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Cycling to and from a stem cell niche: the temporal and spatial odyssey of mitotic male germ cells

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ABSTRACT The development of male germ cells within the prenatal and prepubertal periods in mammals combines multiple biological events that integrate cell cycle regulation, epigenetic reprogramming, and cell migration along temporally and spatially dynamic lines. Germ cells arise from their precursor primordial germ cells in the mid-gestation embryo, forming gonocytes that enter G_0 phase cell cycle arrest within the fetal testis. Cyclin-dependent kinase inhibitors, activated retinoblastoma 1 protein, and increased levels of transforming growth factor beta 2 collectively influence this cell cycle arrest. Gonocyte quiescence persists until shortly after birth, whereupon the cells concomitantly re-enter the cell cycle and migrate towards a niche that establishes and maintains self-renewing spermatogonial stem cells and balances them with differentiating spermatogonia. Platelet-derived growth factor signaling is one of the mechanisms that regulates both mitotic activation and migration in neonatal gonocytes, along with mitogens, 17β-estradiol and retinoic acid, and chemoattractants C-C-motif ligand 9 and members of the ADAM, integrin, and tetraspanin families. Numerous germ cell-intrinsic proteins have been identified that ensure the retention of germ cells within the spermatogonial stem cell niche. Sertoli cells are a significant component of this niche, contributing essential growth factors and chemokines to spermatogonia. This review focuses on the dynamic events that occur to mitotic male germ cells before and during their arrival at this niche, with an emphasis on the cell cycle and directed migration.

KEY WORDS: *spermatogonia, cell cycle, germ cell, testis, gonocyte, stem cell niche*

Introduction

Male germ cell development in mammals is a remarkable process that depends upon precise migratory and cell cycle dynamics. From their precursor primordial germ cells (PGCs) that proliferate while migrating to the fetal gonad, male germ cells undergo a period of mitotic arrest until shortly after birth. They then re-enter the cell cycle and migrate once more to the basement membrane of seminiferous tubules within the testis – a niche that establishes and maintains self-renewing spermatogonial stem cells (SSCs) throughout the lifespan of individual organisms. When appropriate differentiation signals are processed, a subset of spermatogonia then leave the stem cell niche and commit to a multitude of differentiation events comprising spermatogenesis. This balance of SSC self-renewal with differentiation must continue throughout adulthood to ensure the uninterrupted production of spermatozoa.

Nearly six decades of research into how mammalian SSCs, their precursor cells and differentiating progeny are regulated from a cell cycle perspective have contributed to our current understanding of this highly orchestrated process. Beginning with cell counts and ^{3}H-thymidine labeling (Clermont and Leblond, 1953, Monesi, 1962), and progressing to transgenic pulse-labeling and live imaging experiments (Nakagawa *et al.*, 2007, Yoshida *et al.*, 2007), the field of male germ cell biology has uncovered the complexity of the journey taken by these cells from PGCs to differentiating spermatogonia. This development spans fetal and neonatal stages through puberty and into adulthood, with temporal and spatial dynamics that reveal alternating periods of mitotic activity and cell movement, quiescence and repose (Fig. 1). This review will encompass the developmental events that occur during these periods, highlighting the experimental approaches taken to

Abbreviations used in this paper: PGC, primordial germ cell; SSC, spermatogonial stem cell.

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elucidate their regulatory mechanisms. While some studies have been performed using non-human primate and human samples, the majority of experimental analysis has been accomplished with mouse and rat male germ cells. Accordingly, this review will focus on rodent male germ cell mechanisms.

From the site of origin to the gonadal ridge

Mouse primordial germ cells (PGCs) were first identified by light microscopy at 8.5 days post coitum [embryonic day (E)8.5] in the posterior region of the embryo (Chiquoine, 1954). These cells express high levels of alkaline phosphatase (AP), which facilitated their subsequent discovery at the base of the allantois at E8.0 (Ozdzenski, 1967). Cell counting identified approximately 100 PGCs per embryo at E8.5, which increase in number to approximately 25,000 germ cells per embryo at E13.5 (Tam and Snow, 1981). Detection of PGCs at E7.0 was then enabled through the use of whole-mount immunostaining for AP activity (Ginsburg *et al.*, 1990). PGCs specification is initiated from signals coming from the extraembryonic ectoderm and visceral endoderm that encompass the epiblast. These signals include bone morphogenetic proteins (BMPs) 2, 4, and 8b (Lawson *et al.*, 1999, Ying *et al.*, 2000, Ying and Zhao, 2001). BMP signaling within epiblast cells results in the upregulation of *Fragilis* and the expression of *Blimp1* in approximately six PGC precursor cells at E6.25 (Ohinata *et al.*, 2005). *Blimp1* encodes a transcription factor that leads to the upregulation of genes characteristic of germ cells, including *Stella* and *Nanos3*, and the downregulation of mesodermal genes such as fibroblast growth factor 8 (*Fgf8*) and brachyury (*T*).

By E7.5 the proliferating PGCs have begun to migrate, although the specifics of their cellular behavior between E7.25 and E8.5 are not well understood. PGC migration depends upon the localization of the kit receptor (KIT) and the chemokine (C-X-C motif) receptor 4 (CXCR4) in PGCs, responding to kit ligand (Steel, stem cell factor) and C-X-C-motif ligand 12 (CXCL12, Sdf1), respectively (Ara *et al.*, 2003, Doitsidou *et al.*, 2002, Godin *et al.*, 1991, Molyneaux *et al.*, 2003). PGCs respond to the chemoattractants as they traverse their path to the developing gonads. By E8.5, ~100 or so PGCs distribute near the hindgut diverticulum and become transcriptionally silent, undergoing a transient G_2 phase cell cycle arrest. Seki and colleagues performed 5-bromo-2'-deoxyuridine (BrdU) labeling and found that most PGCs did not enter S-phase between E7.5 and E9.0 (Seki *et al.*, 2007). Assessment of cyclin B1 levels, which accumulate in the cytoplasm specifically in the G2 phase, revealed that 80% of PGCs exhibited cyclin B1 between E8.5 and E8.75, decreasing in number after E9.75. Coinciding with this quiescence is extensive epigenetic reprogramming, including the erasure of methylated histone lysine H3K9me2, increase in H3K27me3, and decrease in DNA methylation (Seki *et al.*, 2007). DNA methyltransferases DNMT1, DNMT3A, and DNMT3B are suppressed, along with RNA polymerase II transcription. Between E9.0 and E11.0 the transient cell cycle arrest is reversed, and by E11.0 the migrating PGCs have entered the genital ridge of the developing gonads. PGCs actively proliferate upon their arrival in the gonad, from E11.5 to E12.5 (Tam and Snow, 1981). During this same period, the germ cells are once more subjected to extensive epigenetic reprogramming, including DNA demethylation at imprinted gene loci (Sato *et al.*, 2003). DNMT3A and DNMT3B are suppressed, but unlike during the earlier reprogramming between E7.5 and E9.0, DNMT1 remains active during this post-migratory

