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Chapter I

Molecular Signature and Sub-cellular Machinery of Metazoan Gametogenic Stem Cells

Andrey Shukalyuk[1,*] and Valeria Isaeva[2]

[1]Institute of Biomaterials and Biomedical Engineering,
University of Toronto, Toronto, Ontario, Canada
[2]A. V. Zhirmunsky Institute of Marine Biology of the Far Eastern Branch of the Russian Academy of Sciences, Vladivostok and A. N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Science, Moscow, Russia

Abstract

In organisms with exclusively sexual reproduction, primary germ cells become segregated during embryogenesis, and only germ cells are responsible for the reproduction. However, embryonic germline segregation is not universal in animal kingdom. In animals with asexual reproduction, the line of pluripotent stem cells is maintained continuously throughout the life of an individual or a colony, giving rise to germ cells and all the types (or a wide spectrum) of somatic cells and so providing a cell source for gametogenesis, asexual reproduction and regeneration. Examples of the gametogenic pluri/multipotent stem cells include sponge archaeocytes, cnidarian interstitial cells, planarian neoblasts, ascidian hemoblasts and stem cells of colonial rhizocephalans.

Pluripotent gametogenic stem cells share the evolutionarily conserved features of their morphological and functional organization with germline cells. Pluripotent cells as well as germline cells can be identified by the presence of granular/fibrillar material named nuage or germ granules as a key organelle and a cytoplasmic marker of gametogenic cells across the animal kingdom. Over the years we discovered and studied the colonial organization in *Peltogaster reticulatus* and *Thylacoplethus isaevae,* members of the Crustaceans (Rhizocephala). We also indentified vasa-like protein expression as a

* Corresponding author's email: andrey.shukalyuk@utoronto.ca.

cytoplasmic marker in the gametogenic stem cells of colonial *Polyascus polygenea* and *Peltogasterella gracilis*. It is believed that germ granules act as a cytoplasmic RNA depot of the germ line cells and are actively involved in RNA processing, silencing and degradation. Germ granules contain products of evolutionary conserved genes, including proteins, mRNAs, and noncoding RNAs. We also previously reviewed the macromolecular machinery and network, as well as the molecular signature, of the gametogenic cell across Metazoa, including a set of homologue proteins for Vasa, Piwi/Aubergine, Nanos, Tudor, Pumilio and others. In many species, ranging from sponges and cnidarians to flatworms, annelids, some crustaceans, and chordates, primordial germ cells and pluri/multipotent stem cells are characterized by the expression of a similar overlapping set of genes operating in both gametogenic stem cells types. This conservation led to the hypothesis that germline and pluripotent stem cells share a common gene regulatory program underlying pluripotency and gametogenic potentiality. These cells appear to actively repress differentiation programs and maintain developmental potency through transcriptional and mitotic quiescence. The evolutionary conserved gametogenic program involves *vasa/pl10*, *piwi/auberdine*, *nanos*, *tudor*, some other genes and signaling ways. Pluripotent gametogenic cells are also similar in their potential and their molecular signature to mammalian embryonic stem cells. Recent data indicate the broad and partially overlapping spectrum of gene expression in embryonic stem, germline, and pluri/multipotent stem cells, in particular, the possible inducibility of germline cells *de novo* without continuous expression of molecular markers of the germ line.

In the review, we consider some common principles in the sub-cellular and molecular machinery maintaining gametogenic potentiality in germ and gametogenic pluripotent cells in comparative and evolutionary aspects. We postulate evolutionary conserved molecular mechanisms underlying gametogenic potentiality and pluripotency and suppose that pluripotent stem cells, germline cells and embryonic stem cells share a basic molecular machinery as a core regulatory gene networks overlapping to a large extent, which operates in a similar manner across Metazoa, from sponges to chordates, and might be recruited by other tissue-specific networks ensuring self-preservation as a defense mechanism against apoptosis, cell differentiation and aging.

Introduction

Germ cells, which transmit genetic information from generation to generation, occupy a unique position in animal reproduction, development, and evolution (Goel et al., 2008; Ewen-Campen et al., 2010; Wu et al., 2011). In sexually reproducing animals, only germ cells are responsible for both reproduction of the individual and genetic continuity of the species (Extavour, 2008; Ewen-Campen et al., 2010). Germ cells are the only specialized cell type capable of generating a new whole organism in animals; the everlasting germline cycle continues from one generation to the next; thus the germline cells are potentially immortal (Cinalli et al., 2008).

August Weismann (1883) for the first time observed the emergence of germ cells from undifferentiated stem cells (*Stammzellen*), which retained the properties of embryonic cells as the precursors of primordial germ cells (*Ur-Keimzellen*) in the course of his detailed study of colonial hydroids. These results provided the basis for the theory of the germ plasm (*Keimplasma*), the immortal germ line transmitted from generation to generation with the continuity of the germline way (*Keimbahn*) (Weismann, 1892, 1893; see also Bosch, 2009;

Frank et al., 2009). Weismann (1883, 1893) did not associate the concept of a germ cell line with the early segregation of this line: he observed that germ cells in hydroids differentiate not during embryonic development, but much later, in generations formed by budding (*Knospen-generationen*: Weismann, 1883, p. 279). The idea of early germ cell segregation and of the continuity of these cells in the sequence of generations was first of all formulated by Nussbaum (1880), who believed that interstitial cells of *Hydra* maintain the germline way. It is known now that the interstitial cells of hydroids, stem cells continuously undergoing the mitotic cycle, produce both germ cells and some types of somatic cells (see Bode, 1996; Bosch, 2008; Frank et al., 2009). Thus, hydroids, with their late specification of germline cells, differentiating from the interstitial cells during the entire lifespan and producing also somatic derivates, paradoxically became the main object of the studies that resulted in the emergence of the idea of early segregation of the totipotent germ cell line from somatic cells (Nussbaum, 1880), as well as "germ plasm" theory (Weismann, 1883, 1892, 1893).

By now Weismann's term "germ plasm", which originally denoted the genetic material of the nucleus, is reconsidered and understood metaphorically: according to modern views, *germ plasm* is regarded as a cytoplasmic marker, a key organelle of germline cells structured in the form of germinal granules or more dispersed material, "*nuage*" (Matova, Cooley, 2001; Seydoux, Braun, 2006; Strome, Lehman, 2007). Weismann supposed that germ cells preserve all the factors of inheritance, whereas each somatic cell loses, in the course of differentiation, part of the germ plasm and of the initial potential of the egg (Weissman, 1892, 1893), and Weismann's concept has been criticized in the light of modern biological data (see, e.g., Extavour, 2008; Bosch, 2009; Frank et al., 2009).

Now it is known that in many animals, pluripotent stem cells give rise to both germ and somatic derivates and it is believed that specification of germline cells activates a molecular mechanism, preserving their potential during differentiation (see Isaeva et al., 2004, 2008, 2009; Shukalyuk et al., 2005, 2007; Extavour, 2008; Bosch, 2009; Frank et al., 2009; Ewen-Campen et al., 2010; Isaeva, 2011; Rebscher et al., 2012; Shukalyuk, Isaeva, 2012). In this review, we reveal some common principles in the sub-cellular and molecular machinery maintaining pluripotency and gametogenic potentiality in germ and gametogenic pluripotent cells. We suppose that evolutionary conserved molecular mechanisms underlie gametogenic potentiality and pluripotency in metazoan germline, embryonic stem and other pluripotent stem cells.

Germ Cell Segregation in Embryogenesis

In sexually reproducing metazoans, germline stem cells (GSCs) initiating from primordial germ cells (PGCs) give rise to a continual supply of germ cells and contribute genetically to subsequent generations, so their correct specification during embryonic development is critical for reproductive success and species survival (Extavour, 2008; Ewen-Campen et al., 2010; Juliano et al., 2010; Rebscher et al., 2012). In organisms with exclusively sexual reproduction, primary germ cells become segregated during embryogenesis. Two basic types of molecular mechanisms are responsible for germ-cell specification defined as preformation, or mosaic developmental mode (early specification of PGCs by means of asymmetric distribution of maternal cytoplasmic determinants) and

epigenesis, or regulative mode, i.e. later specification of germ lineage cells by inductive signals by the surrounding tissue (Extavour, Akam, 2003; Travis, 2007; Extavour, 2008; Frank et al., 2009; Gustafson, Wessel, 2010; Juliano et al., 2010; Rebscher et al., 2012). For example, in preformation, in both classical model organisms *Drosophila* and *Caenorhabditis elegans*, germ cells specified very early by maternal cytoplasmatic determinants, stored in the egg and allocated during cleavage to one or few daughter cells (Extavour, Akam, 2003; Frank et al., 2009). In epigenesis, specification of germline cells occurs later in the development, by inductive signals as in annelid *Platynereis* (Rebscher et al., 2007), in parasitic barnacles (Shukalyuk et al. 2005, 2007), and the mouse embryo (see Extavour, Akam, 2003; Extavour, 2008; Frank et al., 2009). Recently, Srouji and Extavour (2011) refer to former "preformation" as "inheritance mode", and former "epigenesis" as "inductive mode". Both in preformation and epigenesis, a population of primordial germ cells is established during embryogenesis by molecular signaling and induction of crucial germline-related molecular cascades. In both cases this molecular cascade is quite similar, with the only difference being that the initial signaling is provided by macromolecular complexes which were accumulated in the egg (preformation) or the same type of signaling was provided by surrounding tissue (epigenesis). The cascade of molecular changes stimulates PGCs to migrate into the developing gonad and become GSCs (Juliano et al., 2010).

Epigenesis is currently considered to constitute the ancestral mechanism, used by Urbilateria to specify the germ line, while preformation might have arisen in evolution several times independently and evolved convergently during the bilaterian radiation (Johnson et al., 2003; Extavour, 2008; Rebscher et al., 2012).

Following egg and sperm fusing, the resulting zygote is totipotent and able to give rise to all cell types of the animal. GSCs are unipotent cells normally producing only oocytes or spermatozoa (Juliano et al., 2010). However, germline cells potentially are multipotent or pluripotent, capable of differentiating into somatic lineages *in vitro* or *in vivo* and causing various germline based embryonic tumorogenesis. In fact, mouse PGCs create teratomas when injected into adult mice and chimeric organisms after injection into blastocysts; isolated human or mouse PGCs can be converted to pluripotent stem cells (see Juliano et al., 2010). The trans differentiation of germ cells into muscle, neurons and intestinal cells can be seen in the mutant *Caenorhabditis elegans* (Ciosk et al., 2006).

Somatic Embryogenesis (Blastogenesis) and Pluripotent Stem Cells

Embryonic germline segregation is not universal. In addition to the germline segregation by preformation and epigenesis (Extavour, 2008; Extavour, Akam, 2003; Gustafson, Wessel, 2010), the third mode, somatic embryogenesis was recognized in asexually reproducing animals, which have stem cells able to differentiate into germ and somatic cells (Buss, 1987, 1999; Blackstone, Jasker, 2003; Frank et al., 2009; Rinckevich et al., 2009; Rosner et al., 2009; Gustafson, Wessel, 2010). Pluri/multi/totipotent stem cells of these animals provide a cell source for gametogenesis, asexual reproduction and regeneration throughout the life of an individual or a colony (Isaeva et al., 2008, 2009; Frank et al., 2009; Sköld et al., 2009; Juliano et al., 2010; Isaeva, 2011; Rebscher et al., 2012; Shukalyuk, Isaeva, 2012).

The term *somatic embryogenesis* was advanced by B.P. Tokin (1959) along with his concept of the development of a whole organism from a group of somatic cells and now is used without any reference to this author. In organisms with alternating sexual and asexual (agamous) development, the organism developed from the zygote is naturally cloned, forming numerous genetically identical individuals or modular units of a colony, and clonal morphogenesis followed by the differentiation of gametes from stem cells is referred to as "somatic embryogenesis" (Blackstone, Jasker, 2003; Rinkevich et al., 2009; Sköld et al., 2009). However, morphogenesis in animal asexual reproduction does not completely recapitulate embryogenesis (Isaeva, 2010), and the pluripotent stem cells are similar rather to germline cells but not differentiated somatic cells (see below). Morphogenesis during asexual reproduction in animals was named blastogenesis (Berrill, 1961; Ivanova-Kazas, 1996). Thus, the term *somatic embryogenesis* is not completely correct, and the term *blastogenesis* (Berrill, 1961) seems preferable for the asexual reproduction in animal kingdom (Isaeva, 2010).

In animals with asexual reproduction, the germ lineage remains non-segregated even in the adult organism, the gametes of which differentiate from stem cells and the line of pluripotent stem cells is maintained continuously throughout the life of an individual or a colony, being predecessors of germ cells and a wide spectrum of somatic cells (Buss, 1987; Blackstone, Jasker, 2003; Isaeva et al., 2008; Rinkevich, 2009; Sköld et al., 2009; Isaeva, 2011; Shukalyuk, Isaeva, 2012). Stem cells having the potential to become both somatic and primary germ cells and morphologically indistinguishable from the latter were defined as "primary stem cells" (Sköld et al., 2009). These cells are stable undifferentiated cells that are set aside and persist beyond the completion of embryogenesis; germ cells are continually segregating from a self-renewing population of adult stem cells (Juliano et al., 2010). Such a reproductive strategy is used in sponges, cnidarians, colonial parasitic barnacles, urochordates and some other animals which have populations of adult multi-, pluri- or totipotent stem cells that continually give rise to both germ and somatic cells. Examples of such gametogenic pluripotent stem cells include sponge archaeocytes, cnidarian interstitial cells, planarian neoblasts, ascidian hemoblasts and stem cells of colonial rhizocephalans (reviews: Isaeva et al., 2008, 2009; Bosch, 2009; Frank et al., 2009; Rinkevich et al., 2009; Sköld et al., 2009; Funayama, 2010; Funayama et al., 2010; Juliano et al., 2010; Isaeva, 2011; Srouji, Extavour, 2011; Shukalyuk, Isaeva, 2012).

So, in many studied cnidarians, germ cells differentiate from a pool of multi/totipotent stem cells (Bosch, 2009; Frank et al., 2009). In Bryozoans, a modular colony develops from buds containing stem cells (Bayer et al. 1994); these buds arise from a primordium developing from a thickening in the ectodermal body wall, including also mesodermal cells (Nielsen, 1971). Ascidians exhibit various ways of budding from different tissue (see Sköld et al., 2009). Particularly, in *Botryllus schlosseri*, blood-borne stem cells are involved in budding and can invade other colonies where they differentiate into many different kinds of cells, including germ cells (Laird et al. 2005; Sköld et al., 2009). Alkaline phosphatase, a marker for vertebrate embryonic stem cells, is expressed in developing buds and in some but not all hematocytes, marking a pluripotent sub-population (Akhmadieva et al. 2007).

Among arthropods, many parasitic rhizocephalan crustaceans (Rhizocephala: Cirripedia: Crustacea) have asexual reproduction, somatic embryogenesis by budding without separation of blastozooids resulting in the emergence of colonial organization (Høeg, Lützen, 1995; Høeg et al., 2005). We have found undifferentiated stem cells taking part in the morphogenesis of the earliest buds and later migrating to the developing ovary as primary

germ cells of the colonial rhizocephalans *Peltogasterella gracilis* (Isaeva et al., 2003; Shukalyuk et al., 2005) and *Polyascus polygenea* (Isaeva et al., 2004; Shukalyuk et al., 2005, 2007).

The width of the potential spectrum of cellular differentiation varies among stem cells in different animal taxa and, accordingly, stem cells are defined as totipotent, pluripotent, multipotent, or oligopotent; this terminology is not unified and its usage is controversial (see Isaeva, 2010, 2011). In most invertebrates, pluripotency equals totipotency due to the lack of extra-embryonic tissues (Frank et al., 2009).

Pluripotency is defined as the capacity of a single cell to generate all cell lineages of the developing and adult organism. In mammals, this is a property of a transient population of unrestricted cells known as the epiblast (Silva et al., 2009; De Los Angeles et al., 2012). It was proposed that two distinct states of pluripotency can be defined in the mammalian embryo: naive (naïve) and primed. The naïve state is a ground one, a tabula rasa for embryogenesis, which is present only transiently in the preimplantation epiblast, which forms the embryo proper in vivo (Nichols, Smith, 2009; Han et al., 2011; De Los Angeles et al., 2012). Naïve pluripotent stem cell lines are distinguished from primed cells by self-renewal in response to LIF signaling and two active X chromosomes in female cells (De Los Angeles et al., 2012). The term "primitive cells" is also used to denote both normal embryonic and adult stem cells (Mac et al., 2011).

Thus, animals from various taxa segregate their germline after embryogenesis from pluri/multipotent stem cells, giving rise to both PGCs and somatic derivates (Juliano et al., 2010; Rebscher et al., 2012). In many animals, the self-renewing reserve of stem cells with broad or unlimited morphogenetic potential maintains continuously throughout the lifespan of an individual or a colony, being predecessors of germ cells and all the types (or a wide spectrum) of somatic cells. Pluripotent gametogenic stem cells are a cellular source in the realization of reproductive strategy including both sexual and asexual reproduction. These data support the proposal made by Buss and Green (1985) that asexual proliferation by budding (''ramet production'') requires the presence of an actively dividing multipotent cell line capable of differentiating into somatic as well as germ cells (Bosch, 2009).

