. Boswell, and P.J. Donovan. 1991. Requirement for mast cell growth factor for primordial germ cell survival in culture. Nature 352: 809–811.

Donnell, D.M. and L.S. Corley, G. Chen, and M.R. Strand. 2004. Caste determination in a polyembryonic wasp involves inheritance of germ cells. Proc. Natl. Acad. Sci. USA 101: 10095–10100.

Donoghue, P.C. and A. Graham, and R.N. Kelsh. 2008. The origin and evolution of the neural crest. Bioessays 30: 530–541.

Donovan, P.J. 1998. The germ cell—the mother of all stem cells. Int. J. Dev. Biol. 42: 1043–1050.

Doolittle, R.F. and A.L. York. 2002. Bacterial actins? An evolutionary perspective. BioEssays 24: 293–296.

Dosch, R. and D.S. Wagner, K.A. Mintzer, G. Runke, A.P. Wiemelt, and M.C. Mullins. 2004. Maternal control of vertebrate development before the midblastula transition: mutants from the zebrafish I. Dev. Cell. 6: 771–780.

Douarin, N.L. and C. Kalcheim. 2009. The Neural Crest. Cambridge University Press, Cambridge.

Drost, J.B. and W.R. Lee. 1998. The developmental basis for germline mosaicism in mouse and *Drosophila melanogaster*. Genetica 102–103: 421–443.

Dunn, C.W. and A. Hejnol, D.Q. Matus, K. Pang, W.E. Browne, S.A. Smith, E. Seaver, G.W. Rouse, M. Obst, G.D. Edgecombe, M.V. Sorensen, S.H. Haddock, A. Schmidt-Rhaesa, A. Okusu, R.M. Kristensen, W.C. Wheeler, M.Q. Martindale, and G. Giribet. 2008. Broad phylogenomic sampling improves resolution of the animal tree of life. Nature 452: 745–749.

Egelman, E.H. 2001. Molecular evolution: actin's long lost relative found. Curr. Biol. 11: R1022–1024.

Ellegren, H. 2007. Characteristics, causes and evolutionary consequences of male-biased mutation. Proc. R. Soc. Lond. B. Biol. Sci. 274: 1–10.

Ephrussi, A. and L.K. Dickinson, and R. Lehmann. 1991. Oskar organizes the germ plasm and directs localization of the posterior determinant *nanos*. Cell 66: 37–50.

Ephrussi, A. and R. Lehmann. 1992. Induction of germ cell formation by *oskar*. Nature 358: 387–392.

Erdelyi, M. and A.M. Michon, A. Guichet, J.B. Glotzer, and A. Ephrussi. 1995. Requirement for *Drosophila* cytoplasmic tropomyosin in *oskar* mRNA localization. Nature 377: 524–527.

Evans, M.J. and M.H. Kaufman. 1981. Establishment in culture of pluripotential cells from mouse embryos. Nature 292: 154–156.

Evsikov, A.V. and D. Solter. 2003. Comment on " 'Stemness': transcriptional profiling of embryonic and adult stem cells" and "a stem cell molecular signature". Science 302: 393.

Ewen-Campen, B. and E.E. Schwager, and C. Extavour. 2009. The molecular machinery of germ line specification. Mol. Reprod. Dev. 77: 3–18.

Extavour, C. and A. García-Bellido. 2001. Germ cell selection in genetic mosaics in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA 98: 11341–11346.

Extavour, C. and M.E. Akam. 2003. Mechanisms of germ cell specification across the metazoans: epigenesis and preformation. Development 130: 5869–5884.

Extavour, C. and K. Pang, D.Q. Matus, and M.Q. Martindale. 2005. *vasa* and *nanos* expression patterns in a sea anemone and the evolution of bilaterian germ cell specification mechanisms. Evol. Dev. 7: 201–215.

Extavour, C.G. 2005. The fate of isolated blastomeres with respect to germ cell formation in the amphipod crustacean *Parhyale hawaiensis*. Dev. Biol. 277: 387–402.

Extavour, C. 2007a. Evolution of the bilaterian germ line: lineage origin and modulation of specification mechanisms. Integr. Comp. Biol. 47: 770–785.

Extavour, C. Urbisexuality: The evolution of bilaterian germ cell specification and reproductive systems. pp. 317–338. *In*: A. Minelli, and G. Fusco. [eds.] 2007b. Evolving Pathways: Key Themes in Evolutionary Developmental Biology. Cambridge University Press, Cambridge.

Falleni, A. and V. Gremigni. 1990. Ultrastructural study of oogenesis in the acoel turbellarian *Convoluta*. Tissue Cell 22: 301–310.

Foe, V.E. and B.M. Alberts. 1983. Studies of nuclear and cytoplasmic behaviour during the five mitotic cycles that precede gastrulation in *Drosophila* embryogenesis. J. Cell Sci. 61: 31–70.

Fortunel, N.O. and H.H. Otu, H.H. Ng, J. Chen, X. Mu, T. Chevassut, X. Li, M. Joseph, C. Bailey, J.A. Hatzfeld, A. Hatzfeld, F. Usta, V.B. Vega, P.M. Long, T.A. Libermann, and B. Lim. 2003. Comment on " 'Stemness': transcriptional profiling of embryonic and adult stem cells" and "a stem cell molecular signature". Science 302: 393.

Freitas, R. and G. Zhang, and M.J. Cohn. 2006. Evidence that mechanisms of fin development evolved in the midline of early vertebrates. Nature 442: 1033–1037.

Fujimura, M. and K. Takamura. 2000. Characterization of an ascidian DEAD-box gene, *Ci-DEAD1*: specific expression in the germ cells and its mRNA localization in the posterior-most blastomeres in early embryos. Dev. Genes Evol. 210: 64–72.

Fujino, R.S. and Y. Ishikawa, K. Tanaka, M. Kanatsu-Shinohara, K. Tamura, H. Kogo, T. Shinohara, and T. Hara. 2006. Capillary morphogenesis gene (CMG)-1 is among the genes differentially expressed in mouse male germ line stem cells and embryonic stem cells. Mol. Reprod. Dev. 73: 955–966.

García-Bellido, A. and P. Ripoll, and G. Morata. 1973. Developmental compartmentalisation of the wing disc of *Drosophila*. Nature New Biology 245: 251–253.