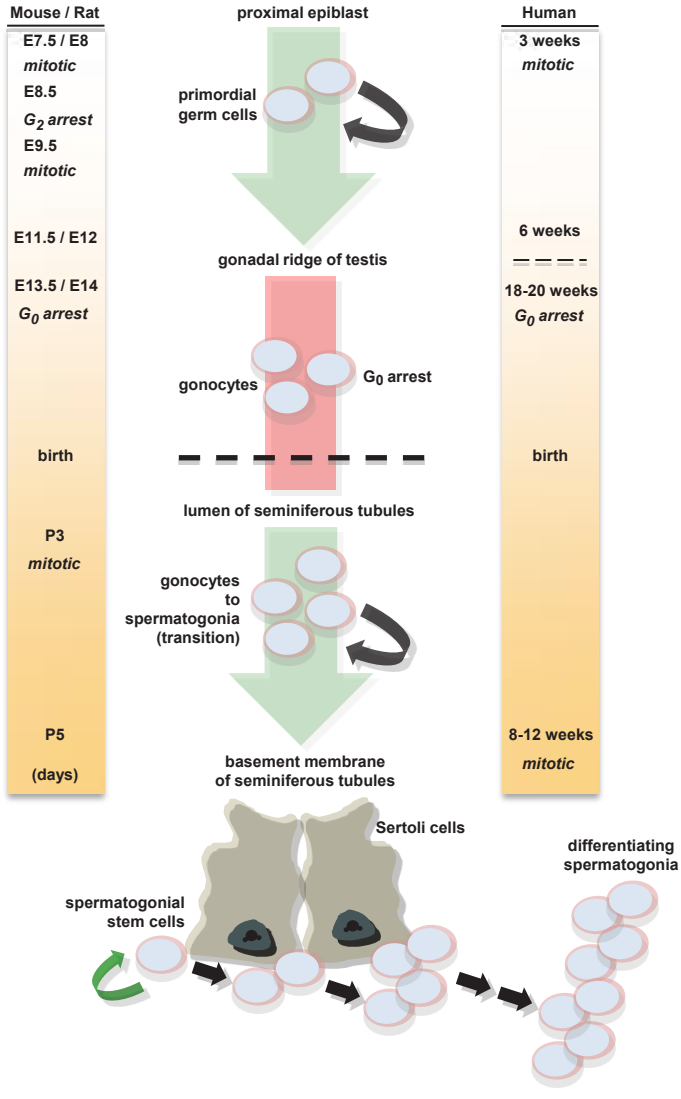

Fig. 1. Overview of mitotic male germ cell development in mammals. *Primordial germ cells (PGCs) originate from epiblast tissue proximal to the extraembryonic ectoderm. Following their specification, PGCs begin active proliferation while migrating through the hindgut to the site of the presumptive gonad. A transient G_2 phase cell cycle arrest occurs in rodent PGCs between embryonic days (E)8-E9.5. Mitotic activity then resumes in rodents after E9.5 as PGCs continue their migration. Upon their arrival at the gonadal ridge, male germ cells continue to proliferate and are now classified as gonocytes. Then, at approximately E13.5 in rodents and 18-20 weeks in humans, male gonocytes undergo G_0 phase cell cycle arrest. These germ cells remain quiescent until shortly after birth. At this time they reside in the lumen of the seminiferous tubules in the testis, surrounded by mitotic Sertoli cells that extend inward from the basement membrane. Upon re-entry into the cell cycle at approximately postnatal day (P)3 in rodents, gonocytes begin to migrate from the lumen to the basement membrane. Once the germ cells have completed their migration, they are classified as spermatogonia and form self-renewing spermatogonial stem cells (SSCs) at approximately P5 in rodents and 8-12 weeks in humans. Sertoli cells comprise a key component of the stem cell niche, which balances SSC maintenance with differentiating spermatogonia in response to multiple germ cell-intrinsic and extrinsic factors.*

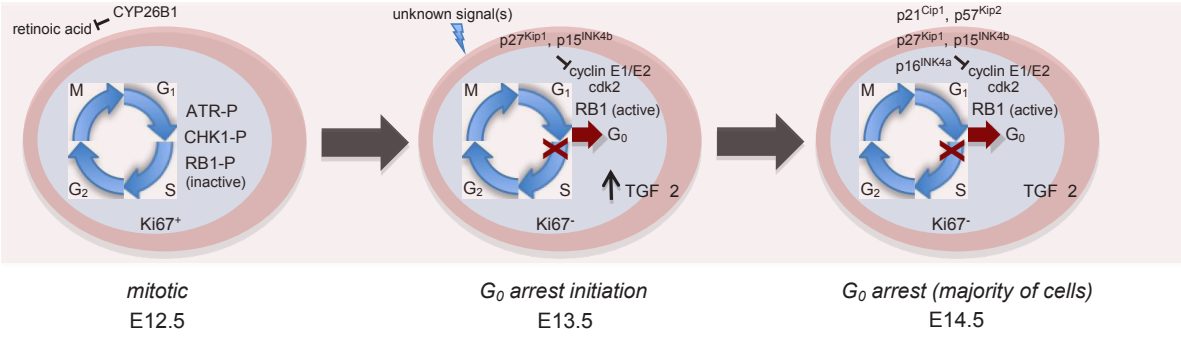

Fig. 2. Mitotic arrest of gonocytes within the fetal mouse testis. *Male gonocytes within the E12.5 testis proliferate actively, expressing the marker Ki67. Unlike female germ cells, they do not respond to retinoic acid (RA) due to the presence of CYP26B1, which degrades RA and prevents meiotic entry. E12.5 gonocytes contain inactive phosphorylated RB1 protein, phosphorylated ATR, and phosphorylated CHK1. When gonocytes are exposed to unknown signals beginning after E12.5, an upregulation in* Cdkn1b *(*$p27^{Kip1}$*) and* Cdkn2b *(*$p15^{INK4b}$*) inhibits Cyclin E-cdk2 complexes, which in turn inhibits the phosphorylation of RB1. The* G_1*-to-S phase checkpoint is activated by hypophosphorylated RB1, leading to cell cycle arrest in the* G_0 *phase. This quiescence is accompanied by an increase in TGFβ2 levels. As the majority of gonocytes become arrested in* G_0 *by E14.5, elevated* $p27^{Kip1}$ *and* $p15^{INK4b}$ *expression is augmented with upregulated* Cdkn1a *(*$p21^{Cip1}$*),* Cdkn1c *(*$p57^{Kip2}$*), and* Cdkn2a *(*$p16^{INK4a}$*), maintaining the block to Cyclin E-cdk2 activity and RB1 phosphorylation.* G_0 *arrest persists until shortly after birth. TGFβ2 levels remain high, and these quiescent gonocytes are Ki67-negative.*

period (Hajkova *et al.*, 2002). PGC arrival at the gonadal ridge also exposes them to inductive signals that results in sex determination. Expression of *Sry* in the surrounding somatic tissue, which develops into Sertoli cells, leads to a cascade of signaling events that culminates in the formation of male germ cells from the PGCs (Wilhelm and Koopman, 2006). Within the developing testis levels of retinoic acid (RA), which are high in the developing ovary and induce female germ cells to enter meiosis, are kept low by the activity of CYP26B1, an enzyme that degrades RA (Bowles *et al.*, 2006, Koubova *et al.*, 2006). This action prevents meiosis from occurring in the fetal testis, and assists in the commitment of male germ cells to the spermatogenic pathway. Other factors involved in this commitment of germ cells to the male program include FGF9 and NANOS2 (Barrios *et al.*, 2010, Bowles *et al.*, 2010, Suzuki and Saga, 2008). Beginning at E11.5, male germ cells rely upon FGF9 for survival, and it has been proposed that FGF9 supports NANOS2 production in these cells to prevent meiotic entry and the activation of events downstream of RA (Barrios *et al.*, 2010, Bowles *et al.*, 2010, DiNapoli *et al.*, 2006, Suzuki and Saga, 2008). Collectively, these factors help to ensure that male germ cells establish their own developmental program that is distinct from that of female germ cells.

Dormancy within the fetal testis

Between E12.5 and E14.5, male germ cells exit the cell cycle and enter a prolonged phase of G_0 arrest until birth (Fig. 2). Now classified as gonocytes, male germ cells during this period respond to unknown signal(s) that induce this quiescence. Various studies had reported that E13.5 gonocytes were partially or fully in cell cycle arrest (Adams and McLaren, 2002, Hilscher *et al.*, 1974), while others had shown gonocytes continuing to divide up to E16.5 (Vergouwen *et al.*, 1991). ^{3}H-thymidine labeling demonstrated that approximately 8% of gonocytes were cycling at E14.5, with almost no mitotic activity at E16.5 (Vergouwen *et al.*, 1991). More recent quantitative studies of BrdU incorporation by Western and colleagues revealed that while roughly 70% of E13.5 gonocytes progressed through S-phase, this percentage dropped to near zero by E14.5 (Western *et al.*, 2008). Indeed, flow cytometry of E14.5 gonocytes revealed that approximately 95% of cells were in G_1/G_0 arrest, while the expression of Ki67, a marker of cycling cells, was observed at E12.5 but no longer detected by E14.5 (Western *et al.*, 2008). Two additional proteins localized in E12.5 gonocytes were phosphorylated ATR (ataxia telangiectasia and Rad3 related), a molecule upstream in cell cycle checkpoint pathways, and its target CHK1 (checkpoint kinase 1), which acts upon cell cycle regulators when phosphorylated (Rhind and Russell, 2000, Shiloh, 2001, Western *et al.*, 2008). These proteins were likewise undetected by E14.5. Direct examination of the cyclin-dependent kinase inhibitors *Cdkn1a* (*$p21^{Cip1}$*), *Cdkn1b* (*$p27^{Kip1}$*), *Cdkn1c* (*$p57^{Kip2}$*), *Cdkn2a* (*$p16^{INK4a}$*), and *Cdkn2b* (*$p15^{INK4b}$*) revealed that *$p27^{Kip1}$* and *$p15^{INK4b}$* undergo enrichment in E13.5 gonocytes, leading to the inhibition of cyclin E1/E2 and cdk2 (cyclin-dependent kinase 2) and cell cycle arrest (Western *et al.*, 2008). By E14.5, the additional inhibitors *$p21^{Cip1}$*, *$p57^{Kip2}$*, and *$p16^{INK4a}$* join *$p27^{Kip1}$* and *$p15^{INK4b}$* in maintaining the cell cycle arrest. Interestingly, the G_1/S phase checkpoint protein retinoblastoma 1 (RB1) is inactive at E12.5, localized in its phosphorylated form (Western *et al.*, 2008). By E13.5 it becomes dephosphorylated, leading to its activation (Fig. 2). This activity is transient, however, and RB1 is degraded once the majority of gonocytes are quiescent (Western *et al.*, 2008). In *Rb1*-deleted male mice, gonocytes show a higher percentage of cycling cells at E14.5, though this activity subsides and delayed quiescence occurs by E16.5 in mutant animals (Spiller *et al.*, 2010). Thus, the temporal role of RB1 in mediating G_0 arrest is compensated a short time later by additional factors, including *$p27^{Kip1}$* and *$p15^{INK4b}$*.

