

Primordial germ cell biology at the beginning of the XXI Century[#]

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ABSTRACT At the *XIV Workshop on the Development and Function of the Reproductive Organs* held at the Congress Centre of the University of Rome Tor Vergata, Monteporzio Catone, Rome, Italy, the introduction to the first session entitled “*Mammalian primordial germ cells*” dedicated to the memory of Anne McLaren, was the occasion for a concise review of the state of art of research on the biology of primordial germ cells (PGCs). This great, unforgettable scientist, who died in a car accident in July 2007, dedicated most of her studies to this field over the last 25 years. Topics briefly reviewed in this Meeting Report are: 1) how the germ line is determined; 2) what are the mechanisms underlying PGC migration; 3) to what extent PGC survival, proliferation and differentiation are cell autonomous or environmentally controlled processes and 4) how the potential for totipotency is retained in PGCs.

KEY WORDS: *primordial germ cell, epigenetics, cell migration, meiosis*

At the *XIV Workshop on the Development and Function of the Reproductive Organs* held at the Congress Centre of the University of Rome Tor Vergata, Monteporzio Catone, Rome, Italy, the introduction to the first session entitled “*Mammalian primordial germ cells*” dedicated to the memory of Anne McLaren, was the occasion for a concise review of the state of art of research on the biology of primordial germ cells (PGCs). This great, unforgettable scientist, who died in a car accident in July 2007, dedicated most of her studies to this field over the last 25 years.

At the beginning of the 1980s, our understanding of the biology of mammalian PGCs was limited to the morphological description of their origin in the gonads, and their migration and differentiation within the gonadal ridges. In the early 1980s, Anne McLaren, as director of the MRC Mammalian Development Unit in London, decided to begin a series of studies aimed at identifying the cellular and the molecular bases of PGC development in the mouse. In an influential 1981 book entitled “*Germ Cells and Soma: a new look to an old problem*”, (McLaren, 1983), Anne put forward and focused many of the main questions about PGC biology which were to be subsequently addressed by her and others working on mouse PGCs during the following years up to the present. Basically, the problems could be classified into the following topics: 1) how the germ line is determined in the early embryo; 2) the mechanism by which PGCs migrate from the site

of their formation to the gonadal ridges; 3) the extent to which PGC survival, proliferation and differentiation is cell autonomous or controlled by environmental signals; the underlying signals and 4) how the potential for totipotency is retained in PGCs.

The period of PGC development in the mouse embryo lasts about 7 days from when around 7 days *post coitum* (dpc), PGC precursors located in the proximal posterior epiblast become committed to the germ line, to when PGCs around 13.5 dpc differentiate within the gonads into oocytes or prospermatogonia (Fig. 1). During this period, crucial processes occur in PGCs at well defined times, including specification, migration, proliferation, entering into meiosis or epigenetic gene regulation, the latter occurring throughout the entire developmental period, so that PGCs can be regarded as a heterogeneous cell population

Abbreviations used in this paper: BMP, bone morphogenetic protein; CXCR4, CXC chemokine receptor 4; ECM, extracellular matrix; KL, KIT ligand; MIS, meiotic inducing substance; PGCs, primordial germ cells; MPS, meiosis-preventing substance; OCT4, octamer4; PRDM1 or -14, PR domain containing 1 or 14; PRMT5, protein arginine methyltransferase5; PTEN, phosphatase and tensin homolog; RA, retinoic acid; SF-1, splicing factor1; SMAD 1-5, mothers against decapentaplegic (MAD); Sox2, SRY sex determining region Y-box 2; SSEA-1, stage specific embryonic antigen -1; STRA8, stimulated by retinoic acid gene-8; TNAP, tissue non-specific alkaline phosphatase.

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undergoing continuous change throughout all stages of their development.