Molecular Signature of Germline Cells

Many data suggest the evolutionary conservation of the morphological and functional organization of the germline cells in all Metazoa (see Extavour, Akam M. 2003; Seydoux, Braun, 2006; Extavour, 2008; Ewen-Campen et al., 2010). The morphology of germline cells of all Metazoa studied is characterized by a high nuclear–cytoplasmic ratio, a relatively round nucleus with diffuse chromatin, a distinct nucleolus, and a basophilic cytoplasm that includes the so-called germ plasm (Houston, King, 2000; Matova, Cooley, 2001; Extavour, Akam, 2003; Kloc et al., 2004; Frank et al., 2009; Ewen-Campen et al., 2010). These features are mainly "default" morphological characteristics of the undifferentiated state (Alié et al., 2011). The cells of the germ line can be identified and retraced during development of an organism owing to the availability of the germ plasm as cytoplasmic markers presented by granular or fibrillar material not surrounded by a membrane. It is believed that such an organelle is acting as a "RNA depot" in the cytoplasm and is actively involved in the RNA processing,

selectively regulating transcripts by secondary modification, silencing and degradation. This specific electron-dense material usually named "nuage" or "germ (germinal) granules" is a key organelle of germline cells considered as a germline hallmark across the animal kingdom (Brown et al, 2009; Eddy et al., 1975; Ikenishi, 1998; Lim, Kai, 2007; Mahowald, 2001; Matova, Cooley, 2001). Many other terms were introduced to designate these structures in different animals (see Mahowald, 1971, 2001; Beams, Kessel, 1974; Matova, Cooley, 2001; Kloc et al., 2004; Seydoux, Braun, 2006; Lim, Kai, 2007; Strome, Lehnam, 2007; Isaeva et al., 2008, 2009; Frank et al., 2009; Isaeva, 2010, 2011).

Germ plasm granules contain products of marker germline genes, including proteins, mRNAs, noncoding RNAs, recognized as the molecular signature of germline cells. Evolutionary conserved germ-cell-specific gene expression marks germline cells and distinguishes them from somatic cells in all studied metazoans (Ewen-Camden et al., 2010; Extavour, 2008; Leatherman, Jongens, 2003; Matova, Cooley, 2001; Seydoux, Braun, 2006; Sroji, Extavour, 2011). During early development, the translational control of either maternally loaded mRNAs or of previously transcribed zygotic mRNAs might be crucial for controlling PGCs fate (see Juliano et al., 2010). RNA-binding proteins in germinal granules are involved in mRNA localization, protection, and translation control. A feature of germ cells that makes their development distinct from the soma is the prominent role played by post-transcriptional controls of mRNA translation in the regulation of proliferation and differentiation (Haag, 2009). Pluripotency in the germ line is maintained by total suppression of mRNA transcription (Berekelya et al. 2005). Germ cells rely upon post-transcriptional control of mRNA regulation by translational repression via various RNP complexes (Seydoux, Braun, 2006), and this translational control is mediated by a number of widely conserved, often germ line-specific proteins (Haag, 2009). Germ cells in *Drosophila*, *C. elegans* and mouse undergo a period of global transcriptional quiescence after their specification (Nakamura, Seydoux, 2008).

The molecular machinery and molecular signature of germline cell specification includes a set of evolutionary conserved proteins such as Vasa, Piwi/Aubergine, Nanos, Tudor, Pumilio, Staufen and some others whose homologues have been identified in all metazoans studied (Extavour, Akam, 2003; Leatherman, Jongens, 2003; Kloc et al., 2004; Parvinen, 2005; Chuma et al., 2006; Lim, Kai, 2007; Extavour, 2008; Gustafson, Wessel, 2010; Flemr et al., 2010; Pepling, 2010; Juliano et al., 2010; Srouji, Extavour, 2011). These proteins are found in the germplasm or nuage at different stages and they determine germ cell fate. Remarkably, their genes are evolutionary conservative in all studied metazoans (Ikenishi, 1998; Matova, Cooley, 2001; Mochizuki et al., 2001; Seydoux, Braun, 2006; Srouji, Extavour, 2011). The set of genes, including *vasa*, *nanos* and *piwi*, appears to specify and maintain PGCs; a high percentage of germline-expressed genes appears to be RNA-binding proteins, indicating the crucial role of post-transcriptional control in this cell type. These cells appear to actively repress differentiation programs and maintain developmental potency through transcriptional and mitotic quiescence (Juliano et al., 2010). Products of the *vasa*- and *piwi*-related genes are the most widely used molecular germline markers for Metazoa (Extavour, Akam, 2003; Ewen-Camden et al., 2010; Gustafson, Wessel, 2010; Alié et al., 2011). Genes related to *vasa (vas)* and other genes of the DEAD family (see Raz, 2000; Extavour, Akam 2003; Sunanaga et al. 2006; Ewen-Campen et al., 2010; Shukalyuk, Isaeva, 2012) and to *piwi/argonaute* family (see Extavour, 2007; Funayama al., 2010; Ewen-Campen et al., 2010) were found in a diverse range of eukaryotes from yeast to plants and animals –

molecular and functional similarities of these genes were found across the kingdoms (see Mochizuki et al., 2001; Watanabe et al. 2009; Funayama al., 2010).

The Vasa protein is a major conserved molecular germ cell marker for the metazoan germ line and the most widely used one (see Castrillon et al., 2000; Raz, 2000; Extavour, Akam 2003; Sunanaga et al. 2006; Sköld et al., 2009; Ewen-Campen et al., 2010; Shukalyuk, Isaeva, 2012). Vasa proteins are ATP-dependent RNA helicases belonging to the DEAD box protein family, which are generally involved in RNA metabolism and can mediate both RNA–RNA and RNA–protein interactions (Raz, 2000; Rocak, Linder, 2004). It is likely that Vasa regulates a broader suite of transcripts. The vasa (vas) locus was first identified in Drosophila; Vasa protein localizes to cytoplasmic granules within pole plasm, and localization of the mRNA, protein, or both to germ plasm and germ cells at some stage of development is a universal characteristic of the *vasa* gene family (see Hay et al., 1988; Toyooka et al., 2000; Ewen-Campen et al., 2010).

The evolutionarily conserved PIWI/Argonaute family of proteins is involved in the specification and/or maintenance of germ cells (see Extavour, 2007; Ewen-Campen et al., 2010; Gustafson, Wessel, 2010). Argonaute proteins form two clades. The PIWI clade includes, in particular, Mili, Miwi1, and Miwi2 in mice, which are required for retrotransposon silencing in the germline and normal progression through prophase I in male meiosis. The second clade is comprised of the AGO proteins, of which there are four in mammals (see Modzelewski et al., 2012). Silencing of transposable elements in the germ line is the well-established role of PIWI-family proteins; this function is mediated through the interaction of PIWI-family proteins and additional factors with a special class of small RNAs, piRNA, that provide sequence specificity to a transcript-silencing complex (see Ewen-Campen et al., 2010; Huang et al., 2011; Watanabe et al., 2011; Modzelewski et al., 2012). Piwi/Argonaute family members serve as epigenetic regulators of stem cells in many systems. Piwi/Ago proteins are an animal germline-specific subclass, highly conserved across eukaryotes, specifically expressed in germ cells and playing a key role in germ cell maintenance and self-renewal, transposon silencing, and RNA silencing. These proteins are at the core of the RNA-silencing machinery that uses small RNA molecules as guides to identify homologous sequences in RNA or DNA. The small RNAs regulate genes at the transcriptional or post transcriptional level affecting either the chromatin structure or mRNA stability and mediating transcriptional gene silencing in germline maintenance (see Gustafson, Wessel, 2010; Peters, Meister, 2007; Thomson, Lin, 2009; Watanabe et al. 2009, 2011; Sroji, Extavour, 2011; Modzelewski et al., 2012).

Piwi-interacting RNAs (piRNAs) are small RNAs of mostly 24–30 nucleotides in length that are expressed in germ cells of animals and bound to the PIWI proteins, which represent a subfamily of the Argonaute protein family (Watanabe et al., 2011). The PIWI-piRNA complex, a germline-specific defense mechanism, represses retrotransposons in germ cells and is generated via multiprotein complexes with distinct subcellular localization (Aravin et al., 2009). One complex localizes to one type of an electron-dense structure known as nuage and transiently associated with aggregated mitochondria in gametogenesis shortly before and during meiosis; the other complex is nearby germinal cytoplasmic granules (see Huang et al., 2011). It was shown recently that piRNA-associated germline nuage (intermitochondrial cement) formation during mammalian spermatogenesis requires lipid signaling on the mitochondrial surface, facilitated by mitochondrial phospholipase D (MitoPLD). The Drosophila MitoPLD homolog Zucchini (Zuc) is required for piRNA generation; Zuc

localizes to mitochondria and has MitoPLD-like activity (see Huang et al., 2011; Watanabe et al, 2011). So mitochondrial-surface lipid signaling generated by MitoPLD/Zuc recruits or activates nuage components critical for piRNA production and spermatogenesis; the data suggest a link between mitochondrial dynamics and the germline ultrastructure necessary for germline development (Huang et al., 2011). Thus, the evidence of the relationship between piRNA localization in nuage/germinal granules material and mitochondrial involvement in germline cell specification is found. We supposed earlier the participation of mitochondria in the biogenesis of the germinal granules, nuage or other germplasm-related perinuclear bodies considered as a specific cytoplasmic regulatory center, maintaining the cell pluripotency and protecting germline cells from somatic fate (Isaeva et al., 2005, 2011; Isaeva, 2011; Shukalyuk, Isaeva, 2012),

Nanos, which contains two CCHC zinc fingers, is a translational repressor that acts with *pumilio* (see Extavour, 2007; Ewen-Campen et al., 2010). Orthologs of Nanos localize to germ cells of nearly all animal taxa studied; the phylogenetically widespread expression of these proteins in germ cells suggests that a germ line function of Nanos may have evolved very early in animals (see Extavour, 2007; Ewen-Campen et al., 2010). *Drosophila* germ cells that lack *nanos* undergo apoptosis, or if apoptosis is repressed, the germ cells are incorporated into somatic tissues (Hayashi et al., 2004). This highlights the importance of *nanos* and of mitotic quiescence in retaining the germ cell fate (Ewen-Campen et al., 2010). During *Drosophila* germ cell development, *nanos* acts as a translational repressor of *cyclin b*, to prevent premature divisions (Asaoka-Taguchi et al., 1999). Cell cycle lengthening is a conserved feature of newly specified PGCs and is exhibited by the sea urchin small micromeres (Tanaka, Dan, 1990). The nuclei of the *Drosophila* pole cells exhibit a prolonged cell cycle (Su et al., 1998). This requires *nanos*-dependent translational repression of the mitotic cyclin, *cyclin B*, which is present in the germ plasm but remains untranslated until just before mitotic divisions resume in the pole cells (Kadyrova et al., 2007; see Juliano et al., 2010). In *Xenopus laevis*, *nanos* and *pumilio* regulate *cyclin B1* translation during oocyte maturation, suggesting that the role of *nanos* as a repressor of *cyclin b* mRNA is conserved (Nakahata et al., 2003). Pumilio, which has orthologs in organisms as diverse as yeast and plants, has been shown to physically interact with Nanos proteins in flies, nematodes, frogs, and humans, implying that this interaction is ancestral in bilaterians (see Ewen-Campen et al., 2010; Juliano et al., 2010). Like *vasa*, *nanos* and *pumilio* were first discovered in Drosophila, where all three genes play essential roles in abdominal patterning and germ cell survival (Nüsslein-Volhard et al., 1987; Kobayashi et al., 1996).

Proteins containing the Tudor domains were found in organisms ranging from yeast to humans; Tudor mutants do not maintain expression of germ granule components Oskar and Vasa; they form abnormal germ granules; and they and fail to produce pole cells. Products of *vasa*, *nanos*, *pumilio*, *piwi*, *tudor* are components of germplasm (germ granules or nuage components); these genes are necessary but not sufficient for germ cell specification and function (see Ewen-Campen et al., 2010).

Although molecular organization of germ plasm is evolutionarily conserved among studied representatives of the animal kingdom some molecular components necessary for germ cell specification evidently do not have universal distribution. For example, the gene *oskar* is restricted to the Diptera (Drosophila), while *bucky ball* found in zebrafish is restricted to vertebrates (Bontems et al., 2009; see Ewen-Campen et al., 2010).

In mammals, the core genes *Oct4*, *Sox2*, and *Nanog* regulate pluripotency and perform an important role in regulating germ cell development (see Akamatsu et al., 2009; Nichols, Smith, 2009; Western et al., 2010). Oct3/4, a POU-family transcription factor, is expressed in the ovum prior to fertilization, and zygotic transcription from the late 2-cell stage results in distribution to all cells during cleavage. In the late blastocyst, Oct4 protein can be detected in cells of the epiblast. Expression of Oct3/4 becomes restricted to the germ cell lineage after gastrulation (see Akamatsu et al., 2009; Nichols, Smith, 2009). Nanog is a transcription factor, homeodomain-containing protein, a core element of the transcription network and regulatory circuits underlying pluripotency and reprogramming. It is essential for early embryonic development, a hallmark of pluripotent cells *in vivo* and *in vitro*, and the developing germline cells of mammals and birds (see Chambers et al., 2003, 2007; Do, Schöler, 2009; Silva et al., 2009). Nanog safeguards pluripotency and self-renewal of germline cells, mediates germline development and prevents progression to commitment (Chambers et al., 2007). As the blastocyst develops, Nanog becomes confined to the ICM and then re-expressed in the epiblast (Chambers et al., 2003; Nichols, Smith, 2009). Nanog is not expressed in mammalian somatic stem cells, and loss of Nanog is an early marker of differentiation; however, expression persists in the primordial germ cells (see Chambers et al., 2003, 2007; Do, Schöler, 2009; Nichols, Smith, 2009). Nanog mediates acquisition of both embryonic and induced pluripotency; it is proposed that Nanog has a unique function in creating the ground state and a secondary role in stabilizing self-renewal during diapause and in ESCs (Silva et al., 2009). It is proposed that regulation of pluripotency in mammalian fetal germ cells involves transcriptional repression and methylation of functional promoter elements that regulate *Nanog* and *Sox2* expression as well as post-transcriptional suppression of *Oct4* (Western et al., 2010). The methylation is presumably erased and the genes reactivated during the rapid paternal demethylation that occurs at fertilization and during early embryonic development (see Western et al., 2010).

Germline cells are relatively transcriptionally and mitotically quiescent during embryogenesis, until the time that gametogenesis begins (Extavour, 2008). The transfer of most of the control of gene expression to the cytoplasmic germ plasm is an important evolutionary conservative acquisition, ensuring plasticity for the germ cell genome (Seydoux, Braun, 2006).

Aside from transcriptional silencing, in some metazoan animals chromatin diminution in somatic cells functions as a mechanism of germline gene regulation that specifically removes a large number of genes involved in gametogenesis and early embryogenesis (see Wang et al., 2012). Soma-specific elimination provides a unique mechanism of gene repression, reminiscent of Weismann's theory of the differentiation between germline and soma (Weissmann, 1893). Chromatin diminution is the programmed elimination of specific DNA sequences during development. Particularly, chromatin diminution in the nematode *Ascaris suum* occurs during early cleavages and leads to the loss of germline genome sequences resulting in the formation of a distinct genome in somatic cells, in which at least 685 genes are eliminated (Wang et al., 2012).

Molecular Signature of Pluripotent Stem Cells

Weismann (1883) wrote about the indistinguishability of gametogentic cells and the undifferentiated cells that retain the "germ plasm"; Rinkevich et al. (2009) also pointed the unclearness of the boundaries between the two types of stem cells in those cases when the germ line is segregated late in ontogeny or is not segregated at all. For example, germ cells that arise from the interstitial cells of hydroids are initially distinguishable from them only by a somewhat larger size (Thomas, Edvards, 1991). The fundamental identity, indistinguishability, and likely evolutionary and ontogenetic relationship between primary germ cells and totipotent (pluripotent) stem cells have been reported (Weissman, 2000; Extavour, Akam, 2003; Travis, 2007; Extavour, 2008; Shukalyuk, Isaeva, 2012). Stem cells of asexually reproducing animals termed "primary stem cells" can serve as the predecessors of primordial germ cells; primary stem cells may be immortal (Sköld rt al., 2009). It was supposed that primordial germ cells and pluri/totipotent stem cells are evolutionarily and ontogenetically related (Weissman, 2000; Extavour, Akam, 2003; Travis, 2007). Pluripotent stem cells as cell source for both blastogenesis and gametogenesis share the evolutionarily conserved features of their morphological and functional organization with germline cells. We believe that ontogenetically and evolutionarily related primary stem and primordial germ cells potentially are able to accomplish a full program of development including gametogenesis and blastogenesis in asexually reproducing animals (Isaeva, 2010, 2011; Shukalyuk, Isaeva, 2012).