The mitotic arrest of fetal male gonocytes has also been investigated in the context of transforming growth factor (TGF) signaling. TGFβ was shown to target rat gonocytes through immunohistochemical studies as well as *in vitro* functional assays (Gautier *et al.*, 1994, Olaso *et al.*, 1998, Teerds and Dorrington, 1993). TGFβ1 also exhibits anti-proliferative properties on mouse PGCs (Godin and Wylie, 1991). More recently, Moreno and colleagues analyzed a conditional *Tgfbr2* gene-targeted mouse and *ex vivo* organ cultures and observed higher levels of gonocyte quiescence when TGFβ2 expression was elevated (Moreno *et al.*, 2010). Addition of exogenous TGFβ2 to gonocytes *in vitro* significantly diminished the number of BrdU-positive gonocytes. Thus, TGFβ2 appears to

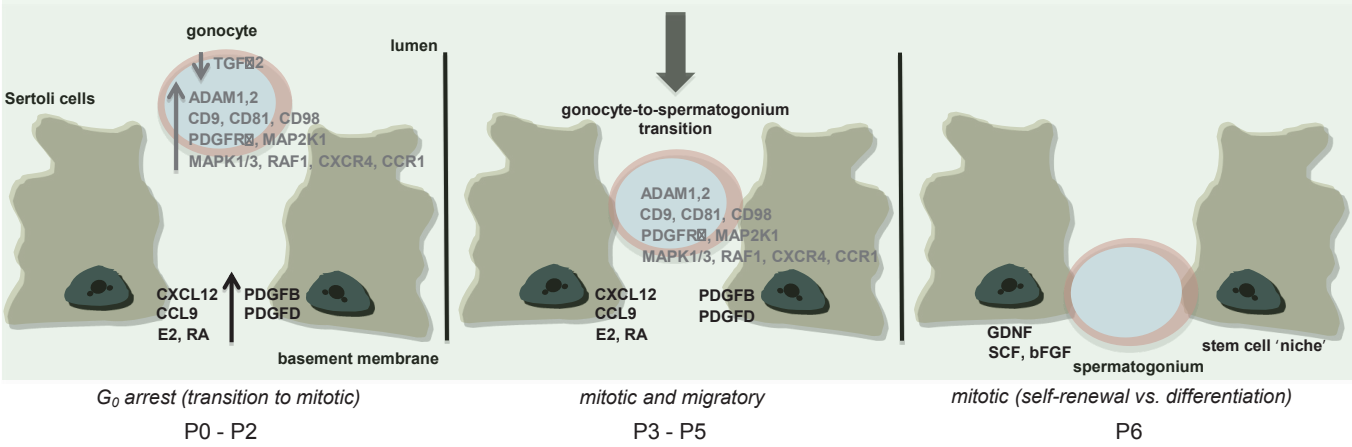

Fig. 3. Migration of perinatal gonocytes to the spermatogonial stem cell niche. *Between birth and P2 in mice, gonocytes remain in G_0 arrest within the lumen of seminiferous tubules. This period of quiescence is accompanied by a decrease in the levels of TGFβ2, and an increase in the gonocyte levels of ADAMs 1 and 2, CD9, CD81, CD98, PDGFRβ, MAP2K1, MAPK1/3, RAF1, CXCR4, and CCR1. Actively proliferating Sertoli cells, meanwhile, exhibit an increase in PDGFB, PDGFD, CXCL12, CCL9, RA (retinoic acid), and E2 (17β-estradiol). By P3, gonocytes re-enter the cell cycle and begin migrating towards the basement membrane, continuing to express molecules that respond to chemoattractants and other factors. Between P4-P6, gonocytes reach the basement membrane and are now classified as spermatogonia. Growth factors GDNF, bFGF, and KIT ligand (Steel; stem cell factor, SCF) are released by Sertoli cells, and are critical components of the stem cell niche that establishes and maintains self-renewing spermatogonial stem cells.*

influence the G_0 arrest in gonocytes after E12.5 (Fig. 2). Activin signaling may also play a role, as inhibin-beta A (*Inhba*) knockout male mice exhibited increased numbers of gonocytes at E15.5 (Mendis *et al.*, 2011). Taken altogether, these results suggest that growth factors, in addition to cyclin-dependent kinase inhibitors, exert anti-mitotic activity on gonocytes from E13.5 until just after birth to maintain male germ cells in their appropriate state. Further research is necessary to identify the signal(s) that initiate these anti-proliferative events, triggering the upregulation of such growth factors and cell cycle inhibitors.

From the lumen to the basement membrane: entering the stem cell niche

Upon birth, perinatal male gonocytes remain quiescent and reside within the lumen of the seminiferous tubules in the testis. Then, at approximately postnatal day (P)3 in both mice and rats, gonocytes re-enter the cell cycle and begin to proliferate once more (Fig. 3). The identity of the factor(s) responsible for this resumption of mitotic activity remained unknown until the past two decades. Li and colleagues discovered that platelet-derived growth factor (PDGF)-BB as well as 17β-estradiol (E2) induced *in vitro* cycling of P3 rat gonocytes by measuring BrdU incorporation (Li *et al.*, 1997). When an estrogen receptor antagonist was introduced to the cells, the effect of E2 was abolished, highlighting the importance of E2 engaging its receptor (Li *et al.*, 1997). Daily injections of E2 into P1 rat pups also increased the number of gonocytes 2-fold by P3 (Vigueras-Villasenor *et al.*, 2006). Both PDGF-BB and E2 are produced in neonatal Sertoli cells, and PDGF receptors (PDGFRs) α, β, and variant truncated V1-PDGFRβ are expressed in neonatal gonocytes (Basciani *et al.*, 2008, Thuillier *et al.*, 2003, Wang and Culty, 2007). Furthermore, gonocytes express RAF1 (v-raf-leukemia viral oncogene 1), MAP2K1 (mitogen-activated protein kinase kinase 1), and MAPK1/3 (mitogen-activated protein kinase 1/3). Thuillier and colleagues reported that inhibiting RAF1 and MAP2K1 blocked both PDGF- and E2-induced gonocyte proliferation, placing these factors downstream in the pathways (Thuillier *et al.*, 2010). Interestingly, E2 did not promote cycling in gonocytes in the absence of PDGF, highlighting its dependence. RA also exerts a positive effect upon P3 rat gonocyte cycling during *ex vivo* organ culture conditions (Livera *et al.*, 2000). P3 gonocyte proliferation increased after three days of RA exposure in gonocyte-Sertoli cell co-cultures, and the number of BrdU-positive P2 gonocytes increased after one day of RA treatment *in vitro* (Boulogne *et al.*, 2003, Zhou *et al.*, 2008a). Thus, a combination of *in vivo*, *ex vivo*, and *in vitro* experiments have demonstrated that levels of PDGFs, E2, and RA increase shortly after birth and act upon gonocytes to stimulate cell cycle progression (Fig. 3).