PGC specification

This is certainly the topic in which the most impressive advances have recently occurred (for a review, see McLaren and Lawson, 2005; Hayashi *et al.*, 2007). Briefly, we now know that at 5-6 dpc, the pluripotent proximal epiblast cells respond to bone morphogenetic protein (BMP) signals from extraembryonic tissues that primed them to become mesoderm cells. Roughly 12 hours later, about 6 clustered E-cadherin/fragilis expressing cells located in the posterior side of the proximal epiblast, receiving the highest dose of BMP-4 and expressing *Smad1-5*, are restricted to the germ cell lineage following *Prdm1* (*Blimp1*) and *Prdm14* expression. Finally, PGC precursors move along extraembryonic mesoderm cells at the base of the allantois where they are specified as PGCs following the expression of stella and other PGC markers such as TNAP, kit and SSEA-1 (Fig. 2).

At the genomic level, PGC specification involves at least three key events: repression of the somatic mesodermal program, reacquisition of potential pluripotency and genome-wide epigenetic reprogramming (Yamaji *et al.*, 2008). PRDM1 associated to the methyltransferase PRMT5 seems to be critical for the repression of the somatic mesodermal program, likely through symmetrical methylation of arginine 3 on histones H4 and H2A. PRDM14 is essential for the reacquisition of potential pluripotency indicated by *Sox2* and *Nanog* expression and genome-wide epigenetic reprogramming marked by several modifications of histones associated with a repressed or active chromatin state. OCT4 activated by a different way cooperates with SOX2 and nanog for pluripotency (Fig. 3).

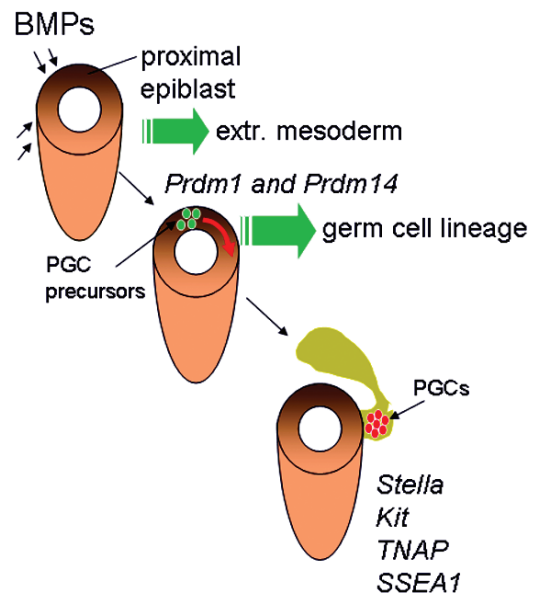


Fig. 2. Schematic representation of the main stages and processes of PGC specification in the mouse embryo.

PGC migration

There is still not a univocal view about the mechanisms of PGC migration and only scant information about their molecular motility machine is available (for reviews, see De Felici *et al.*, 2005; Soto-Suazo and Zorn, 2006). Passive and active movements, single and grouping movements, interaction with the extracellular matrix (ECM), repulsive and attractive forces from the surrounding tissues; all these factors have been shown to be involved and likely play a role in this process. PGC migration can be schematically divided in three stages:

1) 8-9 dpc: PGCs move from the base of the allantois into the developing hindgut epithelium. E-Cad downregulation; *lftim1* repulsive and *lftim3* attractive homing in the hindgut epithelium, are all processes likely to play a role during this stage. 2) 9.5-10.5 dpc: PGCs leave the hindgut and move directly into the developing gonadal ridges. Acquisition/reacquisition of a motile phenotype, E-cadherin upregulation, perhaps mediating intercellular contact among pioneer PGCs, ECM interaction through $\beta 1$ integrin, SF-1/CXR4 and/or KL/*kit* chemoattractive action, are involved in this second phase. 3) 11.5-12.5 dpc: a final round of migration from the hindgut occurs through the dorsal mesentery into the gonads. Most of the factors involved in the second migratory stage continue to play a role. However, the fate of these late migrating PGCs remains uncertain. In particular, it is not known if they contribute to the germ cell population within the gonads, or if they are misallocated in other tissues to die by apoptosis, or if they have some other developmental fate.