On the morphological and gene expression levels germ and stem cells are very similar (Extavour, 2008). Morphological features of germ cells was also shown to be displayed by the potentially gametogenic stem cells of asexually reproducing invertebrates (Isaeva et al., 2008; Frank et al., 2009; Isaeva, 2011; Shukalyuk, Isaeva, 2012; Rinkevich et al., 2009; Sköld et al., 2009). Pluripotent cells often display all of the morphological features commonly used to identify germ cells, creating an inability to distinguish between germ cells and other types of stem cells (Extavour, 2008; Shukalyuk, Isaeva, 2012). The electron-dense nuage material invariably found in germ cells has also been found in stem cell lineages (see Eddy, 1975; Extavour, 2008; Isaeva et al., 2008, 2011; Shukalyuk, Isaeva, 2012). Germ granules or nuage in stem cells of asexually reproducing invertebrates have been observed in archaeocytes of the sponge (Isaeva, Akhmadieva, 2011), in the interstitial cells of the hydra *Pelmatohydra robusta* (Noda, Kanai, 1977) and colonial hydroids *Ectopleura crocea* and *Obelia longissima* (Isaeva et al., 2011), in the planarian neoblasts (Coward, 1974; Hori, 1982; Hori, Kishida, 2003; Isaeva et al., 2005) and the stem cells of colonial rhizocephalan crustaceans (Shukalyuk et al., 2005; Shukalyuk, Isaeva, 2012).

While the germ line and somatic organelles are not identical, there is some overlap in the proteins and RNAs that they contain which suggest the functional analogies between RNA processing organelles in germline cells, pluripotent stem cells and P granules in somatic cells (Ewen-Campen et al., 2010; Shukalyuk, Isaeva, 2012).

The stem cells of asexually reproducing animals, like all stem cells, are characterized by the ability to self-renew and are identified by markers of cellular reproduction, such as a positive reaction to PCNA, telomerase activity, and incorporation of bromodeoxyuridine (see Rinkevich et al., 2009; Sköld et al., 2009; Isaeva, 2011; Shukalyuk, Isaeva, 2012). The

capacity of self-renewal in stem cells is partly reflected in that they may, in contrast to differentiated cells, be immortal due to long telomeres and sustained telomerase activity, a system highly conserved in the animal kingdom (see Sköld al., 2009). Pluripotent stem cells, such as choanocytes in sponges, interstitial cells in cnidarians, neoblasts in planarians, or epiblast cells in mice cells give rise to both PGCs and somatic derivates (Shibata et al., 1999; Mochizuki et al., 2000; Ohinata et al., 2009; Funayama, 2010; Funayama et al., 2010; Rebscher, 2012).

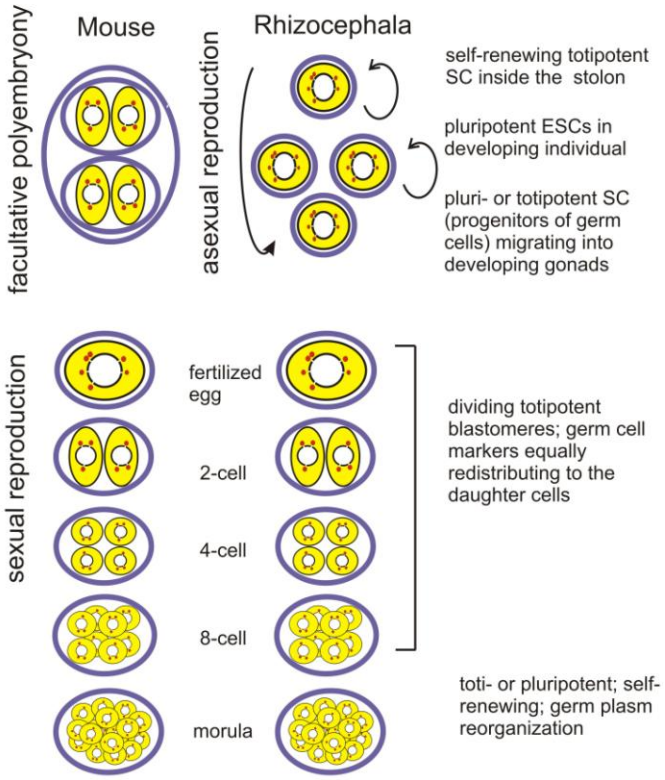

Figure 1. Unique similarity of distribution of germline related material in: (i) the asexually reproducing Rhizocephalans and facultative polyembryony in mouse, and (ii) the sexual reproduction of mammalian (mouse) and colonial arthropods (Rhizocephala). All cells contain germ granules near the nucleus in cytoplasm (red) and germ cell markers are distributed across the cell cytoplasm (yellow).

A high level of alkaline phosphatase activity serves as a classic marker of embryonic stem cells and primary germ cells of mammals and other vertebrates. A method for the identification of mammalian embryonic stem cells was successfully applied to reveal the stem cells of invertebrates; the high activity of alkaline phosphatase proved to be a cytochemical marker of invertebrate stem cells (Isaeva et al., 2003; Laird et al., 2005; Shukalyuk et al., 2005; Akhmadieva et al., 2007; Rinkevich et al., 2009; Sköld et al., 2009; Isaeva et al., 2011; Shukalyuk, Isaeva, 2012). Pluripotent stem cells in various invertebrates with asexual reproduction as well as cells of the germ lineage display the expression of conserved genes related to *vasa*, *piwi* and others which function in the specification and maintenance of both cell types across different metazoan phyla (Agata et al., 2006; Gustafson, Wessel, 2010; Juliano, Wessel, 2010; Rinkevich et al., 2009; Sköld et al., 2009; Juliano et al., 2010; Srouji,

Extavour, 2011; Wu et al., 2011). Besides the default characteristics of undifferentiated cells, these stem cells contain electron-dense perinuclear germ granules and express germline marker genes demonstrating that pluripotent stem cells display all of the morphological and functional features commonly used to identify germ cells.

Molecular signature of pluripotent stem cells includes the same set of genes, which specify and maintain PGCs. In many species, ranging from sponges, and cnidarians, to flatworms, annelids, some crustaceans, sea urchins, and tunicates, primordial germ cells (PGCs) and pluri/multipotent stem cells are characterized by the expression of a similar overlapping set of genes, including *vasa*, *nanos*, *piwi*, and *pl10* operating in both gametogenic stem cells types (Shibata et al., 1999; Mochizuki et al., 2000, 2001; Rebscher et al., 2007, 2012; Isaeva et al., 2008; Voronina et al., 2008; Frank et al., 2009; Gustafson, Wessel, 2009; Rinkevich et al., 2009; Sköld et al., 2009; Juliano et al., 2010; Alie et al., 2011; Isaeva, 2011; Shukalyuk, Isaeva, 2012). This conservation implies the existence of an underlying germline multipotency program in these cell types that has conserved function in maintaining multi/pluripotency and led to the hypothesis that PGCs and stem cells are not only closely related, but also share a common gene regulatory module, the "germline multipotency program" (Ewen-Campen et al., 2010; Juliano et al., 2010). The transcriptional and mitotic quiescence in both germ cells and pluripotent stem cells may serve to protect their DNA. In addition, *piwi*, another gene found in these cell types, contributes to protecting germ cell DNA, in conjunction with a class of small RNAs called piRNAs, by repressing transposons in these immortal cell types (O'Donnell, Boeke, 2007). Recent evidence in mice also indicates a role for *vasa* in transposon suppression by participating in piRNA biogenesis (Kuramochi-Miyagawa et al., 2010). The role of the germline multipotency program may be to protect the DNA, which will contribute to future generations (Juliano et al., 2010).

In the sponge *Ephydatia fluviatilis*, two *piwi* orthologs are specifically expressed in totipotent stem cells, archeocytes and choancytes (Funayama et al., 2010). Cnidarian stem cells selectively express *vasa*, *nanos*, *piwi (*Denker et al., 2008; Rebscher et al., 2008; Bosch, 2009; see also Juliano et al., 2010). Besides, Wnt and Notch pathways are involved in the control of stem cell behavior in Hydra, similar key signaling pathways appear to orchestrate stem cell behavior throughout the animal kingdom from Hydra to man (Bosch, 2009).

It is known an extraordinary ability to regenerate in flatworms owing to a population of pluripotent adult stem cells called neoblasts that gives rise to somatic cells and germ cells. The neoblasts in flatworms express homologues of genes *vasa*, *piwi*, *tudor*, *pumilio* and *bruno* (Shibata et al., 1999; Reddien et al., 2005; Salvetti et al., 2005; Guo et al., 2006; Pfister et al., 2008; De Mulder et al., 2009a, b; Solana et al., 2009; Rouhana et al., 2010; Martín-Duran et al., 2012).

In the oligochaete *Enchytraeus japonensis* (Annelida) reproducing both sexually and asexually expression of two *vasa*-related genes is revealed; both genes were expressed in germline stem cells and germ cells in gonads, while expression of only one gene was detected in neoblasts (Yoshida-Noro, Tochinai, 2010; Sugio et al., 2008). In *E. japonensis*, Ej-piwi is expressed in the developing gonads; during the asexual phase Ej-piwi is expressed in cells distributed throughout the body (Takodoro et al., 2002). Thus, pluripotent stem cells of various asexually reproducing animals share *vasa*, *piwi*, and some other germline marker gene expression.

In echinoderms and chordates, asexual reproduction and regeneration often involve more than one cell type that dedifferentiates into a more uncommitted state (Sköld et al., 2009). The

solution of cell dedifferentiation problem in asexual reproduction and regeneration requires special markers. The high plasticity of the development and fate of cells in colonial animals appears sufficiently justified (Frank et al., 2009; Rinkevich et al., 2009; Sköld et al., 2009).

For example, mature medusa of the hydrozoan *Turritopsis nutricula* is able to transform into a polyp through dedifferentiation of fully differentiated cells (Piraino et al., 1996).

Expression of Germline Marker Genes Beyond Gametogenic Stem Cells

During embryonic development, clusters of specific genes usually express in a specific sequence. However, transient ectopic "germline" gene expression is possible. Many germ plasm components are expressed and required not only in primordial germ cells but also during gametogenesis (see Extavour et al., 2005; Extavour, 2008).

Expression of germline marker genes beyond gametogenic stem cells is observed during cell differentiation in *Hydra magnipapillata*: *pl10* mRNA is expressed not only in undifferentiated stem cells (multipotent interstitial stem cells and germline cells) but also in differentiating somatic cells of the interstitial cell lineage and the ectodermal epithelial cells in the body column; however, none of the *vasa/PL10* genes were expressed in fully differentiated somatic cells in *Hydra* (Mochizuki et al., 2001). In the sea anemone *Nematostella vectensis,* transcripts of *vasa*- and *nanos*-related genes are present not only in presumptive germline cells but also in many somatic domains in the embryo, in the larva, these transcripts accumulate in two patches of cells (Extavour et al., 2005). Authors suggest that these cells are PGCs because gametes are found in the mesenteries of the adult; however, the possibility remains that these cells are precursors to multipotent stem cells that give rise to both somatic and germ cells in the adult. Analyses of the *piwi*-related gene during embryogenesis and medusa formation in the hydrozoan *Podocoryne carnea* have shown this gene expression in somatic stem cells as well as the germ line cells (Seipel et al., 2004). The jellyfish *Podocoryne carnea* exhibits a low level of *piwi* expression in all somatic cells, and this expression increases upon transdifferentiation, thus implicating *piwi* function in reprogramming (Seipel et al., 2004). Derivatives of the interstitial cell lineage exhibit a remarkable plasticity in terms of their differentiation capacity, and that, beside stem cell-based mechanisms, transdifferentiation is involved in normal development and maintenance of cell type complexity in hydroids (Bosch, 2009).

During embryogenesis of the planaria *Schmidtea polychroa,* Tudor-related protein is expressed in differentiating cells rather than neoblasts (Solana et al., 2009).

In the larvae of polychaete annelid *Platynereis dumerilii*, *piwi*-, *vasa*-, *PL10*- and *nanos*-related genes are expressed altogether at the mesodermal posterior growth zone in highly proliferative stem cells providing the somatic mesoderm and the germ line; *vasa*-like gene expression was revealed in the germ line as well as in multiple somatic tissues, including the mesodermal bands, brain, foregut, and posterior growth zone (Rebscher et al., 2007). Finally, *vasa*, *nanos* and *piwi* expression is retained in the newly specified germ cells, and lost in other differentiated tissues (Rebscher et al., 2007). However, while PGCs and the cells of the mesodermal posterior growth zone in *P. dumerilii* are indistinguishable in morphology and both express the germline markers *vasa*, *nanos*, and *piwi*, a distinct cluster of PGCs is

detectable forming independently from the stem cells of the mesodermal growth zone prior to gastrulation (Rebscher et al., 2012). In the developing polychaete *Capitella sp*. (*Capitella teleta*) during larval, and juveniles stages, *vasa* and *nanos* orthologues are expressed transiently in multiple somatic tissues as well as in germline; expression of these genes revealed overlapping but not identical patterns; expression is observed also in the presumptive brain, mesodermal bands, and developing foregut (Dill, Seaver, 2008). Giani and co-workers (2011) shown that in *Capitella teleta piwi* orthologs *Ct-piwi1* and *Ct-piwi2* are expressed throughout the life cycle in a dynamic pattern that includes both somatic and germline cells during embryonic and larval development, gradually becoming restricted to putative primordial germ cells (PGCs) and the posterior growth zone. In juveniles, *Ct-piwi1* is expressed in the presumptive gonads, and in reproductive adults, it is detected in gonads and the posterior growth zone. *Ct-piwi1* is expressed in regenerating tissue, and once segments differentiate, it becomes most prominent in the posterior growth zone and immature oocytes in regenerating ovaries (Giani et al., 2011). So, in *C. teleta*, *vasa*, *nanos* and *piwi* orthologs are all expressed in very similar patterns to one another in both the germline and posterior growth zone, likely in multipotent stem cells (Giani et al., 2011). In both *C. teleta* and the *P. dumerilii*, *piwi* is detected in PGCs and the posterior growth zone in larval and juvenile stages (Rebscher et al., 2007; Giani et al., 2011). During embryonic development of the oligochaete annelid *Tubifex tubifex*, transient *vasa* homologue expression was observed in cells in nongenital segments, and gradually becomes restricted (Oyama, Shimizu, 2007). Possibly, *T. tubifex* generates supernumerary presumptive PGCs during embryogenesis whose number is variable among embryos (Oyama, Shimizu, 2007).

These observations indicate the presence of a compensatory mechanism to produce PGCs from somatic stem cells in the absence of the original germ cells (Giani et al., 2011). Although there are clear species-specific differences in reproductive anatomy and morphogenesis, it appears that in annelids there is conservation of piwi expression in the primordial germ cells, developing gametes, and posterior growth zone (Giani et al., 2011).

In the gastropod mollusk *Haliotis asinina*, spatial and temporal expression of Vasa and Nanos have largely overlapping, but not identical during embryonic and larval development; by the trochophore stage, both HasVasa and HasNanos are expressed in the mesodermal bands of the larva (Kranz et al., 2010). These authors hypothesized that HasVasa is expressed in a population of undifferentiated multipotent cells, from which the PGCs are segregated later during development in *Haliotis asinina*.

In sea urchins, Vasa, Nanos, and Piwi are expressed in descendants of the small micromeres and subsequently become restricted to the coelomic pouches, from which the entire adult rudiment will form, suggesting that these conserved molecular factors are involved in the formation of multipotent progenitor cells that contribute to the generation of the adult body, including both somatic and germ cells (Juliano et al., 2010; Voronina et al., 2008). Following removal of *vasa*-expressing micromeres in the embryo of the sea urchin *Strongylocentrotus purpuratus*, an accumulation of Vasa protein is induced in other cells that presumably give rise to functional PGCs (Voronina et al., 2008) resulting in cell fate transitions (Juliano et al., 2006, 2010). Juliano and co-workers (2010) hypothesized that the small micromeres are embryonic multipotent progenitor cells and the germline is later segregated from these cells in the late larva or developing juvenile.

Among chordates, in the colonial ascidian *Botryllus schlosseri*, *Pl10*, *piwi* and *Oct4* orthologues are highly expressed in differentiating soma cells (Rosner et al., 2009). In the

amphioxus *Branchiostoma floridae*, Vasa and Nanos, in addition to the early localization of their maternal transcripts in the primordial germ cells, are also expressed zygotically in the tail bud, which is the posterior growth zone of amphioxus cells, thus also function in highly proliferating somatic stem cells (Wu et al., 2011). During normal development in the ascidian *Ciona intestinalis*, germ cells in the tailbud of the tadpole stage are absorbed during metamorphosis and persist as PGCs in the young juvenile; however, upon removal of the larval tail prior to metamorphosis, PGCs from another source appear in the gonad rudiment at a later stage (see Nakamura, Seydoux, 2008; Giani et al., 2011; Rebscher et al., 2012).

Giani and co-workers (2011) proposed that under normal circumstances in studied animals, PGCs are segregated from somatic tissues during early development and are responsible for generating all gametes. In altered conditions, such as during regeneration or when PGCs are experimentally removed, multipotent stem cells in somatic tissue may compensate and produce gametes.

A two-step model of germ cell specification was proposed as an ancestral mechanism involving co-specification of germ cells and stem cells: setting aside a population of undifferentiated pluripotent stem cells, which is excluded from somatic differentiation and has the potential to form both somatic and germ cells, from which the primary stem cells are segregated later (Rebscher et al., 2007).