Geigy, R. 1931. Action de l'ultra-violet sur le pôle germinal dans l'oef de *Drosophila melanogaster*. Rev. Suisse Zool. 38: 187–288.

Gilbert, S.F. and S.R. Singer 2006. Developmental Biology. Sinauer Associates, Inc., Sunderland, MA.

Ginsburg, M. and M.H.L. Snow, and A. McLaren. 1990. Primordial germ cells in the mouse embryo during gastrulation. Development pp. 521–528.

Godin, I. and R. Deed, J. Cooke, K. Zsebo, M. Dexter, and C.C. Wylie. 1991. Effects of the *steel* gene product on mouse primordial germ cells in culture. Nature 352: 807–809.

Graham, C.F. Teratocarcinoma cells and normal mouse embryogenesis. pp. 315–394. *In*: Sherman M.I. [ed.] 1977. Concepts in Mammalian Embryogenesis. MIT Monograph, Cambridge.

Grant, P.R. and B.R. Grant, J.N. Smith, I.J. Abbott, and L.K. Abbott. 1976. Darwin's finches: population variation and natural selection. Proc. Natl. Acad. Sci. USA 73: 257–261.

Grant, P.R. 1999. Ecology and Evolution of Darwin's Finches. Princeton University Press, Princeton, NJ.

Grbic, M. and L.M. Nagy, and M.R. Strand. 1998. Development of polyembryonic insects: a major departure from typical insect embryogenesis. Dev. Genes Evol. 208: 69–81.

Gschwentner, R. and P. Ladurner, K. Nimeth, and R. Rieger. 2001. Stem cells in a basal bilaterian. S-phase and mitotic cells in *Convolutriloba longifissura* (Acoela, Platyhelminthes). Cell Tiss. Res. 304: 401–408.

Guan, K. and K. Nayernia, L.S. Maier, S. Wagner, R. Dressel, J.H. Lee, J. Nolte, F. Wolf, M. Li, W. Engel, and G. Hasenfuss. 2006. Pluripotency of spermatogonial stem cells from adult mouse testis. Nature 440: 1199–1203.

Guo, T. and A.H. Peters, and P.A. Newmark. 2006. A Bruno-like gene is required for stem cell maintenance in planarians. Dev. Cell. 11: 159–169.

Guo, Z. and Z. Wang. 2009. The glypican Dally is required in the niche for the maintenance of germline stem cells and short-range BMP signaling in the *Drosophila* ovary. Development 136: 3627–3635.

Gutzeit, H.O. 1985. Oosome formation during in vitro oogenesis in *Bradysia tritici* (syn. *Sciara ocellaris*). Roux' Arch. Dev. Biol. 194: 404–410.

Haig, D. 2004. The (dual) origin of epigenetics. Cold Spring Harbor Symp. Quant. Biol. 69: 67–70.

Halabi, N. and O. Rivoire, S. Leibler, and R. Ranganathan. 2009. Protein sectors: evolutionary units of three-dimensional structure. Cell 138: 774–786.

Haldane, J.B.S. 1935. The rate of spontanous mutation of a human gene. J. Genet. 31: 317–326.

Haldane, J.B.S. 1947. The mutation rate of the gene for haemophilia and its segregation ratios in males and females. Ann. Eugenics 13: 262–271.

Hatano, S.Y. and M. Tada, H. Kimura, S. Yamaguchi, T. Kono, T. Nakano, H. Suemori, N. Nakatsuji, and T. Tada. 2005. Pluripotential competence of cells associated with Nanog activity. Mech. Dev. 122: 67–79.

Hay, B. and L. Ackerman, S. Barbel, L.Y. Jan, and Y.N. Jan. 1988. Identification of a component of *Drosophila* polar granules. Development 103: 625–640.

Hayashi, K. and S.M. de Sousa Lopes, and M.A. Surani. 2007. Germ cell specification in mice. Science 316: 394–396.

Hayashi, K. and M.A. Surani. 2009. Resetting the epigenome beyond pluripotency in the germline. Cell Stem Cell 4: 493–498.

Heerema-McKenney, A. and M.R. Harrison, B. Bratton, J. Farrell, and C. Zaloudek. 2005. Congenital teratoma: a clinicopathologic study of 22 fetal and neonatal tumors. Am. J. Surg. Pathol. 29: 29–38.

Hegner, R.W. 1908. Effects of removing the germ-cell determinants from the eggs of some chrysomelid beetles. Preliminary report. Biol. Bull. 16: 19–26.

Hejnol, A. and M.Q. Martindale. 2008. Acoel development supports a simple planula-like urbilaterian. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 363: 1493–1501.

Hejnol, A. and M. Obst, A. Stamatakis, M. Ott, G.W. Rouse, G.D. Edgecombe, P. Martinez, J. Baguna, X. Bailly, U. Jondelius, M. Wiens, W.E. Muller, E. Seaver, W.C. Wheeler, M.Q. Martindale, G. Giribet, and C.W. Dunn. 2009a. Assessing the root of bilaterian animals with scalable phylogenomic methods. Proc. R. Soc. Lond. B. Biol. Sci. 276: 4261–4270.

Hejnol, A. and M. Obst, A. Stamatakis, M. Ott, G.W. Rouse, G.D. Edgecombe, P. Martinez, J. Baguna, X. Bailly, U. Jondelius, M. Wiens, W.E. Muller, E. Seaver, W.C. Wheeler, M.Q. Martindale, G. Giribet, and C.W. Dunn. 2009b. Assessing the root of bilaterian animals with scalable phylogenomic methods. Proc. Biol. Sci. 276: 4261–4270.

Henry, J.Q. and M.Q. Martindale, and B.C. Boyer. 2000. The unique developmental program of the acoel flatworm, *Neochildia fusca*. Dev. Biol. 220: 285–295.

Hird, S.N. and J.G. White. 1993. Cortical and cytoplasmic flow polarity in early embryonic cells of *Caenorhabditis elegans*. J. Cell Biol. 121: 1343–1355.

Hird, S. 1996. Cortical actin movements during the first cell cycle of the *Caenorhabditis elegans* embryo. J. Cell Sci. 109: 525–533.