Accompanying the resumption of proliferation in P3 rodent gonocytes is a migration from the lumen to the basement membrane of seminiferous tubules (Fig. 3; Clermont and Perey, 1957). Such migration is necessary for the further development of male germ cells, as gonocytes that remain in the lumen after P5 undergo apoptosis (Tres and Kierszenbaum, 2005). Studies on KIT suggest that it may be one of many factors that mediate this process (Orth *et al.*, 1997). Antibodies that blocked KIT on gonocytes *in vitro* decreased the number of cells with detectable pseudopods available for motility (Orth *et al.*, 1997). Additional factors that may influence gonocyte migration include members of the ADAM (A Disintegrin And Metalloprotease), integrin, and tetraspanin families. Specifically, ADAMs 1 and 2, integrins α3β1 and α6β1, and tetraspanins CD9, CD81, and CD98 have been proposed to mediate this cell movement based upon their localization and increasing levels in migratory gonocytes, as well as their known interactions (Tres and Kierszenbaum, 2005). PDGFRβ and V1-PDGFRβ, in addition to regulating gonocyte proliferation, also appear to influence migration. Exposure of neonatal male mice to imatinib, an inhibitor of PDGFRs and KIT, prevented the relocation of gonocytes from the lumen to the basement membrane, resulting in increased cell death (Basciani *et al.*, 2008). The authors also demonstrated that PDGFB and PDGFD induce gonocyte chemotaxis *in vitro*. Collectively, these findings suggest that levels of ADAMs 1/2,

CD9, CD81, CD98, and PDGFRβ increase in pre-migratory and migratory neonatal gonocytes, mediating their movement away from the lumen. Recent experiments performed using P5-P6 male germ cells, comprising the transition period between gonocytes and spermatogonia, showed chemotaxis of undifferentiated germ cells expressing the glial cell line derived neurotrophic factor family receptor alpha 1 (GFRA1) towards the chemokine C-C-motif ligand 9 (CCL9) *in vitro* (Simon *et al.*, 2010). CCL9 is strongly expressed by neonatal Sertoli cells, and its receptor, CCR1, is localized to GFRA1+ germ cells (Simon *et al.*, 2010). Furthermore, neonatal Sertoli cells lacking the gene *Sin3a* exhibit significantly diminished levels of CXCL12, while *Sin3a*-deleted perinatal gonocytes have reduced levels of its receptor, CXCR4, and fail to migrate from the lumen to the basement membrane (Payne *et al.*, 2010; Gallagher *et al.,* 2013). Thus, it is possible that migratory neonatal gonocytes also utilize CCR1 and CXCR4 to migrate towards high levels of CCL9 and CXCL12, respectively, present at the basal surface of Sertoli cells along the basement membrane. In this case, postnatal gonocytes would utilize some of the same molecules that embryonic PGCs use to mediate their migration. Upon reaching the basement membrane, male germ cells enter the spermatogonial stem cell niche, where establishment and self-renewal of the germline stem cell pool occurs in balance with spermatogenesis. The gonocytes have now become spermatogonia.

Balancing self-renewal with differentiation

Spermatogonia were originally classified by their nuclear morphology. The so-called Type A spermatogonia had undetectable levels of heterochromatin using light microscopy, while Intermediate spermatogonia had some heterochromatin, and Type B had larger amounts (Clermont and Leblond, 1953, Monesi, 1962, Roosen-Runge and Giesel, 1950). Two primary models of spermatogonial stem cell (SSC) regulation and maintenance have been proposed during the past forty years. In the first model, the examination of rodent seminiferous tubules by whole-mount microscopy revealed that within the Type A class, the putative stem cell A_{single} (A_s) divides to generate either two additional A_s spermatogonia or A_{paired} (A_{pr}) daughter cells that do not undergo complete cytokinesis and remain connected with intercellular cytoplasmic bridges (Huckins, 1971, Oakberg, 1971). Additional divisions of the A_{pr} cells generate chains of 4, 8, 16, and even 32 interconnected Type A spermatogonia ($A_{a-ligned}$, or A_{al}) that are still classified as undifferentiated. That is, they continue to harbor a similar transcriptome to the A_s spermatogonia, and even retain stem cell potential (Nakagawa *et al.*, 2010). The large chains of A_{al} then become A_1 differentiating spermatogonia, which further divide to generate A_2, A_3, A_4, Intermediate, and Type B spermatogonia. The spermatogonial cell divisions beginning with A_1 are all synchronous, unlike the A_s, A_{pr}, and A_{al} cell divisions.

In contrast to the aforementioned description, the second model of SSC regulation proposes a so-called A_0/A_1 model. Observations of single or pairs of cells with little to no mitotic activity classified them as A_0 spermatogonia, or reserve stem cells much like hematopoietic stem cells (Clermont and Bustos-Obregon, 1968, Clermont and Hermo, 1975, Dym and Clermont, 1970). Active stem cells, then, are comprised of A_1, A_2, A_3, and A_4 spermatogonia (no relation to the first model). A_4 cell division either gives rise to another A_1 spermatogonium as SSC self-renewal, or generates a differentiating intermediate spermatogonium. While similarities exist between the A_0/A_1 model and the so-called A_{dark} and A_{pale} model of SSC regulation in non-human primates and humans, most studies examining the molecular mechanisms of SSC self-renewal and differentiation rely upon the A_s-A_{pr}-A_{al} model.

Within the SSC niche, undifferentiated spermatogonia are exposed to growth factors, cytokines, and other molecules that promote the maintenance of the actual and potential germline stem cells in their undifferentiated state (Fig. 4). The predominant niche component is the Sertoli cell, producing glial cell line derived neurotrophic factor (GDNF) that acts upon GFRA1 and its co-receptor Ret in undifferentiated A_s, A_{pr}, and A_{al} spermatogonia (Meng *et al.*, 2000). When *Gdnf*, *Gfra1*, or *Ret* are deleted in male mice, SSCs establishment fails and germ cells are rapidly depleted (Meng *et al.*, 2000, Naughton *et al.*, 2006). Sertoli cells also release basic FGF (bFGF) and stem cell factor (SCF, kit ligand, Steel) into the milieu to ensure SSC survival (Mullaney and Skinner, 1992, Tajima *et al.*, 1991). Peritubular myoid cells and Leydig cells, meanwhile, exhibit colony-stimulating factor 1 (CSF1) localization, with the CSF1 receptor restricted to the undifferentiated spermatogonia expressing cell surface antigen THY1 (Oatley *et al.*, 2009). Additional germ cell-intrinsic molecules enriched in SSCs and important for their self-renewal include POU domain, class 5, transcription factor 1 (*Pou5f1*, *Oct4*), zinc finger and BTB domain containing 16 (*Zbtb16*, *Plzf*), lin-28 homolog A (*Lin28*), B cell CLL/lymphoma 6, member B (*Bcl6b*), neurogenin 3 (*Neurog3*, *Ngn3*), inhibitor of DNA binding 4 (*Id4*), sal-like 4 (*Sall4*), cadherin 1 (*Cdh1*), and undifferentiated embryonic cell transcription factor 1 (*Utf1*) (Buaas *et al.*, 2004, Costoya