Expression of germline marker genes beyond gametogenic stem cells was observed in *Drosophila*, with *piwi* expression in the somatic cells of the ovary (Cox et al., 1998). Human $CD34^+$ hematopoetic stem cells are *piwi* positive, and lose *piwi* as they differentiate (Sharma et al., 2001).

In humans, widespread synthesis of NANOG and POU5F1 at pharyngula stages was observed (Thomas et al., 2008). Expression remains high in central and peripheral nervous systems but decreases dramatically in most other somatic tissues. Somatic cells from the pharyngula-stage embryo, such as these human neural crest cells, may have a closer ground state to a pluripotent phenotype than do adult somatic cells, with their very low frequency of inducibility (Thomas et al., 2008).

Germline Marker Gene Expression in Neural Development

The primitive murine neural stem cells (NSCs) have the ability to differentiate to non-neural tissues and transition into definitive NSC between embryonic day 7.5 and 8.5 *in vivo*, accompanied by a decrease in non-neural competency (Akamatsu et al., 2009). In mice lacking *germ cell nuclear factor* (GCNF) coding a transcriptional repressor of Oct4, generation of definitive NSCs was dramatically suppressed, accompanied by a sustained expression of Oct4 in the early neuroectoderm. Knockdown of Oct4 in GCNF deficient neural stem cells rescued these cells while overexpression of Oct4 blocked the differentiation of primitive NSCs to definitive NSCs (Akamatsu et al., 2009). These results suggest that primitive NSCs develop into definitive NSCs by means of GCNF induced suppression of Oct4, and the suppression of Oct4 by GCNF is important for restriction of the non-neural competency in the early neural stem cell lineage (Akamatsu et al., 2009). Thus, the authors demonstrated novel roles for Oct4 and GCNF in vertebrate somatic cells.

Mouse embryos lacking GCNF die at embryonic day 10.5 with abnormal development accompanied by a sustained Oct4 expression in the developing neuroectoderm, while in normal development, the expression of Oct4 in the developing early neuroectoderm is suppressed and restricted (see Akamatsu et al., 2009).

The primitive NSCs are dependent on leukemia inhibitory factor (LIF) and are similar to the primitive NSCs from embryonic stem cells; it has been proposed that neural fate specification from mouse ESCs is a default choice (Tropere et al., 2001).

Some proteins classically related to germ line development have been recently found to be involved in neuronal function and development. In the planaria *Schmidtea polychroa*, Tudor-related protein is expressed, beyond germline cells and neoblasts, in the central nervous system (Solana et al., 2009). *pumilio* and *bruno* planarian homologues are expressed similarly in neoblasts and in the central neural system, in perinuclear particles surrounding the nuclei of neurons (Salvetti et al., 2005; Guo et al., 2006). *nanos* and *pumilio* are involved in neuronal excitability, dendrite morphogenesis, and long-term memory in *D. melanogaster* (see Muraro et al., 2008; Solana et al., 2009).

Neural Crest Stem Cells

In vertebrates, neural crest stem cells represent a striking stem cell population. The neural crest cells (NCCs) are considered to be multipotent (Teng, Labosky, 2006; Thomas et al., 2008; Minoux, Rijli, 2010) or pluripotent cells (Trainor et al., 2003; Le Douarin et al., 2004; Thomas et al., 2008). NCCs are a migratory cell population that originates from the neural crest and migrates to different regions of the embryo, giving rise to diverse cell types (Trainor et al., 2003; Le Douarin et al., 2004; Thomas et al., 2008; Minoux, Rijli, 2010). Following induction, NCCs delaminate and migrate to different regions of the embryo, where they differentiate into a broad range of cell types, including peripheral and enteric neurons, glia, melanocytes, endocrine cells and smooth muscle. Rostral cranial NCCs extensively contribute to the frontonasal skeleton and the membranous bones of the skull, whereas more posterior cranial NCCs fill the pharyngeal arches, where they form the jaw, middle ear, hyoid and thyroid cartilages. In the cranial region, NCCs contribute to most of the cartilage and bone of the skull, cephalic tendons, dermis, meninges, vascular smooth muscle and adipocytes; such locations as the enteric ganglia, the dorsal root ganglia, the hair follicle, the tooth and even the bone marrow appear to be later niches for the maintenance of persistent, oligopotent avian and rodent neural crest-derived stem cells (Teng, Labosky, 2006; Thomas et al., 2008; Minoux, Rijli, 2010). The pluripotentiality and multiple roles of the neural crest cells and its derivatives co-exist with a striking level of plasticity of the neural crest cells both during development and even after the derived structures have fully differentiated (Le Douarin et al., 2004).

Multipotent, proliferative human neural crest cells (hNCCs) were derived from the pharyngula stage and self-renewing hNCC lines were maintained (Thomas et al., 2008). It was found that the global hNCC molecular profile is highly similar to that of pluripotent embryonic stem cells. The pluripotency markers NANOG, POU5F1 (Oct3/4) and SOX2 are also expressed by hNCC, and a small subset of transcripts can unambiguously identify hNCC among other cell types. The hNCC molecular profile is thus both unique and globally

characteristic of uncommitted stem cells (Thomas et al. 2008). Somatic cells from the pharyngula-stage embryo, such as these hNCC, may have a state close to a pluripotent phenotype. Pou5f1 (Oct3/4), best known for its expression by the murine germ-cell lineage and derived cell lines, is also expressed by both embryonic ectoderm and neuroepithelium (Thomas et al., 2008). The simultaneous transcription of SOX2, NANOG and POU5F1 is not sufficient to confer ES identity, since all three are found in the hNCC lines. Thus, the shared transcriptional signature between hNCC and human embryonic stem cells was startling (Thomas et al., 2008).

Embryonic Stem Cells

Embryonic stem cells (ESCs) are derived from the inner cell mass of the developing embryo. Mammalian ESCs are considered to be a standard cell culture model for studying pluripotency (Do, Schöler, 2009). We compared our data on invertebrate pluripotent stem cells with the information on the molecular signature of pluripotent ESCs (Isaeva et al., 2003; Shukalyuk, 2009; Shukalyuk, Stanford, 2008; Shukalyuk et al., 2005; Shukalyuk, Isaeva, 2012), taking into consideration that mammalian ESCs *in vitro* are in some sense artifacts of tissue culture (Zwaka, Thomson, 2005; Shostak, 2006).

ES cells, derived from pre-implantation embryo, and embryonic germ (EG) cells, derived from embryonic precursors of gametes, primordial germ cells (PGCs), can differentiate into any cell type in the body (Eguizabal et a., 2009). The expression of Pou5f1 (also known as Oct3/4), a POU and homeobox transcription factor, is essential for maintaining the pluripotential phenotype; Oct3/4, *Sox2*, and NANOG are hallmarks of mouse and human ES cells (see Goel et al., 2008; Yu, Thomson, 2008). *Nanog* are key regulators of pluripotency; the presence of Nanog is considered a key hallmark of pluripotent cells *in vivo* and *in vitro*: primitive germ cells, mammalian ES, EG and also embryonal carcinoma cells (see Chambers et al. 2003, 2007; Goel et al., 2008; Yu, Thomson, 2008; Western et al., 2010). However, the simultaneous transcription of SOX2, NANOG and POU5F1 (Oct3/4) is not sufficient to confer ES identity, since all three were found in the hNCC lines (Thomas et al., 2008).

Induced pluripotent stem cell (iPSC) technology, i.e. reprogramming somatic cells into pluripotent cells that closely resemble embryonic stem cells (ESCs) by introduction of defined transcription factors (TFs), holds great potential in biomedical research and regenerative medicine (see Zhu et al., 2010). The standard method of generating iPSCs involves the coexpression of four defined Yamanaka (Oct4–Sox2–Klf4–c-Myc) or Thomson (Oct4–Sox2–Nanog–Lin28) factors to convert somatic cells into pluripotent stem cells (see Kelley, Lin, 2012). For example, mammalian germ cells grown in culture and treated with fibroblast growth factor (FGF) and leukemia inhibitory factor (LIF) can be induced to become pluripotent EG cells, which are very similar in differentiation potential to ES cells derived from the inner cell mass of the blastocyst. Mouse PGCs acquire the morphological and cytological characteristics of ESC when cultured in the presence of three growth factors (Matsui et al., 1992). iPSCs can be created from adult somatic cells by introducing the combinations of several transcription factors, e.g., Oct3/4, Sox2, Klf4, and Myc (see Hayashi et al., 2012). Using these four factors, direct reprogramming of fibroblasts is possible resulting in presumably either a naive or a primed pluripotent state (Han et al., 2011). Several

small molecules modulating mitochondrial oxidation and glycolytic metabolism also showed effects on reprogramming: i.e., compounds that promote glycolytic metabolism enhance reprogramming (see Zhu et al., 2010).

Recently a novel mechanism of somatic cell reprogramming (SCR) was proposed that is controlled by a single microRNA, miR-302, instead of proteins (Kelley, Lin, 2012). Mature miR-302 localized to the cytoplasm is the most abundant miRNA found in human ESCs and iPSCs, but its expression is absent in differentiated cells. The total targets of miR-302 include over 600 cellular genes, many of which are involved in differentiation and developmental signaling. miR-302 has two parallel functions that occur simultaneously: reprogramming and tumor suppression. It was found that miR-302 is capable of reprogramming human cancer cells into a normal ESC-like state (see Kelley, Lin, 2012). During the first step of SCR, miR-302 forces global demethylation and histone modification; after global demethylation, many genes are accessible to transcription machinery, allowing the expression of ESC-specific transcription factors including Oct4–Sox2–Nanog–Lin28. The interaction between miR-302 and Oct4–Sox2–Nanog/Klf4 may result in a stable iPSC state suitable for stem cell self-renewal without the problem of either tumor formation or early senescence (Kelley, Lin, 2012).

Pluripotent stem cells of epiblast cells in mice can give rise to both PGCs and somatic derivates (Ohinata et al., 2009). EC cells, ES cells, and EG cells were also capable of reverting the differentiated state of somatic cells to that of pluripotent stem cells (Yu, Thomson, 2008). EG, ES and embryonal carcinoma (EC) cells may all have their closest *in vivo* equivalent not in ICM cells but rather in germ cells (Zwaka, Thomson, 2005). ES cells have the capacity to differentiate into PGCs *in vitro*. Reprogramming into germ cells from somatic cells is a way to obtain germ cells. Development of human germ cells from *in vivo* and *in vitro* cultured ESCs/iPSCs is possible (Hayashi et al., 2012). Human germ cells (hEGCs), which can maintain their self-renewal and pluripotency *in vitro*, express alkaline phosphatase (AP), OCT4, SOX2, NANOG as the markers of pluripotent stem cells.

The data on reprogramming of ESCs and some other stem cells into germ cells provide support for our view that embryonic stem cells, germline cells and some pluri/multipotent cell types share a basic molecular machinery underlying pluripotency and gametogenic potentiality.

Gene Regulatory Networks Underlying Gametogenic Potential and Pluripotency

The metazoan development program may be imagined as translation regulatory cascades (Davidson, 2006 Peter, Davidson, 2011). Although some gene regulatory network (GRN) subcircuits are quite ancient, other aspects are highly flexible and thus, in any given genome, more recent (Peter, Davidson, 2011). The specific GRN to maintain the conserved germline program has been conserved during metazoan evolution. Genes *vasa/pl10*, *piwi/auberdine*, *nanos*, *tudor*, *pumilio*, and *staufen*, representing the core of the germline program, show striking evolutionary conservation (Alié et al., 2011; Chuma et al., 2006; Ewen-Camden et al., 2010; Watanabe et al., 2009; Extavour, 2008; Gustafson, Wessel, 2010; Leatherman, Jongens, 2003; Parvinen, 2005; Western et al., 2010; Sroji, Extavour, 2011). This gene

network consists of gene modules whose interactions are highly stable and highly evolutionary conserved, operating in similar ways both in different organisms, and in different places and/or times during the development of an animal organism. Conserved germ cell-specific RNA networks repress transcriptional programs for somatic differentiation and promote germ cell maintenance (Cinalli et al., 2008).

Common "stemness" genes include the cumulative expressions of Piwi, Vasa, Nanos and PCNA within invertebrate and vertebrate stem cells (Rinkevich et al., 2009). RNA silencing of genes is also important for stem cell maintenance for many organisms and the highly conserved Piwi protein that is part of the silencing machinery is expressed in the neoblast stem cells of planarians (Reddien et al. 2005; Sköld et al., 2009).

Interactions between *vasa* and other germline genes have suggested a complex network of positive and negative regulation at multiple levels, including transcription, translation, and post-translational modification, epigenetic control of chromatin architecture mediated gene regulation crucial for the role in development (Cinalli et al., 2008; Ewen-Camden et al., 2010). The post-transcriptional regulation of *vasa* potentially is realized by protein stability, and *nanos*-dependent cell fate via repression of the cell cycle and apoptosis (Juliano et al., 2010). Homologous genes often interact physically with the same conserved molecular partners across the metazoans. Piwi physically interacts with Vasa; in mice, Piwi homologues Miwi and Miwi2 form a complex with Mvh/Ddx4, the mouse vasa homologue (Vagin et al., 2009). Both Nanos and Pumilio proteins bind each other, and can conditionally recruit additional protein factors to regulate target RNAs; in both flies and worms, binding of Nanos and Pumilio orthologs to target RNAs correlates with translational repression; Nanos is also implicated in the maintenance of a specific "chromatin architecture" that is associated with general transcriptional repression (see Ewen-Campen et al., 2010). One Oskar protein has been shown to directly interact with Staufen and Vasa proteins (see Ewen-Campen et al., 2010). The data suggest a connection between the interacting networks of proteins and RNAs in germ granules (Ewen-Campen et al., 2010). While many genes with critical roles in the germ line have been characterized with respect to expression pattern and genetic interactions, it is the molecular interactions of the relevant gene products that are ultimately responsible for germ cell differentiation. Ewen-Campen et al. (2010) suggested a preliminary molecular definition of an ancestral ''pluripotency module'' that could have been modified during metazoan evolution to become specific to the germ line.

Many genes involved in germ cell specification are conserved across evolution; these molecules often also interact biochemically in similar ways in phylogenetically distant animals. The Nanos/Pumilio complex, the Tudor domain/PIWI family interaction, and the Tudor/spliceosome component association may therefore represent evolutionarily ancient interactions. Moreover, some of these molecules, and in some cases their molecular roles, are conserved not just in germ cells but are also found in pluripotent cells of many types, and in RNA-processing bodies of somatic cells (Ewen-Campen et al., 2010). The conserved protein–protein and protein–RNA interactions described above could represent components of an ancestral pluripotency module, which would have likely contained Tudor domain protein, PIWI family members, and a DEAD box helicase. In early multicellular animals where a pluripotent stem cell population produced gametes, those stem cells that entered into gametogenesis would have tailored this module by the addition of unique germ cell genes, such as nanos, vasa, and Aub/Ago3. With the advent of dedicated germ cells in metazoans, this specialized germ line module would have come under the control of cytoplasmic

inheritance or inductive mechanisms that operated exclusively in the germ line, preventing somatic cells (including somatic stem cells) from producing gametes (Ewen-Campen et al., 2010 Juliano et al., 2010; Rebscher et al., 2012).

The vertebrate embryonic stem cells seem to be maintained by complex regulatory networks including the transcription factors oct3/4, sox2, NANOG and such classic markers as presence of alkaline phosphatase and telomerase (see Thomas et al., 2008; Yu, Thomson, 2008; Western et al., 2010; Shukalyuk, Isaeva, 2012). The machinery controlling pluripotency is repressed as male and female germ cells exit the cell cycle, and the data support the conclusion that repression of this core machinery is a robust and early event involved in the differentiation of the germ cell lineage (Western et al., 2012).

One of the predicted consequences of modularity in development is that modules themselves can remain highly conserved throughout evolution, while their upstream effectors and downstream targets can evolve independently. The mechanisms that localize germ line determinants to germ cells appear to be relatively flexible not only on an evolutionary time scale, but also even within developing individuals. Such apparent flexibility in the spatial and temporal deployment of multiple functional germ line factors further supports the notion that such factors may operate as an interacting module, capable of being induced by a variety of upstream signals. The entire suite of genes is linked together biochemically. Many of the powerful GRNs that have been constructed to model aspects of somatic differentiation rely largely on transcriptional regulation (see, e.g., Davidson, 2006). However, most of the molecules for which functional biochemical data are available appear to be involved in translational regulation and protein–protein interactions, suggesting that post-transcriptional gene regulation is particularly crucial in the germ line (see Cinalli et al., 2008). Several other recently discovered mechanisms of gene regulation play a critical, albeit not yet well-defined, role in the germ line. These include piRNA-mediated transposon silencing, RNP formation to repress translation, and chromatin architecture mediated gene regulation. Building GRNs is necessary to create a framework for understanding the molecular network of the germ line (Ewen-Campen et al., 2010).