Hird, S.N. and J.E. Paulsen, and S. Strome. 1996. Segregation of germ granules in living *Caenorhabditis elegans* embryos: cell-type-specific mechanisms for cytoplasmic localisation. Development 122: 1303–1312.

Hoei-Hansen, C.E. and A. Sehested, M. Juhler, Y.-F.C. Lau, N.E. Skakkebaek, H. Laursen, and E. Rajpert-de Meyts. 2006. New evidence for the origin of intracranial germ cell tumours from primordial germ cells: expression of pluripotency and cell differentiation markers. J. Pathol. 209: 25–33.

Hu, D. and R.S. Marcucio, and J.A. Helms. 2003. A zone of frontonasal ectoderm regulates patterning and growth in the face. Development 130: 1749–1758.

Hübner, K. and G. Fuhrmann, L.K. Christenson, J. Kehler, R. Reinbold, R. De La Fuente, J. Wood, J.F. Strauss, 3rd, M. Boiani, and H.R. Scholer. 2003. Derivation of oocytes from mouse embryonic stem cells. Science 300: 1251–1256.

Huettner, A.F. 1923. The origin of the germ cells in *Drosophila melanogaster*. J. Morphol. 2: 385–422.

Humphrey, R.R. 1929. The early position of the primordial germ cells in Urodeles: evidence from experimental studies. Anat. Rec. 42: 301–314.

Illmensee, K. and A.P. Mahowald. 1974. Transplantation of posterior polar plasm in *Drosophila*. Induction of germ cells at the anterior pole of the egg. Proc. Natl. Acad. Sci. USA 4: 1016–1020.

Illmensee, K. and A.P. Mahowald. 1976. The autonomous function of germ plasm in a somatic region of the *Drosophila* egg. Exp. Cell Res. 97: 127–140.

Illmensee, K. and A.P. Mahowald, and M.R. Loomis. 1976. The ontogeny of germ plasm during oogenesis in *Drosophila*. Dev. Biol. 49: 40–65.

Ivanova, N.B. and J.T. Dimos, C. Schaniel, J.A. Hackney, K.A. Moore, and I.R. Lemischka. 2002. A stem cell molecular signature. Science 298: 601–604.

Jankovics, F. and R. Sinka, T. Lukacsovich, and M. Erdelyi. 2002. MOESIN crosslinks actin and cell membrane in Drosophila oocytes and is required for OSKAR anchoring. Curr. Biol. 12: 2060–2065.

Jeffery, W.R. and A.G. Strickler, and Y. Yamamoto. 2004. Migratory neural crest-like cells form body pigmentation in a urochordate embryo. Nature 431: 696–699.

Jeffery, W.R. and T. Chiba, F.R. Krajka, C. Deyts, N. Satoh, and J.S. Joly. 2008. Trunk lateral cells are neural crest-like cells in the ascidian *Ciona intestinalis*: insights into the ancestry and evolution of the neural crest. Dev. Biol. 324: 152–160.

Johnson, A.D. and M. Drum, R.F. Bachvarova, T. Masi, M.E. White, and B.I. Crother. 2003. Evolution of predetermined germ cells in vertebrate embryos: implications for macroevolution. Evol. Dev. 5: 414–431.

Jones, L.J. and R. Carballido-López, and J. Errington. 2001. Control of cell shape in bacteria: helical, actin-like filaments in *Bacillus subtilis*. Cell 104: 913–922.

Jones, J.R. and P.M. Macdonald. 2007. Oskar controls morphology of polar granules and nuclear bodies in *Drosophila*. Development 134: 233–236.

Kabsch, W. and K.C. Holmes. 1995. The actin fold. FASEB J. 9: 167–174.

Kahan, B.W. and B. Ephrussi. 1970. Developmental potentialities of clonal in vitro cultures of mouse testicular teratoma. J. Nat. Cancer Inst. 44: 1015–1036.

Kanatsu-Shinohara, M. and N. Ogonuki, K. Inoue, A. Ogura, S. Toyokuni, T. Honjo, and T. Shinohara. 2003. Allogeneic offspring produced by male germ line stem cell transplantation into infertile mouse testis. Biol. Reprod. 68: 167–173.

Kanatsu-Shinohara, M. and K. Inoue, J. Lee, M. Yoshimoto, N. Ogonuki, H. Miki, S. Baba, T. Kato, Y. Kazuki, S. Toyokuni, M. Toyoshima, O. Niwa, M. Oshimura, T. Heike, T. Nakahata, F. Ishino, A. Ogura, and T. Shinohara. 2004. Generation of pluripotent stem cells from neonatal mouse testis. Cell 119: 1001–1012.

Katoh, K. and H. Toh. 2008. Recent developments in the MAFFT multiple sequence alignment program. Brief. Bioinform. Q: 286–298.

Kawamura, K. and T. Sunanaga. 2010. Hemoblasts in colonial tunicates: are they stem cells or tissue-restricted progenitor cells? Dev. Growth Differ. 52: 69–76.

Kawase, E. and M.D. Wong, B.C. Ding, and T. Xie. 2004. Gbb/Bmp signaling is essential for maintaining germline stem cells and for repressing *bam* transcription in the *Drosophila* testis. Development 131: 1365–1375.

Kee, Y. and B.J. Hwang, P.W. Sternberg, and M. Bronner-Fraser. 2007. Evolutionary conservation of cell migration genes: from nematode neurons to vertebrate neural crest. Genes Dev. 21: 391–396.

Keller, L. (Ed.), 1999. Levels of Selection in Evolution. Princeton University Press, Princeton, NJ.

Kerr, C.L. and M.J. Shamblott, and J.D. Gearhart. 2006. Pluripotent stem cells from germ cells. Methods Enzymol. 419: 400–426.

Khare, A. and G. Shaulsky. 2006. First among equals: competition between genetically identical cells. Nat. Rev. Genet. 7: 577–583.

Kim-Ha, J. and J.L. Smith, and P.M. Macdonald. 1991. *oskar* mRNA is localized to the posterior pole of the *Drosophila* oocyte. Cell 66: 23–35.

Kim-Ha, J. and K. Kerr, and P.M. Macdonald. 1995. Translational regulation of *oskar* mRNA by bruno, an ovarian RNA-binding protein, is essential. Cell 81: 403–412.