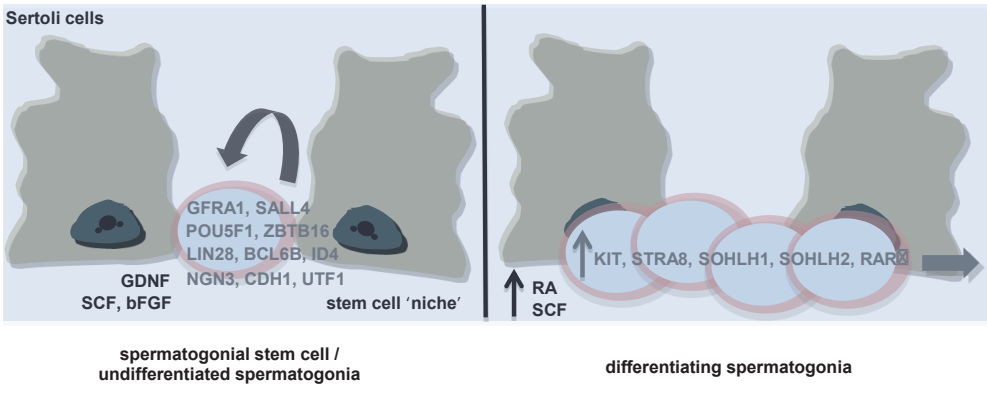

Fig. 4. Maintenance of self-renewing spermatogonial stem cells and differentiating spermatogonia. *Spermatogonial stem cells (SSCs) reside within the SSC niche, expressing intrinsic factors such as GFRA1, POU5F1, ZBTB16, LIN28, SALL4, BCL6B, ID4, NGN3, CDH1, and UTF1. Sertoli cells release growth factors like GDNF, bFGF, and KIT ligand (Steel; stem cell factor, SCF) to maintain SSCs within the niche. In response to RA (retinoic acid) and SCF, spermatogonia commit to differentiation, downregulating SSC molecules and upregulating KIT, STRA8, SOHLH1, SOHLH2, and RARγ (retinoic acid receptor). SSCs are first established shortly after birth, whereas the differentiation signals are expressed beginning at puberty. This balance between SSC self-renewal and differentiation continues throughout adulthood.*

et al., 2004, Dann *et al.*, 2008, Eildermann *et al.*, 2012, Oatley *et al.*, 2006, Oatley *et al.*, 2011, Tokuda *et al.*, 2007, van Bragt *et al.*, 2008, Yoshida *et al.*, 2004, Zheng *et al.*, 2009). All of these factors are expressed in SSCs within the niche, resulting in the self-renewal of undifferentiated spermatogonia (Fig. 4). In culture, mouse SSC division occurs every 5.6 days, and rat SSC division occurs every 3-4 days or every 11 days (Hamra *et al.*, 2005, Kubota *et al.*, 2004, Ryu *et al.*, 2005). Upon the commitment to differentiate, spermatogonia then downregulate these SSC markers and upregulate markers of differentiation.

In contrast to the many factors that promote SSC self-renewal, only a handful of differentiation markers have been identified in A_1-A_4, Intermediate, and Type B spermatogonia. Increased levels of RA appear to coincide with the timing of SSC differentiation, with both A_{al} and A_1 spermatogonia expressing RA receptor gamma (RARγ), critical for transducing RA signals and essential for spermatogonial differentiation (Gely-Pernot *et al.*, 2012, Snyder *et al.*, 2010). In response to RA, spermatogonia upregulate stimulated by retinoic acid gene 8 (*Stra8*), whose expression persists until the germ cells enter meiosis (Zhou *et al.*, 2008a, Zhou *et al.*, 2008b). KIT levels also increase in differentiating spermatogonia, responding to elevated SCF activity in the microenvironment (Ohta *et al.*, 2000, Schrans-Stassen *et al.*, 1999). Two additional factors enriched upon differentiation are SOHLH1 and SOHLH2 (spermatogenesis and oogenesis specific basic helix-loop-helix 1 and 2), which were recently shown to upregulate *Kit* (Barrios *et al.*, 2012). Genetic ablation of either family member results in a loss of KIT^+ spermatogonia (Ballow *et al.*, 2006, Toyoda *et al.*, 2009). Thus, when SSCs commit to the spermatogenic process, levels of RARγ, STRA8, SOHLH1, SOHLH2, and KIT increase within spermatogonia, responding to elevated RA and SCF in the surrounding milieu, and the germ cells leave the stem cell niche (Fig. 4). SSC maintenance occurs from shortly after birth to the extent of adulthood, while spermatogonial differentiation from those undifferentiated cells commences at the onset of puberty.

A unique first wave: rodent spermatogenesis initiation

Once the pool of self-renewing SSCs is established, differentiating spermatogonia emanate from those SSCs. In other words, in an adult mouse, STRA8+ spermatogonia originate from OCT4+, PLZF+, NGN3+ spermatogonia (Fig. 5). An unanswered question within the field, however, was whether the initial set of differentiating spermatogonia, or mitotic cells initiating the 'first wave' of spermatogenesis in rodents, emanated from SSCs like in adults, or directly from gonocytes prior to SSC establishment. This was an important question to address as the first functional mouse spermatozoa appear at P40, and the entire mouse spermatogenic process beginning from A_1 cells takes approximately 35 days. Yoshida and colleagues answered this question through elegant lineage tracing and pulse-labeling experiments, examining mice harboring both Ngn-cre and CAG-CAT-Z reporter transgenes (Yoshida *et al.*, 2006). They found that KIT+ spermatogonia contributing to the first wave never expressed *Ngn3*. Few NGN3+ germ cells exist prior to P4, suggesting that these first-wave differentiating spermatogonia arise directly from NGN- gonocytes (Fig. 5). This finding supports the view of two distinct subsets of rodent gonocytes at P3, when mitotic activity resumes: one subset that remains undifferentiated and establishes the self-renewing SSC pool that is maintained throughout adulthood, and one subset that immediately expresses KIT at P3 and differentiates, initiating the first wave of spermatogenesis (Fig. 5). How these differential subsets of gonocytes are established and regulated is poorly understood, but evidence supports the possibility that events within the fetal testis, prior to or during gonocyte quiescence from E13.5 to P2, may influence these differential cell fates (Payne *et al.*, 2010). Mice harboring *Sin3a*-deleted Sertoli cells, in which the gene is rendered non-functional by E13.5, exhibit a normal initiation of the spermatogenic first wave but do not establish SSCs during the first postnatal week (Payne *et al.*, 2010). Thus, there could be an uncharacterized factor expressed by fetal and perinatal Sertoli cells that influence gonocyte cell fate. Galectin 1 has been proposed to influence whether gonocytes generate SSCs or establish first-wave differentiating spermatogonia, but there are likely other contributing factors (Yoshida *et al.*, 2006).

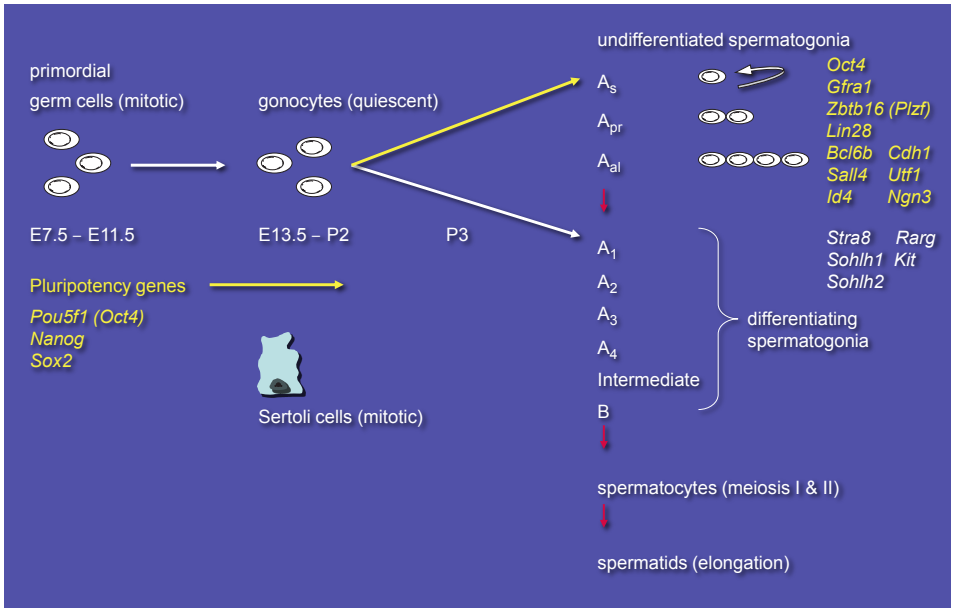

Fig. 5. The establishment of the first spermatogenic wave in rodents. *Fetal male germ cells express pluripotency genes such as* Pou5f1 *(*Oct4*),* Nanog, *and* Sox2. *At P3 in mice, a subset of male germ cells continues to express* Oct4 *and additional stem cell markers* Gfra1, Zbtb16 *(*Plzf*),* Lin28, Bcl6b, Sall4, Id4, Cdh1, Utf1, *and* Ngn3. *These cells collectively are classified as undifferentiated spermatogonia, with Type A single (A_s) spermatogonia comprising the actual stem cells; Type A paired (A_{pr}) and Type A aligned (A_{al}) spermatogonia exhibit stem cell potential and can regenerate the germ cell population of testes if called upon to do so. Another subset of male germ cells at P3 begins to immediately differentiate, upregulating* Kit, Stra8, Sohlh1, Sohlh2, *and* Rarγ. *These differentiating cells form Type A_1-A_4 spermatogonia, which then further differentiate to become Intermediate and Type B spermatogonia, before finally entering meiosis as spermatocytes.*