It was hypothesized that PGCs and stem cells are not only closely related, but also share a common gene regulatory module, the "germline multipotency program" (Ewen-Campen et al., 2010; Schwager, Extavour, 2010; Juliano et al., 2010; Rebscher et al., 2012). Independent and/or alternate functions for such germline multipotency program genes as *piwi* and *nanos* do not preclude them from acting together in a conserved program in multipotent and germline cells. Rather, they may have been independently co-opted by other cells types with similar requirements as multipotent progenitors and germ cells, such as mitotic quiescence and protection from transposons (Juliano et al., 2010).

The expression of *piwi* homologues in both the germline and regions of dividing cells in the posterior growth zone provides a molecular link between germline stem cells and pluripotent somatic stem cells in *C. teleta* (Giani et al., 2011). The similarity in expression of *piwi* to the expression patterns previously observed for *vasa* and *nanos* homologues in *C. teleta* (Dill, Seaver, 2008; Giani et al., 2011) suggests that this core set of stem cell regulators has retained an ancestral role in somatic and germline stem cell production. Such a dual role may reflect an ancestral metazoan feature in which there was a close link between somatic and germline stem cells, and contrasts with the segregation of the germline in animals such as *D. melanogaster* (see Giani et al., 2011).

ES cells maintain self-renewal and pluripotency because of a self-organizing network of transcription factors and intracellular pathways activated by extracellular signalling that together prevent their differentiation and promote their proliferation, and because of epigenetic processes that maintain the chromatin in a plastic differentiation status (De Felici et al., 2009).

Genetic networks and molecular pathways are involved in the numerous cell-to-cell interactions during neural crest cell migration, homing and differentiation (Le Douarin et al., 2004). Thomas et al. (2008) set out to identify molecular networks that were activated in an early human neural crest cell (hNCC) population before they dispersed to their final sites of differentiation. Most genes that are commonly used to characterize NCC in animal studies are expressed by hNCC as well; however, more than 4000 additional Unigene clusters were present only in the human cells. While cross species comparisons showed extensive overlap between human, mouse and avian NCC transcriptomes, some molecular cascades are only active in the human cells, correlating with phenotypic differences. Furthermore, the hNCC transcriptional profile was highly evocative of the molecular signature of human embryonic stem (hES) cells, including but not restricted to the expression of the transcription factors SOX2, NANOG and POU5F1. So cluster and pathway analyses of whole transcriptomes show similarity between hNCC and embryonic stem cells; hNCC express many genes considered to play essential roles in the maintenance of multi-/pluripotency: NANOG, POU5F1 (OCT3/4) and SOX2 (Thomas et al. 2008).

The key element of evolutionary novelty would have been the appearance of genetic regulatory circuitry that underlies the development of “set-aside cell” populations (see below). These cells must be dissociated from the mechanisms that assign immediate terminal fates to all the other cells of the embryo; and they must be endowed with new growth and cell division control systems (Cameron et al., 1998). Davidson and colleagues (1995) proposed that genetic regulatory programs including the homeotic complex in embryogenesis produced in evolution unspecified "set-aside cells" and hierarchical regulatory programs that initially define regions of morphogenetic space in terms of domains of transcription factor expression.

Germ and Stem Cells in Evolution

The true innovation and a crucial step in animal evolution was the generation of a gametogenic lineage with the loss of gametogenic potential from the majority of cells of the organism (Extavour, 2008; Ewen-Campen et al., 2010; Wu et al., 2011). Sequestration of a dedicated germ line early in development makes it possible to develop from only one cell an organism composed of millions. In order to effectively confer the advantage of protection from somatic mutation, such a lineage might show reduction of mitotic activity (since more rounds of DNA replication give more opportunity for mutation through copy error), reduced transcriptional activity (because genes may be more subject to mutation when actively transcribed) and reduced transposable element mobility (see Extavour, 2008). In fact, germ cells are typically mitotically quiescent from the time of their specification during embryogenesis, until the time that gametogenesis begins; they are relatively transcriptionally quiescent during most of embryonic development; RNA-mediated silencing of transposable elements has recently been documented in the germ lines (see Extavour, 2008). Urbilateria

can be assumed to have possessed at least a majority of truly somatic cells, so its reproductive success depended on the successful specification and protection throughout development of a germ line (Extavour, 2008).

It was supposed that the germ line originally evolved from primary stem cells and that the properties of these cells are retained in at least some forms of agametic cloning (Blackstone, Jasker 2003; Agata et al., 2006; Sköld et al., 2003). The data strongly suggest that epigenetic establishment of the germ line was present in Urbilateria (Extavour, 2008). Sponges, cnidarians and acoel flatworms use very similar strategies to obtain gametogenic cells. They all contain a population of pluripotent stem cells (sponge archaeocytes, cnidarian interstitial cells and acoel neoblasts) that can give rise to both somatic cell types and gametes (Extavour, 2008). Urbilateria was unlikely to have had all of its gametogenic cells clustered together in one region, but rather might have had them scattered throughout the body. Evidence from modern molecular and functional comparisons between stem cells and germ cells suggests that Urbilateria's germ cells were a subpopulation of pluripotent stem cells (Extavour, 2008). Thus, Extavour proposed that PGCs have their closest evolutionary equivalent in the pluripotent stem cells that are found in extant non-bilateria and basal bilaterians, and that almost certainly existed in Urbilateria.

A number of major adaptations in animals have been mediated by alteration of germ cells and their immediate derivatives, the gametes (Haag, 2009). Extavour (2008) supposed that Urbilateria used epigenesis to specify the germ line, but preformation as cell-autonomous specification mechanism evolved convergently several times during the bilaterian radiation. In preformation, germ-cell-specific gene products persist through completion of oogenesis in the zygotic cytoplasm, mutations arising in the germ line that affected oocyte cytoskeletal dynamics or mRNA or protein localization of germ cell molecules could allow persistence and/or localization of these molecules in mature oocytes. Changes in the expression timing (heterochrony) and ooplasmic localization (heterotopy) of germ-cell differentiation genes led to early embryonic cytoplasmic inheritance of germ-cell determinants that was both heritable and independent of somatic epigenetic signaling later in embryonic development, resulting in convergent evolution of preformation (Extavour, 2008).

Recently, *piwi* and *vasa* genes have been proposed to be ancestrally associated with stem cell character ("stemness"), rather than solely with germline stem cells (Alié et al., 2011; Giani et al., 2011). It is likely that *piwi* genes, and associated stem cell co-regulators, became restricted to the germline in some taxa during the course of evolution.

Juliano et al. (2010) propose the existence of a highly conserved germline multipotency program that operates in both multipotent cells and germ cells. In many animals, germline segregates from the soma after embryogenesis, similar to the sea urchin. Furthermore, in these cases the germline multipotency program appears to operate in the multipotent progenitor cells, thus allowing for functional comparisons across diverse animal taxa (Juliano et al., 2010). These authors considered two mutually exclusive hypotheses considering post-embryonic germline segregation as ancestral or derived. The first model proposes that post-embryonic germline segregation from a multipotent precursor is ancestral, an idea that has been supported by several investigators (Agata et al., 2006; Extavour, 2007; Funayama, 2010; Rebscher et al., 2007; Ewen-Camden et al., 2010). This model argues that the protostome/deuterostome urbilaterian ancestor segregated its germline through a pluripotent or multipotent stem cell (Agata et al., 2006; Extavour, 2007; Funayama, 2010). Juliano et al. (2010) offered a similar model with the additional proposal that the urbilaterian germ cell

multipotent progenitor was controlled by the germline multipotency program and that dedicated germ cells were segregated post-embryonically. Early segregation of the germline, which is then kept in a quiescent state, allows increased protection from mutations associated with DNA replication and/or additional morphogenetic freedom in subsequent development (Juliano et al, 2010). According to the second model, the urbilaterian segregated its germline by specifying PGCs during embryogenesis using the germline multipotency program; the evolution of multipotent progenitor cells may have occurred relatively recently and were independent acquisitions, for example, during the transition from direct to indirect development in sea urchins with co-optation of the germline multipotency program. Perhaps, the small micromere lineage co-opted the germline multipotency program from the germline cells of the adult (Juliano et al., 2010).

Set-aside Cells and Neural Crest Stem Cells as Evolutionary Innovations

E. Davidson and colleagues have developed the “set-aside cells” hypothesis (Davidson et al., 1995; Peterson et al., 1997; Cameron et al., 1998; Jenner, 2000; Collins, Valentine, 2001; Raff, 2008). It was proposed that set-aside cells exist in larvae of protostomes and deuterostomes, and the adult arises from these set-aside cells. The cells were called "set-aside cells," because they are held out from the early embryonic specification and are developmentally set aside from the embryo-larva differentiation process (Davidson et al., 1995; Peterson et al., 1997). These cells have an essentially unlimited division capacity, and they produce new populations of cells that give rise to the adult body plan in animals with indirect development while most of the larval cells have a dead-end fate. So many adult organs are not derived from cells within larval organs, but from pluripotent cells sequestered, set aside during larval life as primordia from which adult structures form, such as imaginal discs of insects (Collins, Valentine, 2001).

For example, the juvenile sea urchin emerges at metamorphosis from the adult rudiment, which is formed by set-aside cells; thus, the juvenile sea urchin develops from embryonic multipotent cells (Juliano et al., 2010). The small micromeres of the sea urchin *Strongylocentrotus purpuratus* selectively accumulate *vasa*, *nanos* and *piwi* mRNA (Juliano et al., 2006). Descendent of the small micromeres remain relatively quiescent through embryogenesis being set aside for constructing the adult rudiment and then integrating into the larval coelomic pouches where the adult rudiment forms. Once the small micromere descendent are incorporated into the coelomic pouches, they begin to proliferate, which is uncharacteristic of quiescent embryonic PGCs (Juliano et al., 2010).

The “set-aside cells” hypothesis was presented as an explanation of the causal events underlying the "Cambrian explosion" and thus the divergence of large animal body plans with appearance of many recent animal phyla (Davidson et al., 1995; Peterson et al., 1997; Cameron et al., 1998). It was postulated that the latest common ancestor of bilaterians had a larval stage similar to what is found in modern indirectly developing marine organisms. In terms of the genetic regulatory circuitry, this type of embryonic process is much simpler than those by which the adult body plans of most metazoan phyla develop and is considered as the original form of embryonic development (Davidson et al., 1995). This idea is rooted in the

hypothesis that early metazoans were similar to modern larvae, and that the bilaterian adult stage evolved by the innovation of set-aside cells, distinct from the larval cells (Raff, 2008). Thus, the evolution of adult stages through the acquisition of «set-aside» stem cell populations is supposed (Raff, Raff, 2009). Organs and systems of adult animals with indirect development arise from «set-aside» stem cells such as annelid teloblasts or imaginal discs of insects.

In the larvae of colonial rhizocephalan barnacles, groups of undifferentiated cells are visible, including a posterior cluster and other stem cells (Korn et al., 2004; Shulaluk et al., 2005) We suggest that the larvae have a system of set-aside stem cells, which are able, during larval development and in parasitic organism after metamorphosis, to differentiate as somatic cells, or germline cells, or to maintain self-renewal as pluripotent stem cells.

Fuchs and co-workers studied (Fuchs et al., 2011) the spatial expression of 13 developmental genes in the larval stage of the gymnolaemate bryozoan *Bugula neritina*. Bryozoan larvae exhibit blastemic tissues that contribute to build the adult during morphogenesis. The results suggested that the larval blastemas in *Bugula* are pre-patterned according to their future fate in the adult and challenge a view that metazoan larvae share homologous undifferentiated "set-aside cells" (Fuchs et al., 2011).

In vertebrates, an important developmental and evolutionary morphogenetic reserve is the pluripotent stem cell population of the neural crest. They provided a cell source for an increase in organism size and the initiation of a new morphospace (Jenner, 2008). The emergence of a specialized population of migratory neural crest stem cells in a chordate ancestor is regarded as a fundamental event and a key innovation in the evolution of vertebrates (Trainor et al., 2003; Le Douarin et al., 2004). Hall (2000) considered the neural crest as the fourth germ layer in vertebrates. Proliferation, migration and differentiation of neural crest cells are critical for the formation of vertebrate embryos, especially the head. Cells of the neural crest and epidermal placodes give rise to skeletal elements and the nerve ganglia of a "new head" in vertebrates (Northcutt, Gans, 1983), a unique evolutionary invention that facilitated the evolution of highly specialized sensory organs and the skull (Kuratani, Schilling, 2008). The acquisition of the NCC by protochordate ancestors is considered to be a turning point in the evolution of vertebrates (Trainor et al., 2003; Le Douarin et al., 2004). The evolution of the neural crest cells could be reconsidered in terms of the acquisition of new cell properties such as delamination, migration and also pluri/multipotency, which were key innovations that contributed to craniofacial development. It seems clear that alterations of the ancestral gene expression program in NCC were important for craniofacial evolution in vertebrates (Trainor et al., 2003).

In most metazoans, stable undifferentiated cells are set aside and persist beyond the completion of embryogenesis (Juliano et al., 2010). Primordial germ cells are definitively programmed to become PGCs and set aside for future use in the adult animal (Frank et al., 2009). Shostak (2008) proposed that mammalian adult stem cells resemble the blastomeres of planktonic and benthic organisms with small eggs and may have evolved in mature organisms as an adaptation to the growth and maintenance of tissues similarly to set-aside cells.

Generally, we can regard all metazoan stem cells as "set-aside cells", reserve cells.

Thus, the molecular signatures and functional potential of germ cells and pluripotent stem cells suggest a shared evolutionary origin for these cell types and an ancestral pluripotency network including members of Vasa-like and Piwi-like class proteins, which are conserved components of both germ and stem cells across the metazoans (Alié et al., 2011; Ewen-

Campen et al. 2010; Gustafson, Wessel, 2010; Sroji, Extavour, 2011). Based on the literature and our own data analysis, we support the idea that this regulatory gene network is not restricted to the germline cells but is expressed in stem cells that are capable of producing both somatic and germinal derivatives.

The comparative study of germline cells and set-aside stem cells in different tissues, organs and organisms of various metazoan taxa and analysis of evolutionary changes as natural experiments can contribute to the advance of cell engineering.

Conclusion

The data reviewed suggest the existence of an evolutionary conserved basis of pluripotency and "stemness" of germline and pluri/multipotent stem cells. Pluripotent gametogenic stem cells and germline cells share many morphological features and rely on the activity of related genes; their evolutionary and ontogenetic relationship has been proposed (Extavour, 2008; Extavour, Akam, 2003; Rebscher et al., 2007; Sköld et al., 2009; Strouji, Extavour, 2011; Wu et al., 2011; Isaeva, 2011; Shukalyuk, Isaeva, 2012). Since pluri/multipotent stem cells produce germline cells, they might be considered part of the germline (Mochizuki et al., 2001); such "primary" stem cells contributing to the germ line may be immortal, in contrast to somatic tissues (Weismann, 1893; Sköld et al., 2009). A common origin of germ cells and of somatic stem cells has been proposed, which may constitute the ancestral mode of germ cell specification in Metazoa (Rebscher et al., 2007).

Germline stem cells and gametogenic pluripotent stem cells also share a common gene regulation module, the germline multipotency program (Ewen-Campen et al., 2010; Juliano et al., 2010). Even though not everyone shares such a view, focusing on gametogenesis, where the cell indisputably undergoes specification and specialization, we, however, put forth the point of view that germline progenitors must be recognized as pluripotent/multipotent gametogenic stem cells across Metazoa. The evolutionary conserved germline program involves *vasa/pl10*, *piwi/auberdine*, *nanos*, *tudor*, some other genes and signaling ways (Alié et al., 2011; Chuma et al., 2006; Ewen-Camden et al., 2010; Watanabe et al., 2009; Extavour, 2008; Gustafson, Wessel, 2010; Leatherman, Jongens, 2003; Parvinen, 2005; Sroji, Extavour, 2011). This molecular machinery is common for all studied metazoan representatives, from sponges to chordates, and operates at cellular, sub-cellular and molecular levels. In the studied asexually reproducing representatives of Porifera, Cnidaria, Platyhelminthes, Arthropoda and Chordata, stem cells serve as the predecessors of germ and somatic cells and are similar to cells of the germ lineage, displaying evolutionarily conserved features of the morphofunctional organization typical also for cells of the germ line (Ewen-Campen et al. 2010; Extavour, 2008; Funayama et al., 2010; Gustafson, Wessel, 2010; Isaeva et al., 2003, 2008, 2009; Rinkevich et al., 2009; Sköld et al., 2009; Shukalyuk et al., 2005, 2007; Sroji, Extavour, 2011).

The data collected in many animals demonstrate that genes traditionally classified as "germline genes" have a broad role in establishing and maintaining multipotency. An ancient association of "germline genes" with stemness (Watanabe et al. 2009) and "an ancestral gene fingerprint of stemness" (Alié et al. 2011) have been proposed. Since all animals have a common ancestor in single cell organisms it is possible to identify common principles in the

regulatory mechanisms for the transcriptional and epigenetic machinery (Watanabe et al. 2009), in germline cells or beyond the germ line (Gustafson, Wessel, 2010). All animals possess pluripotent gametogenic stem cells in different ontogenetic periods: as a transient state in early cleavage until segregation of germ line (preformation), as a longer state during embryogenesis (epigenesis), or as a continuous state during the entire life of asexually reproducing organisms. Pluripotent stem cells have the capacity to move away from pluripotency towards a special, restricted stem cell identity as germ cells (Sroji, Extavour, 2011) or to restricted identities as somatic multipotent stem cells, oligopotent stem cells and so on.