Kimura, T. and A. Suzuki, Y. Fujita, K. Yomogida, H. Lomeli, N. Asada, M. Ikeuchi, A. Nagy, T.W. Mak, and T. Nakano. 2003. Conditional loss of PTEN leads to testicular teratoma and enhances embryonic germ cell production. Development 130: 1691–1700.

King, N. and S.B. Carroll. 2001. A receptor tyrosine kinase from choanoflagellates: molecular insights into early animal evolution. Proc. Natl. Acad. Sci. USA 98: 15032–15037.

King, M.L. and T.J. Messitt, and K.L. Mowry. 2005. Putting RNAs in the right place at the right time: RNA localization in the frog oocyte. Biol. Cell 97: 19–33.

Klattenhoff, C. and D.P. Bratu, N. McGinnis-Schultz, B.S. Koppetsch, H.A. Cook, and W.E. Theurkauf. 2007. *Drosophila* rasiRNA pathway mutations disrupt embryonic axis specification through activation of an ATR/Chk2 DNA damage response. Developmental Cell 12: 45–55.

Klattenhoff, C. and W. Theurkauf. 2008. Biogenesis and germline functions of piRNAs. Development 135: 3–9.

Kleckowski, E.J. Mutation, apical meristems and developmental selection in plants. pp. 79–83. *In*: J.P. Gustafson. [eds.] 1986. Genetics, Molecules and Evolution. Plenum, New York.

Kleinsmith, L.J. and G.B. Pierce, Jr. 1964. Multipotentiality of single embryonal carcinoma cells. Cancer Res. 24: 1544–1551.

Knaut, H. and F. Pelegri, K. Bohmann, H. Schwarz, and C. Nusslein-Volhard. 2000. Zebrafish *vasa* RNA but not its protein is a component of the germ plasm and segregates asymmetrically before germline specification. J. Cell Biol. 149: 875–888.

Ko, M.S. and A. McLaren. 2006. Epigenetics of germ cells, stem cells, and early embryos. Dev. Cell. 10: 161–166.

Kurosaki, H. and Y. Kazuki, M. Hiratsuka, T. Inoue, Y. Matsui, C.C. Wang, M. Kanatsu-Shinohara, T. Shinohara, T. Toda, and M. Oshimura. 2007. A comparison study in the proteomic signatures of multipotent germline stem cells, embryonic stem cells, and germline stem cells. Biochem. Biophys. Res. Comm. 353: 259–267.

Lander, A. 2009. The 'stem cell' concept: is it holding us back? J. Biol. 8: 70.

Lantz, V.A. and S.E. Clemens, and K.G. Miller. 1999. The actin cytoskeleton is required for maintenance of posterior pole plasm components in the *Drosophila* embryo. Mech. Dev. 85: 111–122.

Lavial, F. and H. Acloque, F. Bertocchini, D.J. Macleod, S. Boast, E. Bachelard, G. Montillet, S. Thenot, H.M. Sang, C.D. Stern, J. Samarut, and B. Pain. 2007. The Oct4 homologue PouV and Nanog regulate pluripotency in chicken embryonic stem cells. Development 134: 3549–3563.

Lavial, F. and H. Acloque, E. Bachelard, M.A. Nieto, J. Samarut, and B. Pain. 2009. Ectopic expression of Cvh (Chicken Vasa homologue) mediates the reprogramming of chicken embryonic stem cells to a germ cell fate. Dev. Biol. 330: 73–82.

Lawson, K.A. and N.R. Dunn, B.A. Roelen, L.M. Zeinstra, A.M. Davis, C.V. Wright, J.P. Korving, and B.L. Hogan. 1999. *Bmp4* is required for the generation of primordial germ cells in the mouse embryo. Genes Dev. 13: 424–436.

Lehmann, R. and C. Nüsslein-Volhard. 1986. Abdominal segmentation, pole cell formation, and embryonic polarity require the localized activity of *oskar*, a maternal gene in *Drosophila*. Cell 47: 144–152.

Lehmann, R. and C. Nusslein-Volhard. 1991. The maternal gene *nanos* has a central role in posterior pattern formation of the *Drosophila* embryo. Development 112: 679–691.

Mahowald, A.P. 1962. Fine structure of pole cells and polar granules in *Drosophila melanogaster*. J. Exp. Zool. pp. 201–215.

Mahowald, A.P. 1968. Polar granules of *Drosophila*. II. Ultrastructural changes during early embryogenesis. J. Exp. Zool. 167: 237–261.

Mahowald, A.P. and K. Illmensee, and F.R. Turner. 1976. Interspecific transplantation of polar plasm between *Drosophila* embryos. J. Cell Biol. 70: 358–373.

Marlow, F.L. and M.C. Mullins. 2008. *Bucky ball* functions in Balbiani body assembly and animal-vegetal polarity in the oocyte and follicle cell layer in zebrafish. Dev. Biol. 321: 40–50.

Marques-Mari, A.I. and O. Lacham-Kaplan, J.V. Medrano, A. Pellicer, and C. Simón. 2009. Differentiation of germ cells and gametes from stem cells. Hum. Reprod. Update 15: 379–390.

Martin, G.R. and M.J. Evans. 1974. The morphology and growth of a pluripotent teratocarcinoma cell line and its derivatives in tissue culture. Cell 2: 163–172.

Martin, G.R. 1975. Teratocarcinomas as a model system for the study of embryogenesis and neoplasia. Cell 5: 229–243.

Martin, G.R. 1981. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc. Natl. Acad. Sci. USA 78: 7634–7638.

Matsui, Y. and D. Toksoz, S. Nishikawa, D. Williams, K. Zsebo, and B.L. Hogan. 1991. Effect of Steel factor and leukaemia inhibitory factor on murine primordial germ cells in culture. Nature 353: 750–752.

Matsui, Y. and K. Zsebo, and B.L. Hogan. 1992. Derivation of pluripotential embryonic stem cells from murine primordial germ cells in culture. Cell 70: 841–847.

McCosh, G.K. 1930. The origin of the germ cells in *Amblystoma maculatum*. J. Morphol. 50: 569–611.

Metschnikoff, E. 1866. Embryologische Studien an Insekten. Zeit. f. wiss. Zool. 16: 389–500.

Michod, R.E. and D. Roze. 2001. Cooperation and conflict in the evolution of multicellularity. Heredity 86: 1–7.