PGC proliferation

Owing to mitotic proliferation during 8.5 to 13.5 dpc, the number of PGCs increases about 400 times from about 50 to 20,000 cells per embryo in 7-8 mitotic cycles (Tam and Snow,

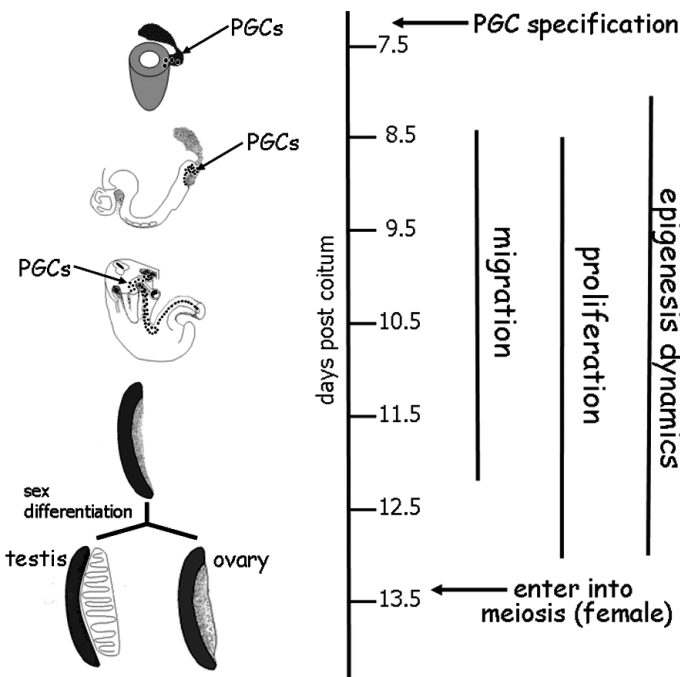


Fig. 1. Timetable of PGC development in the mouse embryo.

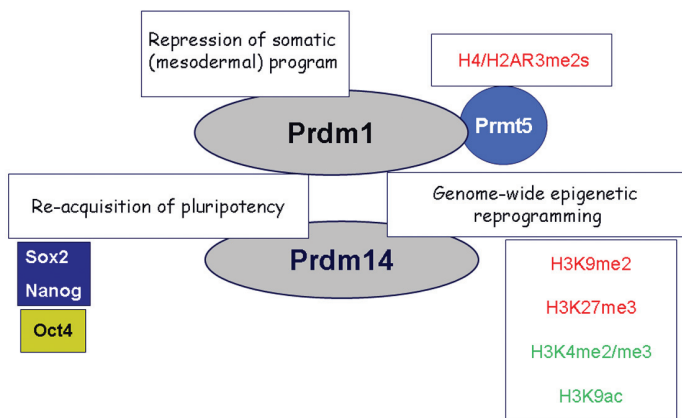


Fig. 3. Schematic representation of the main molecular events identified to be necessary for PGC specification in the mouse embryo. Histones in red and green are associated with repressed or active chromatin, respectively.

1981). PGC growth is sustained by a variety of soluble and membrane-bound growth factors (for a review, see De Felici *et al.*, 2004). These factors prevent PGC apoptosis and/or stimulate their proliferation. PGCs undergo two mitotic blocks: the first at 7.5 up to 9.0 dpc in G₂ both in the female and male (Seki *et al.*, 2007) and the second at 13.5-14.5 dpc in G₁/G₀ in the male (Western *et al.*, 2008). Mutations affecting PGC proliferation as well some intracellular players controlling their mitotic cycle have been identified (De Felici *et al.*, 2004; Sorrentino *et al.*, 2007; Western *et al.*, 2008; Spiller *et al.*, 2009) (Fig. 4).