Recent data indicate the broad and partially overlapping spectrum of gene expression in embryonic stem, germline, and pluri/multipotent stem cells, in particular, the possible inducibility of germline cells *de novo* without continuous expression of molecular markers of the germ line. The data also show a transient molecular signature typical of the germline in broad somatic stem pools during embryogenesis. Embryonic stem, germline and pluripotent stem cells of various metazoans share the expression *piwi, vasa*-related and other germline marker genes. Pluripotent gametogenic cells are similar in their potential and their molecular signature to mammalian embryonic stem cells, although the latter are artificial cell systems cultured *in vitro*. Multipotent neural crest cells use much the same transcriptional machinery as embryonic stem cells to delay differentiation (Thomas et al., 2008).

It is likely that there is a continuum between embryonic PGC and pluri/multipotent stem cells (Juliano er al., 2010; Shukalyuk, Isaeva, 2012). Such a continuum ranges from pluripotent gametogenic stem cells to germline cells and up to multipotent somatic stem cells lacking gametogenic potential. We suppose mainly common and partially overlapping molecular signatures in pluri/multipotent stem cells of a wide range of animals from sponges to chordates. A core regulatory gene network of pluripotent gametogenic stem cells, germline cells and multipotent stem cells evidently overlap to a large extent including also some specific distinctions and fluctuations of key gene expression. Genes of the molecular machinery of stem cells appeared to be interconnected in related pathways that are involved in post-transcriptional regulation and epigenetic modification, acting in a coordinated manner, as part of a complex network of signal cascades that are known to regulate the balance between cell death and survival (Rossi et al., 2007). It is possible a functional diversification of paralogues of *vasa*, *piwi* and other marker "germline" genes fulfilling different functions in germ and other stem cells. In the animal kingdom, *vasa*-like genes are present in numbers from one to four (Shibata et al., 1999; Rebscher et al., 2007; Extavour et al., 2005; Pfister et al., 2008). In mammals, four Argonaute subfamily members have been shown to be involved in the miRNA pathway (Parvinen, 2005; Kotaja et al., 2006). A secondary evolutionary co-optation of germline regulatory gene network to other systems of stem cells was also possible.

Germ granules, chromatoid bodies, nuage, P-bodies, RNP granules and so on are the physical embodiment of overlapping but not identical gene networks. Such organelles concentrate the activity factors normally present in all cells, but especially active in germline cells because of their intensive reliance on post-transcriptional controls of gene expression (Snee, Macdonald, 2004). Basic germ-plasm machinery may exist as discrete granules or bodies, large complexes as Balbiani bodies, dispersed nuage, small particles (for example, P-bodies) or submicroscopic RNP aggregates. In somatic cells, P-granules fulfill functions from RNA localization and decay to translational activation and repression (Anderson, Kedersha, 2006; Seydoux, Braun, 2006). It seems to us that similar networks in mammalians overlapped

with the macromolecular frame of the germinal granules emerging earlier in germline cell evolution.

It is very important for embryonic, germ and pluripotent stem cells as well as for long living neural precursors and neurons to have protection against apoptosis; neuronal precursors evolutionarily obtained unique machinery, which allows them to continue their differentiation but keep their immortality over the lifespan of the individual. It is known that in zebrafish, loss of *piwi*-related gene function results in a progressive loss of germ cells due to apoptosis during larval development (Houwing et al., 2007). In mouse homozygotes that have a *vasa* homologue gene (*Mvh/Ddx4*) with restricted expression, premeiotic germ cells cease differentiation and undergo apoptotic death (Tanaka et al., 2000).

The notion that asexual reproduction is common only among the lower but not the higher animals remains a widely accepted dogma (see, e.g., Blackstone, Jasker, 2003). In some reviews (Blackstone, Jasker, 2003; Sköld et al., 2009) and in modern textbooks, crustaceans, like all other arthropods and the entire Ecdysozoa clade, are considered non-colonial and incapable of cloning, though in many rhizocephalan barnacles (Crustacea: Cirripedia: Rhizocephala) asexual reproduction and resulting colonial organization are described (see Høeg, Lützen, 1995; Glenner et al., 2003; Shukalyuk, Isaeva, 2012; Isaeva et al., 2012). In colonial ascidians, members of the phylum Chordata, stem cells giving rise to the germ and somatic cell lineages were found (Pancer et al., 1995; Weissman, 2000; Laird et al., 2005; Sunanaga et al., 2006; Akhmadieva et al., 2007; Rinkevich et al., 2009). These data on the asexual reproduction in some arthropods and chordates contradict the dogma that asexual reproduction is common exclusively among the lower animals (Isaeva, 2010, 2011). The statement that vertebrates are incapable of natural cloning (Blackstone, Jasker, 2003) is disproved by the long known facts about facultative polyembryony in mammals, which has become obligate in some armadillo species, e.g. in *Dasypus novemcinctus* (see Loughry et al., 1998). Polyembryony, the development of a whole embryo from one of the early blastomeres, i.e. asexual reproduction at an early embryonic stage, is known in members of different taxa in six phyla of animals (Craig et al., 1997; Sköld et al., 2009). For instance, among insects, polyembryony has been long described in some parasitoid members of Hymenoptera and Strepsiptera (Hagan, 1951). The term "somatic embryogenesis" (Buss, 1987; Blackstone, Jasker, 2003) suggests that stem cells, which ensure the asexual reproduction, are recognized as somatic ones; pluripotent stem cells in animals with asexual reproduction are often referred as somatic (Blackstone, Jasker, 2003; Extavour, Akam, 2003; Extavour, 2008; Rinkevich, 2009; Sköld et al., 2009; Funayama et al., 2010). However, pluriponent gametogenic stem cells of asexually reproducing invertebrates, like primary germ cells, do not belong to any germ layer, differentiated tissue, and population of specialized somatic cells or their uni/oligopotent stem cells (Isaeva, 2010, 2011). Such pluripotent stem cells are dispersed in the organism, do not display contact inhibition of cell reproduction and movement and are similar to primary germ cells in their ability to perform amoeboid movements and large-scale migrations within the organism, directed to the localities of asexual reproduction and regeneration or to the gonads, respectively (Isaeva et al., 2008, 2009; Rinkevich et al., 2009; Sköld et al., 2009). We believe that the evolutionarily and ontogenetically related cells of early embryos, pluripotent gametogenic stem cells and germline cells belonging to cell populations capable of realizing the entire developmental program, including gametogenesis (and, potentially, subsequent embryogenesis) are not identical to somatic cells (see Fig. 1) (Isaeva, 2011; Isaeva, Shukalyuk, 2012).

It has been suggested that the adult pluri- or totipotent stem cell systems like those found in many invertebrates have evolved as components of clonal asexual reproduction (Agata et al. 2006; Sköld et al., 2009). The lack of somatic stem cells in such adult metazoans as nematodes, rotifers, gastrotrichs and insects correlates with their very limited regenerative capacity and absence of agametic cloning. It was proposed that the colonial strategy is derived from sexual ancestors and appears independently in different branches of animals (Blackstone, Jasker 2003; Sköld et al., 2009). The ability to reproduce by cloning is reflected in an extensive regenerative capacity and it is clear that the widespread distribution of asexual reproduction as well as the many different modes of clonality within phyla reflect high levels of cell and tissue plasticity (Sköld et al., 2009).

We postulate that pluripotent stem cells, germline cells and some other cell types share a basic molecular machinery as a core regulatory gene networks overlapping to a large extent, which operates in a similar manner across Metazoa and might be recruited by other tissue-specific networks ensuring self-preservation as a defense mechanism against apoptosis, cell differentiation and aging.

References

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

Akamatsu W., DeVeale B., Okano H., Cooney A.J., van der Kooy D. (2009). Suppression of Oct4 by Germ Cell Nuclear Factor Restricts Pluripotency and Promotes Neural Stem Cell Development in the Early Neural Lineage. *J. Neurosci.*, vol. 29, pp. 2113–2124.

Akhmadieva A.V., Shukalyuk A.I., Aleksandrova Ya.N., Isaeva V.V. (2007). Stem Cells in Asexual Reproduction of the Colonial Ascidian *Botryllus tuberatus. Russ. J. Mar. Biol.*, vol. 33, pp. 217–222.

Alié A., Leclère L., Jager M., Dayraud C., Chang P., Le Guyader H., Quéinnec E., Manuel M. (2011). Somatic stem cells express Piwi and Vasa genes in an adult ctenophore: Ancient association of "germline genes" with stemness. *Dev. Biol.*, vol. 350, pp. 183-197.

Anderson P., Kedersha N. (2006). RNA granules. J. Cell Biol., vol. 172, pp. 803-808.

Aravin A.A., van der Heijden G.W., Castaneda J., Vagin V.V., Hannon G.J., Bortvin A. (2009). Cytoplasmic compartmentalization of the fetal piRNA pathway in mice. *PLoS Genet.*, vol. 5, p. e1000764.

Asaoka-Taguchi M., Yamada M., Nakamura A., Hanyu K., Kobayashi S. (1999). Maternal Pumilio acts together with Nanos in germline development in Drosophila embryos. *Nat. Cell Biol.* vol. 1, pp. 431-437.

Bayer M.M., Cormack R.M., Todd C.D. (1994). Influence of food concentration on polypide regression in the marine bryozoan *Electra-Pilosa* (L) (Bryozoa, Cheilostomata). *J. Exp. Mar. Biol. Ecol.*, vol. 178, pp. 35–50.

Beams H.W., Kessel R.G. (1974). The problem of germ cell determinants. *Intern. Rev. Cytol.*, vol. 39, pp. 413–479.

Berekelya L.A., Ponomarev M.B., Mikryukov A.A., Luchinskaya N.N., Belyavsky A.V. (2005). Molecular mechanisms of germ line cell determination in animals. *Mol. Biol.*, vol. 39, pp. 572–584.

Berrill N.J. (1961). *Growth, Development, and Pattern.* San Francisco; London: Freeman and Company, p. 555.

Blackstone N.W., Jasker B.D. (2003). Phylogenetic Consideration of Clonality, Coloniality, and Mode of Germline Development in Animals. *J. Exp. Zool.*, vol. 287, pp. 35–47.

Bontems F., Stein A., Marlow F., Lyautey J., Gupta T., Mullins M.C., Dosch R. (2009). Bucky ball organizes germ plasm assembly in zebrafish. *Curr. Biol.*, vol. 19, pp. 414–422.

Bode, H.R. (1996). The Interstitial Cell Lineage of Hydra: a Stem Cell System that Arose Early in Evolution, *Journal of Cell Science*, vol. 109, pp. 1155-1164.

Bosch T.C.G. (2008). Stem Cells in Immortal *Hydra.*In: *Stem Cells: from Hydra to Man.* Bosch T.G.H., Ed. Berlin: Springer + Business Media, pp. 37–57.

Bosch T.C.G. (2009). Hydra and the evolution of stem cells. *BioEssays*, vol. 31, pp. 478–486.

Brown F.D., Keeling E.L., Le A.D., Swalla B.J. (2009). Whole body regeneration in a colonial ascidian, *Botrylloides violaceus*. J. Exp. Zool., *B Molec. Devel. Evol.*, vol.312, pp.885-900.

Buss, L.W. (1987). *The Evolution of Individuality*. Princeton University Press: Princeton, NJ, p. 203.

Buss L.W. (1999). Slime Molds, Ascidians and the Utility of Evolutionary Theory. *Proc. Natl. Acad. Sci.* USA, vol. 96, pp. 8801–8803.

Buss L.W., Green D R. (1985). Histoincompatibility in vertebrates: the relict hypothesis. *Dev. Comp. Immunol.*, vol. 9, pp. 191–201.

Cameron R.A., Peterson K.J., Davidson E.H. (1998). Developmental Gene Regulation and the Evolution of Large Animal Body Plans. *Amer. Zool.*, vol. 38, pp. 609-620.

Castrillon D.H., Quade B.J., Wang T.Y., Quigley C., Crum C.P. 2000. The human VASA gene is specifically expressed in the germ cell lineage. *Proc Natl Acad Sci USA.*, vol. 97, pp. 9585–9590.

Chambers I., Colby D., Robertson M., Nichols J., Lee S., Tweedie S., Smith A. (2003). Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. *Cell,* vol. 113, pp. 643–55.

Chambers I., Silva J., Colby D., Nichols J., Nijmeijer B., Robertson M., Vrana J., Jones K., Grotewold L., Smith A. (2007). Nanog safeguards pluripotency and mediates germline development. *Nature*, vol. 450, pp. 1230-1235.

Chuma S., Hosokawa M., Kitamura K., Kasai S., Fujioka M., Hiyoshi M., Takamune K., Noce T., Nakatsuji N. (2006). Tdrd1/Mtr-1, a tudor-related gene, is essential for male germ-cell differentiation and nuage/germinal granule formation in mice. *PNAS USA*, vol. 103, pp. 15894-15899.

Cinalli, R.M., Rangan, P. & Lehmann, R. (2008). Germ Cells Are Forever. *Cell*, vol. 132, pp. 559-562.

Ciosk R., DePalma M., Priess J.R. (2006). Translational regulators maintain totipotency in the Caenorhabditis elegans germline. *Science*, vol. 311, pp. 851-853.

Collins A.G., Valentine J.W. (2001). Defining Phyla: Evolutionary Pathways to Metazoan Body Plans, *Evol. Devel.*, vol. 3, no 6, pp. 432-442.

Coward S.J. (1974). Chromatoid Bodies in Somatic Cells of the Planarian: Observation on Their Behavior During Mitosis. *Anat. Rec., vol*. 180, pp. 533–546.

Cox D.N., Chao A., Baker J., Chang L., Qiao D., Lin H. (1998). A novel class of evolutionarily conserved genes defined by piwi are essential for stem cell self-renewal. *Genes Dev.* 12, pp. 3715-3727.

Craig S.F., Slobodkin L.B., Wray G.A., and Biermann C.H. (2005). The 'Paradox' of Polyembryony: A Review of the Cases and a Hypothesis for Its Evolution. *Evol. Devel.*, vol. 11, pp. 127–143.

Davidson E.H., Peterson K.J., Cameron R.A. (1995). Origin of bilaterian body plans: evolution of developmental regulatory mechanisms. *Science,* vol. 270, pp. 1319-1325.

Davidson E.H. (2006). *The Regulatory Genome: Gene Regulatory Networks in Development and Evolution*. San Diego: Academic Press, p. 289.

De Felici M., Farini D., Dolci S. (2009). In or out stemness: comparing growth factor signalling in mouse embryonic stem cells and primordial germ cells. *Curr. Stem Cell Res. Ther.*, vol. 4, pp. 87-97.

De Los Angeles A., Loh Y.-H., Tesar PJ., Daley G.Q. (2012). Accessing naïve human pluripotency. *Curr. Opin. Genet. Devel.*, vol. 22, pp.. 272–282.

De Mulder K., Kuales G., Pfister D., Willems M., Egger B., Salvenmoser W., Thaler M., Gorny AK., Hrouda M., Borgonie G., Ladurner P. (2009a). Characterization of the stem cell system of the acoel *Isodiametra pulchra*. *BMC Dev. Biol.*, vol. 9, pp. 69.

De Mulder K., Pfister D., Kuales G., Egger B., Salvenmoser W., Willems M., Steger J., Fauster K., Micura R., Borgonie G., Ladurner P. (2009b). Stem cells are differentially regulated during development, regeneration and homeostasis in flatworms. *Dev. Biol.*, vol. 334, pp. 198-212.

Denker E., Manuel M., Leclère L., Le Guyader H., Rabet N. (2008). Ordered progression of nematogenesis from stem cells through differentiation stages in the tentacle bulb of *Clytia hemisphaerica* (Hydrozoa, Cnidaria). *Dev. Biol.*, vol. 315, pp. 99–113.

Dill K.K., Seaver E.C. (2008). Vasa and nanos are coexpressed in somatic and germ line tissue from early embryonic cleavage stages through adulthood in the polychaete Capitella sp.I. *Dev. Genes. Evol.*, vol. 218, pp. 453-463.

Do J.T., Schöler H.R. (2009). Regulatory circuits underlying pluripotency and reprogramming. *Trends Pharmacol. Sci.*, vol. 30, pp. 296-302.

Eddy E.M. (1975). Germ plasm and the differentiation of the germ cell line. *Intern. Rev. Cytol.*, vol. 43, pp. 229–280.

Eguizabal C., Shovlin T.C., Durcova-Hills G., Surani A., McLaren A. (2009). Generation of primordial germ cells from pluripotent stem cells. *Differentiation,* vol. 78, pp. 116-23.

Extavour C.G.M. (2007). Evolution of the bilaterian germ line: lineage origin and modulation of specification mechanisms. *Integr. Comp. Biol.*, vol. 47, pp. 770-785.

Extavour C.G.M. (2008). Urbisexuality: The Evolution of Bilaterian Germ Cell Specification and Reproductive Systems. In: *Evolving Pathways. Key Themes in Evolutionary Developmental Biology*. Minelli A., Fusco G., Eds. Cambridge: Cambridge Univ. Press, pp. 321–342.