Michod, R. 2005. On the transfer of fitness from the cell to the multicellular organism. Biol. Philos. 20: 967–987.

Michod, R.E. 2007. Evolution of individuality during the transition from unicellular to multicellular life. Proc. Natl. Acad. Sci. USA 104 Suppl 1: 8613–8618.

Micklem, D.R. and J. Adams, S. Grünert, and D. St Johnston. 2000. Distinct roles of two conserved Staufen domains in *oskar* mRNA localization and translation. EMBO J. 19: 1366–1377.

Milkman, R. 1967. Genetics and developmental studies on *Botryllus schlosseri*. Biol. Bull. 132: 229–243.

Mintz, B. and K. Illmensee. 1975. Normal genetically mosaic mice produced from malignant teratocarcinoma cells. Proc. Natl. Acad. Sci. USA 72: 3585–3589.

Mitsui, K. and Y. Tokuzawa, H. Itoh, K. Segawa, M. Murakami, K. Takahashi, M. Maruyama, M. Maeda, and S. Yamanaka. 2003. The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. Cell 113: 631–642.

Miyata, T. and H. Hayashida, K. Kuma, K. Mitsuyasu, and T. Yasunaga. 1987. Male-driven molecular evolution: a model and nucleotide sequence analysis. Cold Spring Harb. Symp. Quant. Biol. 52: 863–867.

Mochizuki, K. and C. Nishimiya-Fujisawa, and T. Fujisawa. 2001. Universal occurrence of the *vasa*-related genes among metazoans and their germline expression in *Hydra*. Dev. Genes Evol. 211: 299–308.

Modrell, M. and A.L. Price, J. Havemann, C. Extavour, M. Gerberding, and N.H. Patel. submitted. Germline replacement following ablation of the primordial germ cells in *Parhyale hawaiensis*. Science. (submitted).

Morata, G. and P. Ripoll. 1975. Minutes: Mutants of *Drosophila* autonomously affecting cell division rate. Dev. Biol. pp. 211–221.

Moreno, E. and K. Basler, and G. Morata. 2002. Cells compete for *decapentaplegic* survival factor to prevent apoptosis in *Drosophila* wing development. Nature 416: 755–759.

Moreno, E. and K. Basler. 2004. dMyc transforms cells into super-competitors. Cell 117: 117–129.

Mukai, H. and H. Watanabe. 1976. Studies on the formation of germ cells in a compound ascidian, *Botryllus primigenus* Oka. J. Morphol. 148: 337–362.

Müller, W.E. 2006. The stem cell concept in sponges (Porifera): Metazoan traits. Sem. Cell Dev. Biol. 17: 481–491.

Newman, S.A. and R. Bhat. 2009. Dynamical patterning modules: a "pattern language" for development and evolution of multicellular form. Int. J. Dev. Biol. 53: 693–705.

Nichols, S.A. and W. Dirks, J.S. Pearse, and N. King. 2006. Early evolution of animal cell signaling and adhesion genes. Proc. Natl. Acad. Sci. USA 103: 12451–12456.

Nieuwkoop, P.D. 1947. Experimental observations on the origin and determination of the germ cells, and on the development of the lateral plates and germ ridges in the urodeles. Arch. Neerl. Zool. 8: 1–205.

Nieuwkoop, P.D. and L.A. Sutasurya. 1981. Primordial Germ Cells in the Invertebrates: From Epigenesis to Preformation. Cambridge University Press, Cambridge.
Niki, Y. and A.P. Mahowald. 2003. Ovarian cystocytes can repopulate the embryonic germ line and produce functional gametes. Proc. Natl. Acad. Sci. USA 100: 14042–14045.
Nishida, H. 1987. Cell lineage analysis in ascidian embryos by intracellular injection of a tracer enzyme. III. Up to the tissue restricted stage. Dev. Biol. 121: 526–541.
Niwa, H. 2007. How is pluripotency determined and maintained? Development 134: 635–646.
Noda, K. and C. Kanai. 1977. An ultrastructural observation of *Pelmatohydra robusta* at sexual and asexual stages, with a special reference to "germinal plasm". J. Ultrastruct. Res. 61: 284–294.
Noden, D.M. 1975. An analysis of migratory behavior of avian cephalic neural crest cells. Dev. Biol. 42: 106–130.
Nüsslein-Volhard, C. and H.G. Frohnhöfer, and Ruth Lehmann. 1987. Determination of anteroposterior polarity in *Drosophila*. Science 238: 1675–1681.
Odintsova, N.A. 2009. Stem cells of marine invertebrates: regulation of proliferation and differentiation processes in vitro. Tsitologiia 51: 367–372.
Oertel, M. and A. Menthena, M.D. Dabeva, and D.A. Shafritz. 2006. Cell competition leads to a high level of normal liver reconstitution by transplanted fetal liver stem/progenitor cells. Gastroenterology 130: 507–520.
Ohinata, Y. and H. Ohta, M. Shigeta, K. Yamanaka, T. Wakayama, and M. Saitou. 2009. A signaling principle for the specification of the germ cell lineage in mice. Cell 137: 571–584.
Okada, M. and I.A. Kleinman, and H.A. Schneiderman. 1974. Restoration of fertility in sterilized *Drosophila* eggs by transplantation of polar cytoplasm. Dev. Biol. 37: 43–54.
Okita, K. and T. Ichisaka, and S. Yamanaka. 2007. Generation of germline-competent induced pluripotent stem cells. Nature 448: 313–317.
Okita, K. and M. Nakagawa, H. Hyenjong, T. Ichisaka, and S. Yamanaka. 2008. Generation of mouse induced pluripotent stem cells without viral vectors. Science 322: 949–953.
Oliver, E.R. and T.L. Saunders, S.A. Tarlé, and T. Glaser. 2004. Ribosomal protein L24 defect in belly spot and tail (Bst), a mouse Minute. Development 131: 3907–3920.
Olsen, C.E. and R. Aasland, and A. Fjose. 1997. A *vasa*-like gene in zebrafish identifies putative primordial germ cells. Mech. Dev. 66: 95–105.
Ota, K.G. and S. Kuraku, and S. Kuratani. 2007. Hagfish embryology with reference to the evolution of the neural crest. Nature 446: 672–675.
Pan, L. and S. Chen, C. Weng, G. Call, D. Zhu, H. Tang, N. Zhang, and T. Xie. 2007. Stem cell aging is controlled both intrinsically and extrinsically in the *Drosophila* ovary. Cell Stem Cell 1: 458–469.
Pelegri, F. and H. Knaut, H.M. Maischein, S. Schulte-Merker, and C. Nüsslein-Volhard. 1999. A mutation in the zebrafish maternal-effect gene *nebel* affects furrow formation and *vasa* RNA localization. Curr. Biol. 9: 1431–1440.
Pires-daSilva, A. and R.J. Sommer. 2003. The evolution of signalling pathways in animal development. Nat. Rev. Genet. 4: 39–49.
Pokrywka, N.J. and E.C. Stephenson, 1995. Microtubules are a general component of mRNA localization systems in *Drosophila* oocytes. Dev. Biol. 167: 363–370.
Queller, D.C. and J.E. Strassmann. 2009. Beyond society: the evolution of organismality. Phil. Trans. Roy. Soc. London B. 364: 3143–3155.
Ramalho-Santos, M. and H. Willenbring. 2007. On the origin of the term "stem cell". Cell Stem Cell 1: 35–38.
Rangan, P. and M. DeGennaro, and R. Lehmann. 2008. Regulating gene expression in the *Drosophila* germ line. Cold Spring Harb. Symp. Quant. Biol. 73: 1–8.
Rangan, P. and M. DeGennaro, K. Jaime-Bustamante, R.-X. Coux, R.G. Martinho, and R. Lehmann. 2009. Temporal and spatial control of germ-plasm RNAs. Curr. Biol. 19: 72–77.