Conclusion

Mitotic male germ cells undergo a remarkable journey from their initial development from PGCs to their entry into meiosis. Recurring waves of cell cycle dynamics, epigenetic reprogramming, and migration play fundamental, overlapping roles in shaping the identity of these unique cells. Like the epic journey of adventure described by Homer, prenatal male germ cells are, in a sense, destined for the stem cell niche that will house them between birth and adulthood. It is there they will reside until they are called into service once more, to differentiate and begin the spermatogenic process. The critical window of fetal gonocyte quiescence is also where dysregulation can occur, leading to the formation of carcinoma *in situ*, the precursor of testicular germ cell tumors that develop after puberty. Such events will be covered in more detail in the accompanying articles within this special issue. Suffice it to say that both gonocyte quiescence and SSC niche establishment are crucially important biological events, yet remain insufficiently characterized with respect to the molecular mechanisms that initiate and regulate them. When these events do not proceed properly, cancer or infertility can result. Many decades of innovative, exciting research have provided insight into the cellular events of the premeiotic male germline. Undoubtedly, further investigation of these events will continue for many years to come.

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References

ADAMS, I.R. and MCLAREN, A. (2002). Sexually dimorphic development of mouse primordial germ cells: switching from oogenesis to spermatogenesis. *Development* 129: 1155-1164.

ARA, T., NAKAMURA, Y., EGAWA, T., SUGIYAMA, T., ABE, K., KISHIMOTO, T., MATSUI, Y. and NAGASAWA, T. (2003). Impaired colonization of the gonads by primordial germ cells in mice lacking a chemokine, stromal cell-derived factor-1 (SDF-1). *Proc Natl Acad Sci USA* 100: 5319-5323.

BALLOW, D., MEISTRICH, M.L., MATZUK, M. and RAJKOVIC, A. (2006). Sohlh1 is essential for spermatogonial differentiation. *Dev Biol* 294: 161-167.

BARRIOS, F., FILIPPONI, D., CAMPOLO, F., GORI, M., BRAMUCCI, F., PELLEGRINI, M., OTTOLENGHI, S., ROSSI, P., JANNINI, E.A. and DOLCI, S. (2012). SOHLH1 and SOHLH2 control Kit expression during postnatal male germ cell development. *J Cell Sci* 125: 1455-1464.

BARRIOS, F., FILIPPONI, D., PELLEGRINI, M., PARONETTO, M.P., DI SIENA, S., GEREMIA, R., ROSSI, P., DE FELICI, M., JANNINI, E.A. and DOLCI, S. (2010). Opposing effects of retinoic acid and FGF9 on Nanos2 expression and meiotic entry of mouse germ cells. *J Cell Sci* 123: 871-880.

BASCIANI, S., DE LUCA, G., DOLCI, S., BRAMA, M., ARIZZI, M., MARIANI, S., ROSANO, G., SPERA, G. and GNESSI, L. (2008). Platelet-derived growth factor receptor beta-subtype regulates proliferation and migration of gonocytes. *Endocrinology* 149: 6226-6235.

BOULOGNE, B., HABERT, R. and LEVACHER, C. (2003). Regulation of the proliferation of cocultured gonocytes and Sertoli cells by retinoids, triiodothyronine, and intracellular signaling factors: differences between fetal and neonatal cells. *Mol Reprod Dev* 65: 194-203.

BOWLES, J., FENG, C.W., SPILLER, C., DAVIDSON, T.L., JACKSON, A. and KOOPMAN, P. (2010). FGF9 suppresses meiosis and promotes male germ cell fate in mice. *Dev Cell* 19: 440-449.

BOWLES, J., KNIGHT, D., SMITH, C., WILHELM, D., RICHMAN, J., MAMIYA, S., YASHIRO, K., CHAWENGSAKSOPHAK, K., WILSON, M.J., ROSSANT, J. *et al.,* (2006). Retinoid signaling determines germ cell fate in mice. *Science* 312: 596-600.

BUAAS, F.W., KIRSH, A.L., SHARMA, M., MCLEAN, D.J., MORRIS, J.L., GRISWOLD, M.D., DE ROOIJ, D.G. and BRAUN, R.E. (2004). Plzf is required in adult male germ cells for stem cell self-renewal. *Nat Genet* 36: 647-652.

CHIQUOINE, A.D. (1954). The identification, origin, and migration of the primordial germ cells in the mouse embryo. *Anat Rec* 118: 135-146.

CLERMONT, Y. and BUSTOS-OBREGON, E. (1968). Re-examination of spermatogonial renewal in the rat by means of seminiferous tubules mounted “in toto”. *Am J Anat* 122: 237-247.

CLERMONT, Y. and HERMO, L. (1975). Spermatogonial stem cells in the albino rat. *Am J Anat* 142: 159-175.

CLERMONT, Y. and LEBLOND, C.P. (1953). Renewal of spermatogonia in the rat. *Am J Anat* 93: 475-501.

CLERMONT, Y. and PEREY, B. (1957). Quantitative study of the cell population of the seminiferous tubules in immature rats. *Am J Anat* 100: 241-267.

COSTOYA, J.A., HOBBS, R.M., BARNA, M., CATTORETTI, G., MANOVA, K., SUKHWANI, M., ORWIG, K.E., WOLGEMUTH, D.J. and PANDOLFI, P.P. (2004). Essential role of Plzf in maintenance of spermatogonial stem cells. *Nat Genet* 36: 653-659.

DANN, C.T., ALVARADO, A.L., MOLYNEUX, L.A., DENARD, B.S., GARBERS, D.L. and PORTEUS, M.H. (2008). Spermatogonial stem cell self-renewal requires OCT4, a factor downregulated during retinoic acid-induced differentiation. *Stem Cells* 26: 2928-2937.

DINAPOLI, L., BATCHVAROV, J. and CAPEL, B. (2006). FGF9 promotes survival of germ cells in the fetal testis. *Development* 133: 1519-1527.

DOITSIDOU, M., REICHMAN-FRIED, M., STEBLER, J., KOPRUNNER, M., DORRIES, J., MEYER, D., ESGUERRA, C.V., LEUNG, T. and RAZ, E. (2002). Guidance of primordial germ cell migration by the chemokine SDF-1. *Cell* 111: 647-659.

DYM, M. and CLERMONT, Y. (1970). Role of spermatogonia in the repair of the seminiferous epithelium following x-irradiation of the rat testis. *Am J Anat* 128: 265-282.

EILDERMANN, K., AECKERLE, N., DEBOWSKI, K., GODMANN, M., CHRISTIANSEN, H., HEISTERMANN, M., SCHWEYER, S., BERGMANN, M., KLIESCH, S., GROMOLL, J. *et al.,* (2012). Developmental Expression of the Pluripotency Factor Sal-Like Protein 4 in the Monkey, Human and Mouse Testis: Restriction to Premeiotic Germ Cells. *Cells Tissues Organs* 196: 206-220.

GALLAGHER, S.J., KOFMAN, A.E., HUSZAR, J.M., DANNENBERG, J.H., DEPINHO, R.A.,BRAUN, R.E. and PAYNE, C.J. (2013). Distinct requirements for Sin3a in perinatal gonocytes and differentiating spermatogonia. *Dev Biol* 373: 83-94.

GAUTIER, C., LEVACHER, C., AVALLET, O., VIGIER, M., ROUILLER-FABRE, V., LECERF, L., SAEZ, J. and HABERT, R. (1994). Immunohistochemical localization of transforming growth factor-beta 1 in the fetal and neonatal rat testis. *Mol Cell Endocrinol* 99: 55-61.

GELY-PERNOT, A., RAVERDEAU, M., CELEBI, C., DENNEFELD, C., FERET, B., KLOPFENSTEIN, M., YOSHIDA, S., GHYSELINCK, N.B. and MARK, M. (2012). Spermatogonia differentiation requires retinoic acid receptor gamma. *Endocrinology* 153: 438-449.

GINSBURG, M., SNOW, M.H. and MCLAREN, A. (1990). Primordial germ cells in the mouse embryo during gastrulation. *Development* 110: 521-528.