Extavour, C.G., Akam M. (2003). Mechanisms of Germ Cell Specification Across the Metazoans: Epigenesis and Preformation. *Development,* vol. 130, pp. 5869–5884.

Extavour C.G., Pang K., Matus D.Q., Martindale M.Q. (2005). *vasa* and *nanos* Expression Patterns in a Sea Anemone and the Evolution of Bilaterian Germ Cell Specification Mechanisms. *Evol. Dev.*, vol. 7, pp. 201–215.

Ewen-Campen B., Schwager E., Extavour C.G.M. (2010). The Molecular Machinery of Germ Line Specification. *Mol. Reprod. Dev.*, vol. 77, pp. 3–18.

Flemr M., Ma J., Schultz R.M., Svoboda P. (2010). P-Body loss is concomitant with formation of a messenger RNA storage domain in mouse oocytes. *Biol. Reprod.*, vol. 82, pp. 1008-1017.

Frank U., Plickert G., Müller W.A. (2009). Cnidarian Interstitial Cells: the Dawn of Stem Cell Research, In: *Stem Cells in Marine Organisms.* Eds. Rinkevich B., Matranga V., Springer: Dordrecht, Heidelberg, London, New York, pp. 33-59.

Funayama N. (2010). The stem cell system in demosponges: insights into the origin of somatic stem cells. *Dev. Growth Differ.*, vol. 52, pp. 1-14.

Funayama N., Nakatsukasa M., Mohri K., Masuda Y., Agata K. (2010). Piwi expression in archaecytes and choanocytes in demosponges: insights into the stem cell system in demosponges. *Evol. Dev.*, vol. 12, pp. 275-287.

Fuchs J., Martindale M.Q., Hejnol A. (2011). Gene expression in bryozoan larvae suggest a fundamental importance of pre-patterned blastemic cells in the bryozoan life-cycle. *EvoDevo.*, vol. 2:13, pp. 1-15.

Giani V.C., Yamaguchi E. Boyle M.J., Seaver E.C. (2011). Somatic and germline expression of *piwi* during development and regeneration in the marine polychaete annelid *Capitella teleta. EvoDevo*., vol. **2**:10, pp.1-10.

Glenner H., Lützen J., Takahashi T. (2003). Molecular and Morphological Evidence for a Monophyletic Clade of Asexually Reproducing Rhizocephala: *Polyascus*, New Genus (Cirripedia). *J. Crust. Res.*, vol. 23, pp. 548–557.

Goel S., Fujihara M., Minami N., Yamada M. and Imai H. (2008). Expression of NANOG, but not POU5F1, points to the stem cell potential of primitive germ cells in neonatal pig testis. *Reproduction, vol*. 135, pp. 785–795.

Guo T., Peters A.H., Newmark P.A. (2006). A Bruno-like gene is required for stem cell maintenance in planarians. *Dev. Cell,* vol. 11, pp. 159-169.

Gustafson E.A., Wessel G.M. (2010). Vasa genes: Emerging roles in the germ line and in multipotent cells. *Bioassays,* vol. 32, pp. 626-637.

Haag E.C. (2009). *Caenorhabditis* Nematodes as a Model for the Adaptive Evolution of Germ Cells. *Curr. Top. Dev. Biol.*, vol. 86, pp. 43-66.

Hagan H.R. (1951). *Embryology of the Viviparous Insects,* New York: Ronald Press Co., p. 472.

Han D.W., Greber B., Wu G., Tapia N., Araúzo-Bravo M.J., Ko K., Bernemann C., Stehling M., Schöler H.R. (2011). Direct reprogramming of fibroblasts into epiblast stem cells. *Nature Cell Biol.*. vol. 13, pp. 66-71.

Hay B., Jan L.Y., Jan Y.N. (1988). A Protein Component of *Drosophila* Polar Granules is Encoded by *vasa* and Has Extensive Sequence Similarity to ATP-dependent Helicases. *Cell,* vol. 55, pp. 577–587.

Hayashi Y., Hayashi M., Kobayashi S. (2004). Nanos suppresses somatic cell fate in Drosophila germ line. *Proc. Natl. Acad. Sci.* USA, vol. 101, pp. 10338-10342.

Hayashi Y., Saitou M., Yamanaka S. (2012). Germline development from human pluripotent stem cells toward disease modeling of infertility. *Fertil. Steril.*, vol. 97, pp. 1250-1259.

Hori I. (1982). An Ultrastructural Study of the Chromatoid Body in Planarian Regenerative Cells. *J. Electron Microsc.*, vol. 31, pp. 63–72.

Hori I., Kishida Y. (2003). Quantitative Changes in Nuclear Pores and Chromatoid Bodies Induced by Neuropeptides During Cell Differentiation in the Planarian *Dugesia japonica*. J. Submicrosc. *Cytol. Pathol.*, vol. 35, pp. 439–444.

Houston D.W., King M.L. (2000). Germ Plasm and Molecular Determinants of Germ Cell Fate, *Curr. Top. Dev. Biol.*, vol. 50, pp. 155–181.

Houwing S., Kamminga L. M., Berezikov E., Cronembold D., Girard A., van den Elst H., Filippov D. V., Blaser H., Raz E., Moens C. B. et al. (2007). A role for Piwi and piRNAs in germ cell maintenance and transposon silencing in Zebrafish. *Cell*, vol. 129, pp. 69-82.

Høeg J.T., Lützen J. (1995). Life cycle and reproduction in the Cirripedia Rhizocephala. Oceanogr. *Marine Biol.: Ann. Rev.*, vol. 33, pp. 427–485.

Høeg J.T., Glenner H., Shields J.D. (2005). Cirripedia, Thoracica and Rhizocephala (barnacles). In: *Marine Parasites.* Eds. Rohde K. Wallingford, U.K., CABI, and Collingwood, Victoria, Australia: CSIRO, pp. 154–165.

Huang H., Gao Q., Peng X., Choi S.-Y., Sarma K., Ren H., Morris A.J. and Frohman M.A. (2011). piRNA-associated germline nuage formation and spermatogenesis require MitoPLD profusogenic mitochondrial-surface lipid signaling. *Develop. Cell*, vol. 20, pp. 376–387.

Ikenishi K. (1998). Germ plasm in *Caenorabditis elegans*, *Drosophila* and *Xenopus*, *Devel. Growth Differ., vol.* 40, pp. 1–10.

Isaeva V.V. (2010). The diversity of ontogeny in animals with asexual reproduction and plasticity of early development. *Russ. J. Devel. Biol.*, vol. 41, pp. 285-295

Isaeva V.V. (2011). Pluripotent gametogenic stem cells of asexually reproducing invertebrates. In: *Embryonic Stem Cells – Basic Biology to Bioengineering.* Ed. Kallos M.S. Rijeka: Intech, pp. 449-478.

Isaeva V.V., Akhmadieva A.V. (2011). Germinal granules in archaeocytes of the sponge *Oscarella malakhovi* Ereskovski 2006. *Russ. J. Marine Biol.*, vol. 37, pp. 209-216.

Isaeva V.V., Shukalyuk A.I., Akhmadieva A.V. (2008). Stem Cells in Reproductive Strategy of Asexually Reproducing Invertebrates. *Russ. J. Marine Biol.*, vol. 34, pp. 1–8.

Isaeva V.V., Akhmadieva A.V., Aleksandrova Y.N., Shukalyuk A.I. (2009). Morphofunctional Organization of Reserve Stem Cells Ensuring Asexual and Sexual Reproduction of Invertebrates, *Russ. J. Devel. Biol.*, vol. 40, pp. 57–68.

Isaeva V.V., Akhmadieva A.V., Shukalyuk A.I. (2012). The hidden coloniality at the parasitic stage in *Peltogaster reticulatus* (Crustacea: Rhizocephala). *J. Mar. Biol. Assoc.* UK, vol. 2, pp. 457-462.

Isaeva V.V., Akhmadieva A.V., Aleksandrova Y.N., Shukalyuk A.I., Chernyshev A.V. (2011). Germinal granules in interstitial cells of the colonial hydroids *Obelia longissima* Pallas, 1766 and *Ectopleura crocea* Agassiz, 1862. *Russ. J. Marine Biol.*, vol. 37, pp. 303-310.

Isaeva V., Alexandrova Y., Reunov A. (2005). The Interaction Between Chromatoid Bodies and Mitochondria in Neoblasts and Gonial Cells of Asexual and Spontaneously Sexualized Planarian *Girardia* (*Dugesia*) *tigrina. Invertebr. Reprod. Dev*., vol. 48, pp. 119–128.

Isaeva V.V., Shukalyuk A.I., Kizilova E.A. (2003). Revealing of Stem Cells in Colonial Interna of the Rhizocephalans *Peltogasterella gracilis* and *Sacculina polygenea* at the Parasitic Stage of Their Life Cycle. *Tsitologiya*, vol. 45, no. 8, pp. 758–763.

Isaeva V.V., Shukalyuk A.I., Korn O.M., Rybakov A.V. (2004). Development of Primordial Externae in the Colonial Interna of Polyascus Polygenea (Crustacea: Cirripedia: Rhizocephala). *Crust. Res.*, no. 33, pp. 61–71.

Ivanova-Kazas O.M. (1996). Blastogenesis, Cormogenesis, and Evolution. *Russ. J. Marine Biol.*, vol. 22, no. 5, pp. 285–294.

Jenner R.A. (2000). Evolution of Animal Body Plan: the Role of Metazoan Phylogeny at the Interface between Pattern and Process. *Evol. Devel.*, vol. 2, no 4, pp. 208-221.

Jenner R. Evo-devo's identity: from model organisms to developmental types. (2008). In: *Evolving Pathways: Key Themes in Evolutionary Developmental Biology.* Eds. Minelli A., Fusco G. Cambridge, New York e.a.: Cambridge University Press, pp. 100-120.

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

Juliano C.E., Voronina E., Stack C., Aldrich M., Cameron A.R., Wessel G.M. (2006). Germ line determinants are not localized early in sea urchin development, but do accumulate in the small micromere lineage. *Dev. Biol.*, vol. 300, pp. 406-415.

Juliano C.E., Swartz S.Z., Wessel G.M. (2010). A conserved germline multipotency program. *Development*, vol. 137, pp. 4113-4126.

Juliano C., Wessel G. (2010). Versatile germline genes. *Science*, vol. 329, pp. 640–641.

Kadyrova L.Y., Habara Y., Lee T.H., Wharton R.P. (2007). Translational control of maternal Cyclin B mRNA by Nanos in the Drosophila germline. *Development*, vol. 134, pp. 1519-1527.

Kelley K., Lin S.-L. (2012). Induction of somatic cell reprogramming using the microRNA miR-302. *Progress in Molecular Biology*, Elsevier Inc. and Translational Science, vol. 111, pp. 83-107.

Kloc M., Bilinski S., Etkin L.D. (2004). The Balbiani Body and Germ Cell Determinants: 150 Years Later. *Curr. Top. Dev. Biol.*, vol. 59, pp. 1–36.

Kobayashi S., Yamada M., Asaoka M., Kitamura T. (1996). Essential role of the posterior morphogen nanos for germline development in Drosophila. *Nature*, vol. 380, pp. 708–711.

Korn O.M., Shukalyuk A.I., Trofimova A.V., Isaeva V.V. (2004). Reproductive stage of the life cycle in the rhizocephalan barnacle Polyascus polygenea (Crustacea: Cirripedia). *Russ. J. Marine Biol.*, vol. 30, pp. 328-340.

Kotaja N., Bhattacharyya S.N., Jaskiewicz L., Kimmins S., Parvinen M., Filipowicz W., Sassone-Corsi P. (2006). The chromatoid body of male germ cells: Similarity with processing bodies and presence of Dicer and microRNA pathway components. *PNAS*, vol. 103, pp. 2647-2652.

Kranz A.M., Tollenaere A., Norris B.J., Degnan B.M., Degnan S.M. (2010). Identifying the germline in an equally cleaving mollusc: Vasa and Nanos expression during embryonic and larval development of the vetigastropod Haliotis asinina. *J. Exp. Zool. B. Mol. Dev. Evol.*, vol. 314, pp. 267-279.

Kuramochi-Miyagawa S., Watanabe T., Gotoh K., Takamatsu K., Chuma S., Kojima-Kita K., Shiromoto Y., Asada N., Toyoda A., Fujiyama A. et al. (2010). MVH in piRNA processing and gene silencing of retrotransposons. *Genes Dev.*, vol. 24, pp. 887-892.

Kuratani S., Schilling T. (2008). Head segmentation in vertebrates. *Integr. Comp. Biol.*, vol. 48, pp. 604-610.

Laird D.J., De Tomaso, A.W., Weissman I.L. (2005). Stem Cells Are Units of Natural Selection in a Colonial Ascidian. *Cell,* vol. 123, pp. 1351–1360.

Leatherman J.L., Jongens T.A. (2003). Transcriptional Silencing and translational control: Key features of early germline development. *BioEssays,* vol. 25, pp. 326-335.

Le Douarin N.M., Creuzet S., Couly G., Dupin E. (2004). Neural crest cell plasticity and its limits. *Development,* vol. 131, pp. 4637-4650.

Lim A.K., Kai T. (2007). Unique germ-line organelle, nuage, functions to repress selfish Genetic elements in *Drosophila melanogaster*. *Proc. Nat. Acad. Sci.* USA, vol. 104, pp. 6714-6719.

Loughry W.C., Prodöhl P.A., McDonough C.M., Avise J.C. (1998). Polyembryony in Armadillos, *Am. Sci.*, vol. 86, pp. 274–279.

Mak A.B., Blakely K.M., Williams R.A., Penttilä P.-A., Shukalyuk A.I., Osman K.T., Kasimer D., Ketela T., Moffat J. (2011). CD133 Protein *N*-Glycosylation Processing Contributes to Cell Surface Recognition of the Primitive Cell Marker AC133 Epitope. *J. Biol. Chem.*, vol. 286, pp. 41046-41056.

Mahowald A.P. (1971). Polar granules in *Drosophila.* III. The continuityof polar granules during the life cycle of *Drosophil. J. Exp. Zool.*, vol. 170, pp. 551–563.

Mahowald A.P. (2001). Assembly of the *Drosophila* germ plasm. *Intern. Rev. Cytol.*, vol. 203, pp. 187-213.

Martín-Durán J.M., Monjo F., Romero R. (2012). Planarian embryology in the era of comparative developmental biology *Int. J. Dev. Biol.*, vol. 56, pp. 39-48.

Matova N., Cooley L. (2001). Comparative Aspects of Animal Oogenesis. *Dev. Biol.*, vol. 16, pp. 1–30.

Matsui Y., Zsebo K., Hogan B. L. (1992). Derivation of pluripotential embryonic stem cells from murine primordial germ cells in culture. *Cell,* vol. 70, pp. 841-847.

Minoux M., Rijli F.M. (2010). Molecular mechanisms of cranial neural crest cell migration and patterning in craniofacial development *Development,* vol. 137, pp. 2605-2621.

Mochizuki K., Nishimiya-Fujisawa C., Fujisawa T. (2001). Universal Occurrence of the *vasa*-Related Genes Among Metazoans and Their Germline Expression in *Hydra*. Dev. Genes Evol., vol. 211, pp. 299–308.

Mochizuki K., Sano H., Kobayashi S., Nishimiya-Fujisawa C., Fujisawa T. (2000). Expression and evolutionary conservation of nanos-related genes in Hydra. *Dev. Genes Evol.*, vol. 210, pp. 591-602.

Modzelewski A.J., Holmes R.J., Hilz S., Grimson A., and Cohen P.E. (2012). AGO4 regulates entry into meiosis and influences silencing of sex chromosomes in the male mouse germline. *Develop. Cell,* vol. 23, pp. 251–264.

Muraro N.I., Weston A.J., Gerber A.P., Luschnig S., Moffat K.G., Baines R.A. (2008). Pumilio binds para mRNA and requires Nanos and Brat to regulate sodium current in *Drosophila* motoneurons. *J. Neurosci.*, vol. 28, pp. 2099–2109.

Nakahata S., Kotani T., Mita K., Kawasaki T., Katsu Y., Nagahama Y., Yamashita M. (2003). Involvement of Xenopus Pumilio in the translational regulation that is specific to cyclin B1 mRNA during oocyte maturation. *Mech. Dev.*, vol. 120, pp. 865-880.

Nakamura A., Seydoux G. (2008). Less is more: specification of the germline by transcriptional repression. *Development*, vol. 135, pp. 3817-3827.

Nichols J., Smith A. (2009). Naive and Primed Pluripotent States. *Cell Stem Cell*, vol. 4, pp. 487-492.

Nielsen C. (1971). Entoproct life-cycles and the entoproct/ectoproct relationship. Ophelia, vol. 9, pp. 209–341.

Noda K., Kanai C. (1977). An Ultrastructural Observation of *Pelmatohydra robusta* at Sexual and Asexual Stages, with a Special Reference to "Germinal Plasm". *J. Ultrastruct. Res.*, vol. 61, pp. 284–294.

Northcutt R.G., Gans C. (1983). The genesis of neural crest and epidermal placodes: a reinterpretation of vertebrate origins. *Quart. Rev. Biol.*, vol. 58, pp. 1–28.