Raz, E. 2003. Primordial germ-cell development: the zebrafish perspective. Nat. Rev. Genet. 4: 690–700.

Reddien, P.W. and N.J. Oviedo, J.R. Jennings, J.C. Jenkin, and A. Sánchez Alvarado. 2005. SMEDWI-2 is a PIWI-like protein that regulates planarian stem cells. Science 310: 1327–1330.

Remane, A. 1952. Die Grundlagen des natürlichen Systems, der vergleichenden Anatomie und der Phylogenetik. Akademische Verlagsgesellschaft, Berlin.

Remsen, J. and P. O'Grady. 2002. Phylogeny of Drosophilinae (Diptera: Drosophilidae), with comments on combined analysis and character support. Mol. Phylogenet. Evol. 24: 249–264.

Resnick, J.L. and L.S. Bixler, L. Cheng, and P.J. Donovan. 1992. Long-term proliferation of mouse primordial germ cells in culture. Nature 359: 550–551.

Ressom, R.E. and K.E. Dixon. 1988. Relocation and reorganization of germ plasm in *Xenopus* embryos after fertilization. Development 103: 507–518.

Rhiner, C. and B. Díaz, M. Portela, J.F. Poyatos, I. Fernández-Ruiz, J.M. López-Gay, O. Gerlitz, and E. Moreno. 2009. Persistent competition among stem cells and their daughters in the *Drosophila* ovary germline niche. Development 136: 995–1006.

Robb, D.L. and J. Heasman, J. Raats, and C. Wylie. 1996. A Kinesin-like protein is required for germ plasm aggregation in *Xenopus*. Cell pp. 823–831.

Rohr, K.B. and D. Tautz, and K. Sander. 1999. Segmentation gene expression in the mothmidge *Clogmia albipunctata* (Diptera, Psychodidae) and other primitive dipterans. Dev. Genes Evol. 209: 145–154.

Rohwedel, J. and U. Sehlmeyer, J. Shan, A. Meister, and A.M. Wobus. 1996. Primordial germ cell-derived mouse embryonic germ (EG) cells in vitro resemble undifferentiated stem cells with respect to differentiation capacity and cell cycle distribution. Cell Biol. Int. 20: 579–587.

Rosner, A. and E. Moiseeva, Y. Rinkevich, Z. Lapidot, and B. Rinkevich. 2009. Vasa and the germ line lineage in a colonial urochordate. Dev. Biol. 331: 113–128.

Russo, C.A. and N. Takezaki, and M. Nei. 1995. Molecular phylogeny and divergence times of drosophilid species. Mol. Biol. Evol. 12: 391–404.

Sabbadin, A. and G. Zaniolo. 1979. Sexual differentiation and germ cell transfer in the colonial ascidian *Botryllus schlosseri*. J. Exp. Zool. 207: 289–304.

Sanchez Alvarado, A. and H. Kang. 2005. Multicellularity, stem cells, and the neoblasts of the planarian *Schmidtea mediterranea*. Exp. Cell Res. 306: 299–308.

Sandstedt, S.A. and P.K. Tucker. 2005. Male-driven evolution in closely related species of the mouse genus *Mus*. J. Mol. Evol. 61: 138–144.

Sauka-Spengler, T. and D. Meulemans, M. Jones, and M. Bronner-Fraser. 2007. Ancient evolutionary origin of the neural crest gene regulatory network. Dev. Cell 13: 405–420.

Schluter, D. 2000. The Ecology of Adaptive Radiation. Oxford University Press, Oxford.

Schultz, S.T. and D.G. Scofield. 2009. Mutation accumulation in real branches: fitness assays for genomic deleterious mutation rate and effect in large-statured plants. Am. Nat. 174: 163–175.

Shamblott, M.J. and J. Axelman, S. Wang, E.M. Bugg, J.W. Littlefield, P.J. Donovan, P.D. Blumenthal, G.R. Huggins, and J.D. Gearhart. 1998. Derivation of pluripotent stem cells from cultured human primordial germ cells. Proc. Natl. Acad. Sci. USA 95: 13726–13731.

Sharma, V.K. and C. Carles, and J.C. Fletcher. 2003. Maintenance of stem cell populations in plants. Proc. Natl. Acad. Sci. USA 100 Suppl 1: 11823–11829.

Shimmin, L.C. and B.H. Chang, and W.H. Li. 1993. Male-driven evolution of DNA sequences. Nature 362: 745–747.

Shostak, S. 2006. (Re)defining stem cells. BioEssays 28: 301–308.

Smith, J.L. and J.E. Wilson, and P.M. Macdonald. 1992. Overexpression of oskar directs ectopic activation of nanos and presumptive pole cell formation in *Drosophila* embryos. Cell 70: 849–859.