Epigenetic dynamics

As we have seen before, extensive genome-wide epigenetic reprogramming occurs in specified PGCs involving several modifications of histones associated with the repressed or active chromatin state (Seki *et al.*, 2007; Hajkova *et al.*, 2008). DNA dimethylation, X reactivation in female PGCs and erasure of parental imprinting are other critical and unique epigenetic modifications occurring in migratory and post migratory PGCs. Little is yet known about the exact significance of such changes and the

mechanisms involved, except that they are apparently active processes.

Entering into meiosis

Around 13.5 dpc in the fetal testis, PGCs stop mitotic division and enter a period of mitotic quiescence in G₀. Such quiescent male germ cells are now called prospermatogonia or gonocytes. At the same time, in the fetal ovary, the PGC cycle shifts from mitosis to meiosis. Two main views about meiotic entry have been put forward: 1) entering into meiosis occurs spontaneously and cell-autonomously in female PGCs after a certain number of mitotic cycles; in the fetal testis, meiosis is blocked by the action of a meiotic preventing substance (MPS) produced by somatic cells; 2) entering into meiosis in female PGCs is induced by a meiotic inducing substance (MIS) and it doesn't occur in fetal testis due to the absence of MIS and/or the presence of MPS.

Recent evidence indicates that retinoic acid (RA) produced by the mesonephros exerts MIS action in the fetal ovary, inducing PGCs to enter meiosis in an anterior-posterior direction. In the male, the P450 enzyme, active in somatic cells of the fetal testis, degrades RA and prevents PGCs entering into meiosis. An important target of RA is STRA8, a protein of unknown function whose action seems, however, crucial for the G₁ mitotic-meiotic shift in female PGCs. It remains to be seen if RA is absolutely necessary for induction of meiosis in PGCs and if STRA8 really plays a role in the shift from mitosis to meiosis (for a review, see Bowles and Koopman, 2007) (Fig. 5).

Stem cells from PGCs, and PGCs from stem cells

Finally, two interesting aspects of PGC development have raised major interest among scientists in recent decades due to their implications for stem cell biology and for the formation of certain types of tumors called teratoma and teratocarcinoma. It is possible to derive ES (embryonic stem)-like cells called EG (embryonic germ)-cells from PGCs in culture and to produce PGCs from EG or ES cells in culture (for reviews, see Aflatoonian and Moore, 2006; Donovan and De Miguel, 2003). The molecular mechanisms of both processes have not been fully elucidated. We know, however, that LIF, KI and bFGF (or forskolin or RA) are

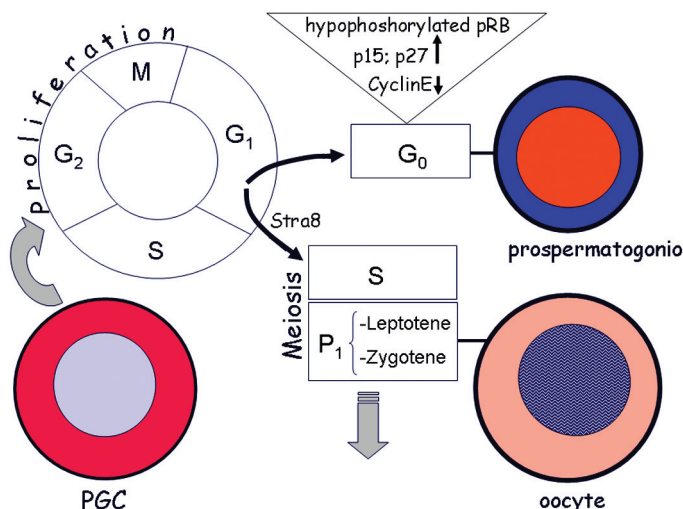


Fig. 4 (Left). The main processes and identified molecules controlling the arrest of the mitotic cell cycle in male PGCs into prospermatogonia or the shift from mitosis into meiosis in female PGCs.