Nussbaum M. (1880). Zur Differenzierung des Geschlechts im Tierreich. *Arch. Mikr. Anat.* B., vol. 18, pp. 1–121.

Nüsslein-Volhard C., Frohnhüfer H.G., Lehmann R. (1987). Determination of anteroposterior polarity in Drosophila. *Science*, vol. 238, pp. 1675–1681.

O'Donnell K.A., Boeke J. D. (2007). Mighty Piwis defend the germline against genome intruders. *Cell*, vol. 129, pp. 37-44.

Ohinata Y., Hiroshi O., Shigeta M., Yamanaka K., Wakayama T., Saitou M. (2009). A signaling principle for the specification of the germ cell lineage in mice. *Cell*, vol. 137, pp. 571-584.

Oyama A., Shimizu T. (2007). Transient occurrence of vasa-expressing cells in nongenital segments during embryonic development in the oligochaete annelid *Tubifex tubifex*. *Dev. Genes Evol.*, vol. 217, pp. 675-90.

Pancer Z., Gershon H., Rinkevich B. (1995). Coexistence and Possible Parasitism of Somatic and Germ Cell Lines in Chimeras of the Colonial Urochordate *Botryllus schlosseri*. *Biol. Bull.*, vol. 189, pp. 106–112.

Parvinen M. (2005). The chromatoid body in spermatogenesis. *Int. J. Androl.*, vol. 28, pp. 189-201.

Pepling M.E. (2010). A novel maternal mRNA storage compartment in mouse oocytes. *Biol. Reprod*, vol. 82, pp. 807-808.

Peter I.S., Davidson E.H. (2011). Evolution of gene regulatory networks controlling body plan development. *Cell*, vol. 144, pp. 970-985.

Peters L., Meister G. (2007). Argonaute proteins: mediators of RNA silencing. *Mol. Cell*, vol. 26, pp. 611–623.

Peterson K.J., Cameron R.A., Davidson E.H. (1997). Set-aside cells in maximal indirect development: evolutionary and developmental significance. *BioEssays.*, vol. 19, pp. 623-631.

Pfister D., De Mulder K., Hartenstein V., Kuales G., Borgonie G., Marx F., Morris J., Ladurner P. (2008). Flatworm stem cells and the germ line: developmental and evolutionary implications of macvasa expression in Macrostomum lignano. *Dev. Biol.*, vol. 319, pp. 146-159.

Piraino S., Boero F., Aeschbach B., Schmid V. (1996). Reversing the life cycle: Medusae transforming into polyps and cell transdifferentiation in *Turritopsis nutricula* (Cnidaria, Hydrozoa). *Biol. Bull.*, vol. 190, pp 302–312.

Raff R.A. (2008). Origins of the other metazoan body plans: the evolution of larval forms. *Philos. Trans. R. Soc. Lond. B. Biol Sci.*, vol. 363, pp 1473-1479.

Raff R.A., Raff E.C. (2009). Evolution in the light of embryos: seeking the origins of novelties in ontogeny. In: *Form and Function in Developmental Evolution* / Eds. Laubichler M.D., Maienschein J. Cambridge e a.: Cambridge University Press, pp. 83-111.

Raz E. (2000). The Function and Regulation of the *vasa*-like Genes in Germ-Cell Development. *Genome Biology*, vol. 3, pp. 1017.1-1017.6.

Rebscher N., Lidke A.K., Ackermann C.F. (2012). Hidden in the crowd: primordial germ cells and somatic stem cells in the mesodermal posterior growth zone of the polychaete Platynereis dumerillii are two distinct cell populations. *EvoDevo.*, vol. 3:9, pp.1-10.

Rebscher N., Volk C., Teo R., Plickert G. (2008). The germ plasm component vasa allows tracing of the interstitial stem cells in the cnidarian Hydractinia echinata. *Dev. Dyn.*, vol. 237, pp. 1736-1745.

Rebscher N., Zelada-González F., Banish T., Raible F., Arendt D. (2007). Vasa Unveils a Common Origin of Germ Cells and of Somatic Stem Cells from the Posterior Embryonic Stem Cells Growth Zone in the Polychaete *Platynereis dumerlii*. *Dev. Biol.*, vol. 306, pp. 599-611.

Reddien P.W., Oviedo N.J., Jennings J.R., Jenkin J.C., Sánchez Alvarado A. (2005). SMEDWI-2 is a PIWI-like protein that's regulates planarian stem cells. *Science*, vol. 310, pp 1327–1330.

Rinkevich Y., Matranga V., Rinkevich B. (2009). Stem Cells in Aquatic Invertebrates: Common Premises and Emerging Unique Themes. In: *Stem Cells in Marine Organisms.* Dordrecht; Heidelberg: Springer, pp. 61–104.

Rocak S., Linder P. (2004). DEAD-box proteins: the driving forces behind RNA methabolism. *Rev. Molec. Cell Biol.*, vol. 5, pp. 232–240.

Rosner, A., Moiseeva, E., Rinkevich, Y., Lapidot, Z., Rinkevich, B. (2009). Vasa and the germ line lineage in a colonial urochordate. *Devel. Biol.*, vol. 331, pp. 113-128.

Rossi L., Salvetti A., Marincola F.M., Lena A., Deri P., Mannini L., Batistoni R., Wang E., Gremigni V. (2007). Deciphering the molecular machinery of stem cells: a look at the neoblast gene expression profile. *Genome Biol.*, vol. 8, pp. R62.

Rouhana L., Shibata N., Nishimura O., Agata K. (2010). Different requirements for conserved post-transcriptional regulators in planarian regeneration and stem cell maintenance. *Dev. Biol.*, vol. 341, pp. 429-443.

Salvetti A., Rossi L., Lena A., Batistoni R., Deri P., Rainaldi G., Locci M. T., Evangelista M., Gremigni V. (2005). DjPum, a homologue of Drosophila Pumilio, is essential to planarian stem cell maintenance. *Development*, vol. 132, pp. 1863-1874.

Schwager E.E., Extavour C.G.M. (2010). The molecular machinery of germ line specification. *Mol. Reprod. Dev.*, vol. 77, pp 3-18.

Seipel K., Yanze N., Schmid V. (2004). The germ line and somatic stem cell gene Cniwi in the jellyfish *Podocoryne carnea*. *Int. J. Dev. Biol.*, vol. 48, pp. 1-7.

Seydoux G., Braun R.E. (2006). Pathway to Totipotency: Lessons from Germ Cells. *Cell*, vol. 127, pp. 891-904.

Sharma A.K., Nelson M.C., Brandt J.E., Wessman M., Mahmud N., Weller K.P., Hoffman R. (2001). Human CD34(+) stem cells express the hiwi gene, a human homologue of the Drosophila gene piwi. *Blood,* vol. 97, pp. 426-434.

Shibata N., Umesono Y., Orii H., Sakurai T., Watanabe K., Agata K. (1999). Expression of vasa(vas)-related genes in germline cells and totipotent somatic stem cells of planarians. *Dev. Biol.,* vol. 206, pp. 73-87.

Shostak S. (2008). Speculation on the evolution of stem cells. *Breast Dis.,* vol. 29, pp. 3-13.

Shukalyuk A., Isaeva V.V. (2012). Molecular and sub-cellular gametogenic machinery of stem and germline cells across Metazoa. Current Frintiers and Perspestives in Cell. *Biology.* Ed. Najman S. Rijeka: Intech, pp. 449-478.

Shukalyuk A.I., Stanford W.L. (2008). Germ plasm signature in embryonic stem cells. *Proceeding of the International Society for Stem Cell Research, 6th Annual Meeting,* June 11-14, Philadelphia, USA, p. 353 N 238.

Shukalyuk A.I., Golovnina K., Baiborodin S., Gunbin K., Blinov A., Isaeva V. (2007). *vasa*-related genes and their expression in stem cells of colonial parasitic rhizocephalan barnacle *Polyascus polygenea* (Arthropoda: Crustacea: Cirripedia: Rhizocephala). *Cell Biology International,* vol. 31, no. 2, pp. 97-108.

Shukalyuk A.I., Isaeva V.V., Kizilova E., Baiborodin S. (2005). Stem cells in reproductive strategy of colonial rhizocephalan crustaceans (Crustacea: Cirripedia: Rhizocephala). *Invert. Reprod. Devel.,* vol. 48, pp. 41-53.

Silva J., Jennifer N., Theunissen T.W., Guo G., van Oosten A.L., Barrandon O., Wray J., Yamanaka S., Chambers I., Smith A. (2009). Nanog is the gateway to the pluripotent ground state. *Cell,* vol. 138, pp. 722–737.

Sköld H.N., Obst M., Sköld M., Åkesson B. (2009). Stem Cells in Asexual Reproduction of Marine Invertebrates In: *Stem Cells in Marine Organisms,* Dordrecht; Heidelberg: Springer, pp. 105–137.

Snee M.J., Macdonald P.M. (2004). Live imaging of nuage and polar granules: evidence against a precursor-product relationship and a novel role for Oskar in stabilization of polar granule components. *J. Cell Sci.,* vol. 117, pp. 2109-2120.

Strome S., Lehman R. (2007). Germ Versus Soma Decisions: Lessons from Flies and Worms, *Science,* vol. 316, pp. 392–393.

Solana J., Lasko P., Romero R. (2009). Spoltud-1 is a chromatoid body component required for planarian long-term stem cell self-renewal. *Dev. Biol.,* vol. 328, pp. 410-421.

Srouji J., Extavour C. (2011). Redefining Stem Cells and Assembling Germ Plasm: Key Transition in the Evolution of the Germ Plasm. In: *Key transition in animal evolution,* Eds. DeSalle R., Schierwater B. Science Publishers: New York, Abingdon, pp. 360-397.

Su T.T., Campbell S.D., O'Farrell P.H. (1998). The cell cycle program in germ cells of the Drosophila embryo. *Dev. Biol.,* vol. 196, pp. 160-170.

Sugio M., Takeuchi K., Kutsuna J., Tadokoro R., Takahashi Y., Yoshida-Noro C., Tochinai S. (2008). Exploration of embryonic origins of germline stem cells and neoblasts in *Enchytraeus japonensis* (Oligochaeta, Annelida). *Gene Expr. Patterns,* vol. 8, pp. 227-36.

Sunanaga N., Saito Y., Kawamura K. (2006). Postembryonic Epigenesis of Vasa_Positive Germ Cells from Aggregated Hemoblasts in the Colonial Ascidian, *Botryllus primigenus*. *Devel. Growth. Differ.,* vol. 48, pp. 87–100.

Tadokoro R., Sugio M., Kutsuna J., Tochinai S., Takahashi Y. (2002). Early segregation of germ and somatic lineages during gonadal regeneration in the annelid *Enchytraeus japonensis*. *Curr. Biol.,* vol. 16, pp. 1012-1017.

Takamura K., Fujimara M., Yamagushi Y. (2002). Primordial germ cells originate from the endodermal strand cells in the ascidian Ciona intestinalis. *Dev. Genes Evol.,* vol. 212, pp. 11-18.

Tanaka S., Dan K. (1990). Study of the lineage and cell cycle of small micromeres in embryos of the sea urchin, *Hemicentrotus pulcherrimus*. *Dev. Growth Differ.,* vol. 32, pp. 145-156.

Tanaka S.S., Toyooka Y., Akasu R., Katoh-Fukui Y., Nakahara Y., Suzuki R., Yokoyama M., Noce T. (2000). The mouse homolog of *Drosophila Vasa* is required for the development of male germ cells *Genes Dev.,* vol. 14, pp. 841-853.

Teng L., Labosky P.A. (2006). Neural crest stem cells. *Adv. Exp. Med. Biol.,* vol. 589, pp. 206-12.

Thomas M.B., Edwards N.C. (1991). Cnidaria: Hydrozoa. In: *Microscopic Anatomy of Invertebrates*, vol. 2: Placozoa, Porifera, Cnidaria, and Ctenophora. Harrison F.W., Westfall J.A., Eds. New York: Wiley_Liss, pp. 91–183.

Thomas S., Thomas M., Wincker P., Babarit C., Xu P., Speer M.C., Munnich A., Lyonnet S., Vekemans M., Etchevers H.C. (2008). Human neural crest cells display molecular and phenotypic hallmarks of stem cells. *Human Molec. Genet.,* vol. 17, pp. 3411–3425.

Thomson T., Lin H. (2009). The biogenesis and function of PIWI proteins and piRNAs: progress and prospect. *Ann. Rev. Cell Dev. Biol.,* vol. 25, pp. 355-376.

Tokin, B.P. (1959). *Regeneratsiya i Somaticheskii Embriogenez (in Russian): Regeneration and Somatic Embryogenesis.* Leningrad: Leningrad. State Univ., (OCoLC) 621073447, p. 267.

Toyooka Y., Tsunekawa N., Akasu R., Noce N. (2003). Embryonic stem cells can form germ gells *in vitro*. *PNAS* USA, vol. 100, pp. 11457–11462.

Trainor P.A., Melton K.R., Manzanares M. (2003). Origins and plasticity of neural crest cells and their roles in jaw and craniofacial evolution. *Int. J. Dev. Biol.,* vol. 47, pp. 541-553.

Travis J. (2007). A Close Look at Urbisexuality. *Science,* vol. 316, pp. 390–391.

Tropere V., Hitoshi S., Sigard C., Mak T.W., Rossant J., van der Kooy D. (2001). Direct neural fate specification from embryonic stem cells: a primitive mammalian neural stem cell stage acquired through a default mechanism. *Neuron,* vol. 30, pp. 65-78.

Vagin V.V., Wohlschlegel J., Qu J., Jonsson Z., Huang X., Chuma S., Girard A., Sachidanandam R., Hannon G.J., Aravin A.A. (2009). Proteomic analysis of murine Piwi proteins reveals a role for arginine methylation in specifying interaction with Tudor family members. *Genes Dev.,* vol. 23, pp. 1749–1762.

Voronina E., Lopez M., Juliano C.E., Gustafson E., Song J.L., Extavour C., George S., Oliveri P., McClay D., Wessel G. (2008). Vasa protein expression is restricted to the small micromeres of the sea urchin, but is inducible in other lineages early in development. *Devel. Biol.,* vol. 314, pp. 276-286.

Wang J., Mitreva M., Berriman M., Thorne A., Magrini V., Koutsovoulos G., Kumar S., Blaxter M.L., Davis R.E. (2012). Silencing of germline-expressed genes by DNA elimination in somatic Cells. *Develop. Cell,* vol. 23, pp. 1072–1080.

Watanabe T., Chuma S., Yamamoto Y., Kuramochi-Miyagawa S., Totoki Y., Toyoda A., Hoki Y., Fujiyama A., Shibata T., Sado T., Noce T., Nakano T., Nakatsuji N., Lin H.,

Sasaki1 H. (2011). MITOPLD is a mitochondrial protein essential for nuage formation and piRNA biogenesis in the mouse germline. *Develop. Cell,* vol. 20, pp. 364–375.

Watanabe H., Hoang V.T., Mättner R., Holstein T.W. (2009). Immortality and the base of multicellular life: Lessons from chidarian stem cells. *Semin. Cell Devel. Biol.,* vol. 20, pp. 1114-1125.

Weismann A. (1883). *Die Entstehung der Sexualzellen bei den Hydromedusen.* Zugleich ein Beitrag zur Kenntniss des Baues und der Lebenserscheinungen dieser Gruppe, Jena: Fischer, p.295.

Weismann A. (1892). *Das Keimplasma. Eine Theorie der Vererbung*, Jena: Verlag von Gustav Fischer, p. 628.

Weismann A. (1893). *The Germ Plasm.* A Theory of Heredity, New York: Charles Scribner's Sons, p. 477.

Weissman I.L. (2000). Stem Cells: Units of Development, Units of Regeneration, and Units in Evolution, *Cell,* vol. 100, pp. 157–168.

Western P.S., van den Bergen J.A., Miles D.C., Sinclair A.H. (2010). Male fetal germ cell differentiation involves complex repression of the regulatory network controlling pluripotency. *FASEB J.,* vol. 24, pp. 3026-3035.

Wu H.R., Chen Y.T., Su Y.H., Luo Y.J., Holland L.Z., Yu J.K. (2011). Asymmetric localization of germline markers Vasa and Nanos during early development in the amphioxus Branchiostoma floridae. *Dev. Biol.,* vol. 353, pp. 147-59.

Yoshida-Noro C., Toshinai S. (2010). Stem cell system in asexual and sexual reproduction of Enchytraeus japonensis (Oligochaeta, Annelida). *Dev. Growth Differ.,* vol. 52, pp. 43-55.

Yu J., Thomson J.A. (2008). Pluripotent stem cell lines. *Genes Dev.,* vol. 22, pp. 1987–1997.

Zhu S., Li W., Zhou H., Wei W., Ambasudhan R., Lin T., Kim J., Zhang K., Ding S. (2010). Reprogramming of Human Primary Somatic Cells by OCT4 and Chemical Compounds *Cell Stem Cell,* vol. 7, pp. 651-655.

Zwaka T.P., Thomson J.A. (2005). A germ cell origin of embryonic stem cells? *Development,* vol. 132, pp. 227-233.