Sonnenblick, B.P. 1941. Germ cell movements and sex differentiation of the gonads in the *Drosophila* embryo. Proc. Natl. Acad. Sci. USA 27: 484–489.

Sperger, J.M. and X. Chen, J.S. Draper, J.E. Antosiewicz, C.H. Chon, S.B. Jones, J.D. Brooks, P.W. Andrews, P.O. Brown, and J.A. Thomson. 2003. Gene expression patterns in human embryonic stem cells and human pluripotent germ cell tumors. Proc. Natl. Acad. Sci. USA 100: 13350–13355.

Spicer, G.S. 1988. Molecular evolution among some *Drosophila* species groups as indicated by two-dimensional electrophoresis. J. Mol. Evol. 27: 250–260.

Spradling, A.C. and Y. Zheng. 2007. Developmental biology. The mother of all stem cells? Science 315: 469–470.

Stadtfeld, M. and M. Nagaya, J. Utikal, G. Weir, and K. Hochedlinger. 2008. Induced pluripotent stem cells generated without viral integration. Science 322: 945–949.

Stahl, Y. and R. Simon. 2009. Plant primary meristems: shared functions and regulatory mechanisms. Curr. Opin. Plant. Biol. 13: 53–58.

Stevens, L.C. and C.C. Little. 1954. Spontaneous testicular teratomas in an inbred strain of mice. Proc. Natl. Acad. Sci. USA 40: 1080–1087.

Stevens, L.C. 1967. Origin of testicular teratomas from primordial germ cells in mice. J. Natl. Cancer Inst. 38: 549–552.

Stewart, T.A. and B. Mintz. 1981. Successive generations of mice produced from an established culture line of euploid teratocarcinoma cells. Proc. Natl. Acad. Sci. USA 78: 6314–6318.

Stewart, T.A. and B. Mintz. 1982. Recurrent germ-line transmission of the teratocarcinoma genome from the METT-1 culture line to progeny in vivo. J. Exp. Zool. 224: 465–469.

Stoner, D.S. and I.L. Weissman. 1996. Somatic and germ cell parasitism in a colonial ascidian: possible role for a highly polymorphic allorecognition system. Proc. Natl. Acad. Sci. USA 93: 15254–15259.

Stoner, D.S. and B. Rinkevich, and I.L. Weissman. 1999. Heritable germ and somatic cell lineage competitions in chimeric colonial protochordates. Proc. Natl. Acad. Sci. USA 96: 9148–9153.

Strome, S. and W.B. Wood. 1982. Immunofluorescence visualization of germ-line-specific cytoplasmic granules in embryos, larvae, and adults of *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA 79: 1558–1562.

Strome, S. and R. Lehmann. 2007. Germ versus soma decisions: lessons from flies and worms. Science 316: 392–393.

Sun, Y. and H. Li, Y. Liu, S. Shin, M.P. Mattson, M.S. Rao, and M. Zhan. 2007. Cross-species transcriptional profiles establish a functional portrait of embryonic stem cells. Genomics 89: 22–35.

Sunanaga, T. and Y. Saito, and K. Kawamura. 2006. Postembryonic epigenesis of Vasa-positive germ cells from aggregated hemoblasts in the colonial ascidian, *Botryllus primigenus*. Dev. Growth and Differ. 48: 87–100.

Sunanaga, T. and A. Watanabe, and K. Kawamura. 2007. Involvement of *vasa* homolog in germline recruitment from coelomic stem cells in budding tunicates. Dev. Genes Evol. 217: 1–11.

Sunanaga, T. and M. Satoh, and K. Kawamura. 2008. The role of Nanos homologue in gametogenesis and blastogenesis with special reference to male germ cell formation in the colonial ascidian, *Botryllus primigenus*. Dev. Biol. 324: 31–40.

Sweasy, J.B. and J.M. Lauper, and K.A. Eckert. 2006. DNA polymerases and human diseases. Rad. Res. 166: 693–714.

Takahashi, K. and S. Yamanaka. 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126: 663–676.

Takahashi, K. and K. Tanabe, M. Ohnuki, M. Narita, T. Ichisaka, K. Tomoda, and S. Yamanaka. 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131: 861–872.

Takamura, K. and M. Fujimura, and Y. Yamaguchi. 2002. Primordial germ cells originate from the endodermal strand cells in the ascidian *Ciona intestinalis*. Dev. Genes Evol. 212: 11–18.

Tam, P.P.L. and M.H.L. Snow. 1981. Proliferation and migration of primordial germ cells during compensatory growth in mouse embryos. J. Emb. Exp. Morph. 64: 133–147.

Tam, P.P. and S.X. Zhou. 1996. The allocation of epiblast cells to ectodermal and germ-line lineages is influenced by the position of the cells in the gastrulating mouse embryo. Dev. Biol. 178: 124–132.

Tamura, K. and S. Subramanian, and S. Kumar. 2004. Temporal patterns of fruit fly (*Drosophila*) evolution revealed by mutation clocks. Mol. Biol. Evol. 21: 36–44.

Thomson, J.A. and J. Itskovitz-Eldor, S.S. Shapiro, M.A. Waknitz, J.J. Swiergiel, V.S. Marshall, and J.M. Jones. 1998. Embryonic stem cell lines derived from human blastocysts. Science 282: 1145–1147.

Toyooka, Y. and N. Tsunekawa, R. Akasu, and T. Noce. 2003. Embryonic stem cells can form germ cells in vitro. Proc. Natl. Acad. Sci. USA 100: 11457–11462.

Tsunekawa, N. and M. Naito, Y. Sakai, T. Nishida, and T. Noce. 2000. Isolation of chicken *vasa* homolog gene and tracing the origin of primordial germ cells. Development 127: 2741–2750.

Turner, F.R. and A.P. Mahowald. 1976. Scanning Electron Microscopy of *Drosophila* Embryogenesis. 1. The Structure of the Egg Envelopes and the Formation of the Cellular Blastoderm. Dev. Biol. pp. 95–108.

Urven, L.E. and T. Yabe, and F. Pelegri. 2006. A role for non-muscle myosin II function in furrow maturation in the early zebrafish embryo. J. Cell Sci. 119: 4342–4352.

van den Ent, F. and L.A. Amos, and J. Löwe. 2001. Prokaryotic origin of the actin cytoskeleton. Nature 413: 39–44.