GODIN, I., DEED, R., COOKE, J., ZSEBO, K., DEXTER, M. and WYLIE, C.C. (1991). Effects of the steel gene product on mouse primordial germ cells in culture. *Nature* 352: 807-809.

GODIN, I. and WYLIE, C.C. (1991). TGF beta 1 inhibits proliferation and has a chemotropic effect on mouse primordial germ cells in culture. *Development* 113: 1451-1457.

HAJKOVA, P., ERHARDT, S., LANE, N., HAAF, T., EL-MAARRI, O., REIK, W., WALTER, J. and SURANI, M.A. (2002). Epigenetic reprogramming in mouse primordial germ cells. *Mech Dev* 117: 15-23.

HAMRA, F.K., CHAPMAN, K.M., NGUYEN, D.M., WILLIAMS-STEPHENS, A.A., HAMMER, R.E. and GARBERS, D.L. (2005). Self renewal, expansion, and transfection of rat spermatogonial stem cells in culture. *Proc Natl Acad Sci USA* 102: 17430-17435.

HILSCHER, B., HILSCHER, W., BULTHOFF-OHNOLZ, B., KRAMER, U., BIRKE, A., PELZER, H. and GAUSS, G. (1974). Kinetics of gametogenesis. I. Comparative histological and autoradiographic studies of oocytes and transitional prospermatogonia during oogenesis and prespermatogenesis. *Cell Tissue Res* 154: 443-470.

HUCKINS, C. (1971). The spermatogonial stem cell population in adult rats. I. Their

morphology, proliferation and maturation. *Anat Rec* 169: 533-557.

KOUBOVA, J., MENKE, D.B., ZHOU, Q., CAPEL, B., GRISWOLD, M.D. and PAGE, D.C. (2006). Retinoic acid regulates sex-specific timing of meiotic initiation in mice. *Proc Natl Acad Sci USA* 103: 2474-2479.

KUBOTA, H., AVARBOCK, M.R. and BRINSTER, R.L. (2004). Culture conditions and single growth factors affect fate determination of mouse spermatogonial stem cells. *Biol Reprod* 71: 722-731.

LAWSON, K.A., DUNN, N.R., ROELEN, B.A., ZEINSTRA, L.M., DAVIS, A.M., WRIGHT, C.V., KORVING, J.P. and HOGAN, B.L. (1999). Bmp4 is required for the generation of primordial germ cells in the mouse embryo. *Genes Dev* 13: 424-436.

LI, H., PAPADOPOULOS, V., VIDIC, B., DYM, M. and CULTY, M. (1997). Regulation of rat testis gonocyte proliferation by platelet-derived growth factor and estradiol: identification of signaling mechanisms involved. *Endocrinology* 138: 1289-1298.

LIVERA, G., ROUILLER-FABRE, V., DURAND, P. and HABERT, R. (2000). Multiple effects of retinoids on the development of Sertoli, germ, and Leydig cells of fetal and neonatal rat testis in culture. *Biol Reprod* 62: 1303-1314.

MENDIS, S.H., MEACHEM, S.J., SARRAJ, M.A. and LOVELAND, K.L. (2011). Activin A balances Sertoli and germ cell proliferation in the fetal mouse testis. *Biol Reprod* 84: 379-391.

MENG, X., LINDAHL, M., HYVONEN, M.E., PARVINEN, M., DE ROOIJ, D.G., HESS, M.W., RAATIKAINEN-AHOKAS, A., SAINIO, K., RAUVALA, H., LAKSO, M. *et al.,* (2000). Regulation of cell fate decision of undifferentiated spermatogonia by GDNF. *Science* 287: 1489-1493.

MOLYNEAUX, K.A., ZINSZNER, H., KUNWAR, P.S., SCHAIBLE, K., STEBLER, J., SUNSHINE, M.J., O'BRIEN, W., RAZ, E., LITTMAN, D., WYLIE, C. *et al.,* (2003). The chemokine SDF1/CXCL12 and its receptor CXCR4 regulate mouse germ cell migration and survival. *Development* 130: 4279-4286.

MONESI, V. (1962). Autoradiographic study of DNA synthesis and the cell cycle in spermatogonia and spermatocytes of mouse testis using tritiated thymidine. *J Cell Biol* 14: 1-18.

MORENO, S.G., ATTALI, M., ALLEMAND, I., MESSIAEN, S., FOUCHET, P., COFFIGNY, H., ROMEO, P.H. and HABERT, R. (2010). TGFbeta signaling in male germ cells regulates gonocyte quiescence and fertility in mice. *Dev Biol* 342: 74-84.

MULLANEY, B.P. and SKINNER, M.K. (1992). Basic fibroblast growth factor (bFGF) gene expression and protein production during pubertal development of the seminiferous tubule: follicle-stimulating hormone-induced Sertoli cell bFGF expression. *Endocrinology* 131: 2928-2934.

NAKAGAWA, T., NABESHIMA, Y. and YOSHIDA, S. (2007). Functional identification of the actual and potential stem cell compartments in mouse spermatogenesis. *Dev Cell* 12: 195-206.

NAKAGAWA, T., SHARMA, M., NABESHIMA, Y., BRAUN, R.E. and YOSHIDA, S. (2010). Functional hierarchy and reversibility within the murine spermatogenic stem cell compartment. *Science* 328: 62-67.

NAUGHTON, C.K., JAIN, S., STRICKLAND, A.M., GUPTA, A. and MILBRANDT, J. (2006). Glial cell-line derived neurotrophic factor-mediated RET signaling regulates spermatogonial stem cell fate. *Biol Reprod* 74: 314-321.

OAKBERG, E.F. (1971). Spermatogonial stem-cell renewal in the mouse. *Anat Rec* 169: 515-531.

OATLEY, J.M., AVARBOCK, M.R., TELARANTA, A.I., FEARON, D.T. and BRINSTER, R.L. (2006). Identifying genes important for spermatogonial stem cell self-renewal and survival. *Proc Natl Acad Sci USA* 103: 9524-9529.

OATLEY, J.M., OATLEY, M.J., AVARBOCK, M.R., TOBIAS, J.W. and BRINSTER, R.L. (2009). Colony stimulating factor 1 is an extrinsic stimulator of mouse spermatogonial stem cell self-renewal. *Development* 136: 1191-1199.

OATLEY, M.J., KAUCHER, A.V., RACICOT, K.E. and OATLEY, J.M. (2011). Inhibitor of DNA binding 4 is expressed selectively by single spermatogonia in the male germline and regulates the self-renewal of spermatogonial stem cells in mice. *Biol Reprod* 85: 347-356.

OHINATA, Y., PAYER, B., O'CARROLL, D., ANCELIN, K., ONO, Y., SANO, M., BARTON, S.C., OBUKHANYCH, T., NUSSENZWEIG, M., TARAKHOVSKY, A. *et al.,* (2005). Blimp1 is a critical determinant of the germ cell lineage in mice. *Nature* 436: 207-213.

OHTA, H., YOMOGIDA, K., DOHMAE, K. and NISHIMUNE, Y. (2000). Regulation of proliferation and differentiation in spermatogonial stem cells: the role of c-kit and its ligand SCF. *Development* 127: 2125-2131.

OLASO, R., PAIRAULT, C., BOULOGNE, B., DURAND, P. and HABERT, R. (1998). Transforming growth factor beta1 and beta2 reduce the number of gonocytes by increasing apoptosis. *Endocrinology* 139: 733-740.

ORTH, J.M., QIU, J., JESTER, W.F., JR. and PILDER, S. (1997). Expression of the c-kit gene is critical for migration of neonatal rat gonocytes in vitro. *Biol Reprod* 57: 676-683.

OZDZENSKI, W. (1967). Observations on the origin of the primordial germ cells in the mouse. *Zool Polon* 17: 367-379.

PAYNE, C.J., GALLAGHER, S.J., FOREMAN, O., DANNENBERG, J.H., DEPINHO, R.A. and BRAUN, R.E. (2010). Sin3a is required by sertoli cells to establish a niche for undifferentiated spermatogonia, germ cell tumors, and spermatid elongation. *Stem Cells* 28: 1424-1434.

RHIND, N. and RUSSELL, P. (2000). Chk1 and Cds1: linchpins of the DNA damage and replication checkpoint pathways. *J Cell Sci* 113 (Pt 22): 3889-3896.

ROOSEN-RUNGE, E.C. and GIESEL, L.O., JR. (1950). Quantitative studies on spermatogenesis in the albino rat. *Am J Anat* 87: 1-30.