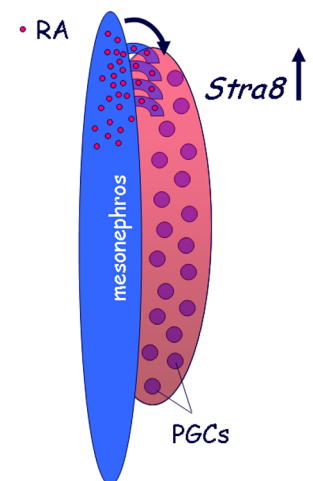


Fig. 5 (Right). The recent model of entering into meiosis in female PGCs stimulated in an antero-posterior direction by retinoic acid (RA) produced by the mesonephros.

necessary for the transformation of PGCs in EG cells and that *Blimp1* downregulation, *Stat3* induction, PTEN inhibition or ablation, likely *E-Ras* upregulation and bFGF-dependent upregulation and cytoplasmic-nuclear translocation of FGF receptor 3 favor, or are required for, this process (Durcova-Hills *et al.*, 2006; our unpublished observations). While it is likely that all these factors cooperate to establish the self renewal and apoptotic independence of PGCs, their interrelationship and link to PGC intrinsic pluripotency have not been elucidated.

Concluding remarks

Progress in our understanding of PGC biology certainly benefited from the availability of modern techniques allowing the isolating and culturing of PGCs, to observe live cells moving within the embryo, to analyze gene expression in single cells, or the expression of thousands of genes in microarrays and to knock-down genes in a cell type specific manner. I remember, however, the amazingly simple instruments which Anne used for her studies in her personal-office-lab: an old stereomicroscope, a pair of forceps, some scissors and pipettes, were all that seemed she needed. For many years everywhere around the world, scientists discussing PGCs are going to remember the results and intuitions of Anne McLaren. Following in her footsteps, we'll continue to face the exciting challenge of unraveling the secrets of germ cells "the most fascinating cells of all" as Anne loved to define them.

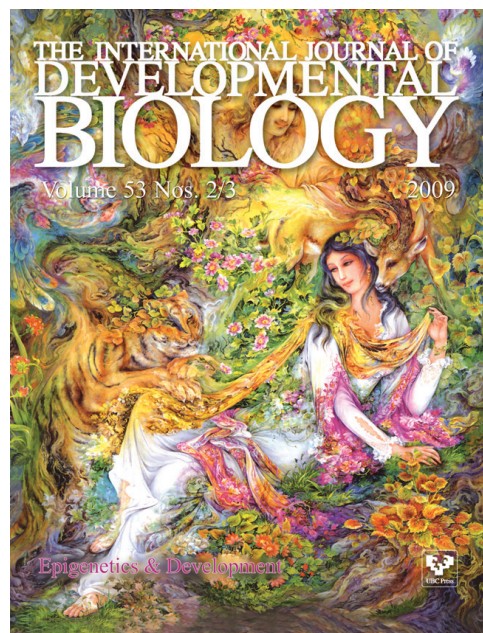
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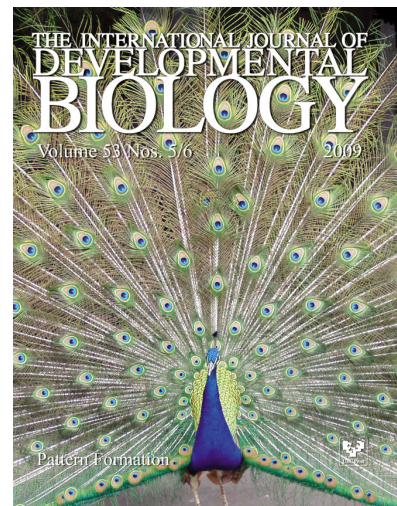
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