Wada, H. 2001. Origin and evolution of the neural crest: a hypothetical reconstruction of its evolutionary history. Dev. Growth Differ. 43: 509–520.

Wallace, A.R. 1885. On the law which has regulated the introduction of new species. Ann. Mag. Nat. Hist. 16: 184–196.

Waterhouse, A.M. and J.B. Procter, D.M. Martin, M. Clamp, and G.J. Barton. 2009. Jalview Version 2—a multiple sequence alignment editor and analysis workbench. Bioinformatics 25: 1189–1191.

Webster, P.J. and J. Suen, and P.M. Macdonald. 1994. *Drosophila virilis oskar* transgenes direct body patterning but not pole cell formation or maintenance of mRNA localization in *D. melanogaster*. Development 120: 2027–2037.

Weismann, A. 1892. The Germ-Plasm: A Theory of Heredity. Walter Scott, Ltd., London.

Weissman, I.L. 2000. Stem cells: units of development, units of regeneration, and units in evolution. Cell 100: 157–168.

Whitham, T.G. and C.N. Slobodchikoff. 1981. Evolution by individuals, plant-herbivore interactions, and mosaics of genetic variability: the adaptive significance of somatic mutations in plants. Oecologia 49: 287–292.

Wilkinson, R.N. and C. Pouget, M. Gering, A.J. Russell, S.G. Davies, D. Kimelman, and R. Patient. 2009. Hedgehog and Bmp polarize hematopoietic stem cell emergence in the zebrafish dorsal aorta. Dev. Cell 16: 909–916.

Willensdorfer, M. 2008. Organism size promotes the evolution of specialized cells in multicellular digital organisms. J. Evol. Biol. 21: 104–110.

Wolpert, L. and R. Beddington, T. Jessell, P. Lawrence, E. Meyerowitz, and J. Smith. 2002. Principles of Development. Oxford University Press, Oxford.

Wolpert, L. and T. Jessell, P. Lawrence, E. Meyerowitz, E. Robertson, and J. Smith 2007. Principles of Development. Oxford University Press Ltd., Bath.

Yamanaka, Y. and A. Ralston, R.O. Stephenson, and J. Rossant. 2006. Cell and molecular regulation of the mouse blastocyst. Dev. Dyn. 235: 2301–2314.

Yamanaka, S. 2007. Strategies and new developments in the generation of patient-specific pluripotent stem cells. Cell Stem Cell 1: 39–49.

Ying, Y. and X.M. Liu, A. Marble, K.A. Lawson, and G.Q. Zhao. 2000. Requirement of *Bmp8b* for the generation of primordial germ cells in the mouse. Mole. Endocrinol. 14: 1053–1063.

Ying, Y. and G.Q. Zhao. 2001. Cooperation of endoderm-derived BMP2 and extraembryonic ectoderm-derived BMP4 in primordial germ cell generation in the mouse. Dev. Biol. 232: 484–492.

Yoon, C. and K. Kawakami, and N. Hopkins. 1997. Zebrafish *vasa* homologue RNA is localized to the cleavage planes of 2- and 4-cell-stage embryos and is expressed in the primordial germ cells. Development 124: 3157–3166.

Zamboni, L. and H. Merchant. 1973. The fine morphology of mouse primordial germ cells in extragonadal locations. Am. J. Anat. 137: 299–335.

Zhang, X.G. and X.G. Hou. 2004. Evidence for a single median fin-fold and tail in the Lower Cambrian vertebrate, *Haikouichthys ercaicunensis*. J. Evol. Biol. 17: 1162–1166.

Zhang, J. and L. Li. 2005. BMP signaling and stem cell regulation. Dev. Biol. 284: 1–11.

Zhou, Y. and M.L. King. 2004. Sending RNAs into the future: RNA localization and germ cell fate. IUBMB Life 56: 19–27.

Zhurov, V. and T. Terzin, and M. Grbic. 2004. Early blastomere determines embryo proliferation and caste fate in a polyembryonic wasp. Nature 432: 764–769.

Zimyanin, V.L. and K. Belaya, J. Pecreaux, M.J. Gilchrist, A. Clark, I. Davis, and D. St Johnston. 2008. *In vivo* imaging of *oskar* mRNA transport reveals the mechanism of posterior localization. Cell 134: 843–853.

Chapter 16

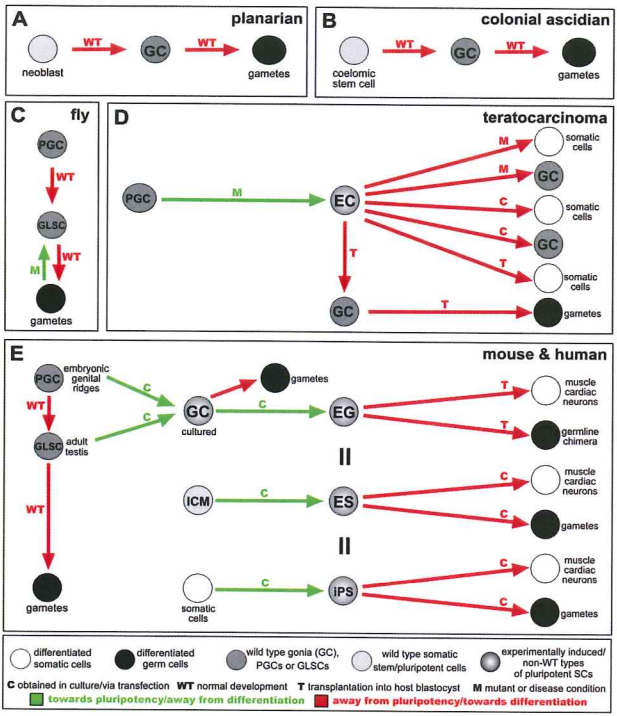

Fig. 1. Stem cell and germ cell relationships and transitions. (A) In platyhelminths, pluripotent stem cells called neoblasts can differentiate into gonia (GC), which generate gametes. **(B)** In colonial ascidians, pluripotent stem cells circulate in the haemolymph, and may differentiate into gonia (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 germ cell 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 gonia (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*.