RYU, B.Y., KUBOTA, H., AVARBOCK, M.R. and BRINSTER, R.L. (2005). Conservation of spermatogonial stem cell self-renewal signaling between mouse and rat. *Proc Natl Acad Sci USA* 102: 14302-14307.

SATO, S., YOSHIMIZU, T., SATO, E. and MATSUI, Y. (2003). Erasure of methylation imprinting of Igf2r during mouse primordial germ-cell development. *Mol Reprod Dev* 65: 41-50.

SCHRANS-STASSEN, B.H., VAN DE KANT, H.J., DE ROOIJ, D.G. and VAN PELT, A.M. (1999). Differential expression of c-kit in mouse undifferentiated and differentiating type A spermatogonia. *Endocrinology* 140: 5894-5900.

SEKI, Y., YAMAJI, M., YABUTA, Y., SANO, M., SHIGETA, M., MATSUI, Y., SAGA, Y., TACHIBANA, M., SHINKAI, Y. and SAITOU, M. (2007). Cellular dynamics associated with the genome-wide epigenetic reprogramming in migrating primordial germ cells in mice. *Development* 134: 2627-2638.

SHILOH, Y. (2001). ATM and ATR: networking cellular responses to DNA damage. *Curr Opin Genet Dev* 11: 71-77.

SIMON, L., EKMAN, G.C., GARCIA, T., CARNES, K., ZHANG, Z., MURPHY, T., MURPHY, K.M., HESS, R.A., COOKE, P.S. and HOFMANN, M.C. (2010). ETV5 regulates sertoli cell chemokines involved in mouse stem/progenitor spermatogonia maintenance. *Stem Cells* 28: 1882-1892.

SNYDER, E.M., SMALL, C. and GRISWOLD, M.D. (2010). Retinoic acid availability drives the asynchronous initiation of spermatogonial differentiation in the mouse. *Biol Reprod* 83: 783-790.

SPILLER, C.M., WILHELM, D. and KOOPMAN, P. (2010). Retinoblastoma 1 protein modulates XY germ cell entry into G1/G0 arrest during fetal development in mice. *Biol Reprod* 82: 433-443.

SUZUKI, A. and SAGA, Y. (2008). Nanos2 suppresses meiosis and promotes male germ cell differentiation. *Genes Dev* 22: 430-435.

TAJIMA, Y., ONOUE, H., KITAMURA, Y. and NISHIMUNE, Y. (1991). Biologically active kit ligand growth factor is produced by mouse Sertoli cells and is defective in SId mutant mice. *Development* 113: 1031-1035.

TAM, P.P. and SNOW, M.H. (1981). Proliferation and migration of primordial germ cells during compensatory growth in mouse embryos. *J Embryol Exp Morphol* 64: 133-147.

TEERDS, K.J. and DORRINGTON, J.H. (1993). Localization of transforming growth factor beta 1 and beta 2 during testicular development in the rat. *Biol Reprod* 48: 40-45.

THUILLIER, R., MAZER, M., MANKU, G., BOISVERT, A., WANG, Y. and CULTY, M. (2010). Interdependence of platelet-derived growth factor and estrogen-signaling pathways in inducing neonatal rat testicular gonocytes proliferation. *Biol Reprod* 82: 825-836.

THUILLIER, R., WANG, Y. and CULTY, M. (2003). Prenatal exposure to estrogenic compounds alters the expression pattern of platelet-derived growth factor receptors alpha and beta in neonatal rat testis: identification of gonocytes as targets of estrogen exposure. *Biol Reprod* 68: 867-880.

TOKUDA, M., KADOKAWA, Y., KURAHASHI, H. and MARUNOUCHI, T. (2007). CDH1 is a specific marker for undifferentiated spermatogonia in mouse testes. *Biol Reprod* 76: 130-141.

TOYODA, S., MIYAZAKI, T., MIYAZAKI, S., YOSHIMURA, T., YAMAMOTO, M., TASHIRO, F., YAMATO, E. and MIYAZAKI, J. (2009). Sohlh2 affects differentiation

of KIT positive oocytes and spermatogonia. *Dev Biol* 325: 238-248.

TRES, L.L. and KIERSZENBAUM, A.L. (2005). The ADAM-integrin-tetraspanin complex in fetal and postnatal testicular cords. *Birth Defects Res C Embryo Today* 75: 130-141.

VAN BRAGT, M.P., ROEPERS-GAJADIEN, H.L., KORVER, C.M., BOGERD, J., OKUDA, A., EGGEN, B.J., DE ROOIJ, D.G. and VAN PELT, A.M. (2008). Expression of the pluripotency marker UTF1 is restricted to a subpopulation of early A spermatogonia in rat testis. *Reproduction* 136: 33-40.

VERGOUWEN, R.P., JACOBS, S.G., HUISKAMP, R., DAVIDS, J.A. and DE ROOIJ, D.G. (1991). Proliferative activity of gonocytes, Sertoli cells and interstitial cells during testicular development in mice. *J Reprod Fertil* 93: 233-243.

VIGUERAS-VILLASENOR, R.M., MORENO-MENDOZA, N.A., REYES-TORRES, G., MOLINA-ORTIZ, D., LEON, M.C. and ROJAS-CASTANEDA, J.C. (2006). The effect of estrogen on testicular gonocyte maturation. *Reprod Toxicol* 22: 513-520.

WANG, Y. and CULTY, M. (2007). Identification and distribution of a novel platelet-derived growth factor receptor beta variant: effect of retinoic acid and involvement in cell differentiation. *Endocrinology* 148: 2233-2250.

WESTERN, P.S., MILES, D.C., VAN DEN BERGEN, J.A., BURTON, M. and SINCLAIR, A.H. (2008). Dynamic regulation of mitotic arrest in fetal male germ cells. *Stem Cells* 26: 339-347.

WILHELM, D. and KOOPMAN, P. (2006). The makings of maleness: towards an integrated view of male sexual development. *Nat Rev Genet* 7: 620-631.

YING, Y., LIU, X.M., MARBLE, A., LAWSON, K.A. and ZHAO, G.Q. (2000). Requirement of Bmp8b for the generation of primordial germ cells in the mouse. *Mol Endocrinol* 14: 1053-1063.

YING, Y. and ZHAO, G.Q. (2001). Cooperation of endoderm-derived BMP2 and extraembryonic ectoderm-derived BMP4 in primordial germ cell generation in the mouse. *Dev Biol* 232: 484-492.

YOSHIDA, S., SUKENO, M. and NABESHIMA, Y. (2007). A vasculature-associated niche for undifferentiated spermatogonia in the mouse testis. *Science* 317: 1722-1726.

YOSHIDA, S., SUKENO, M., NAKAGAWA, T., OHBO, K., NAGAMATSU, G., SUDA, T. and NABESHIMA, Y. (2006). The first round of mouse spermatogenesis is a distinctive program that lacks the self-renewing spermatogonia stage. *Development* 133: 1495-1505.

YOSHIDA, S., TAKAKURA, A., OHBO, K., ABE, K., WAKABAYASHI, J., YAMAMOTO, M., SUDA, T. and NABESHIMA, Y. (2004). Neurogenin3 delineates the earliest stages of spermatogenesis in the mouse testis. *Dev Biol* 269: 447-458.

ZHENG, K., WU, X., KAESTNER, K.H. and WANG, P.J. (2009). The pluripotency factor LIN28 marks undifferentiated spermatogonia in mouse. *BMC Dev Biol* 9: 38.

ZHOU, Q., LI, Y., NIE, R., FRIEL, P., MITCHELL, D., EVANOFF, R.M., POUCHNIK, D., BANASIK, B., MCCARREY, J.R., SMALL, C. *et al.,* (2008a). Expression of stimulated by retinoic acid gene 8 (Stra8) and maturation of murine gonocytes and spermatogonia induced by retinoic acid in vitro. *Biol Reprod* 78: 537-545.

ZHOU, Q., NIE, R., LI, Y., FRIEL, P., MITCHELL, D., HESS, R.A., SMALL, C. and GRISWOLD, M.D. (2008b). Expression of stimulated by retinoic acid gene 8 (Stra8) in spermatogenic cells induced by retinoic acid: an *in vivo* study in vitamin A-sufficient postnatal murine testes. *Biol Reprod* 79: 35-42.

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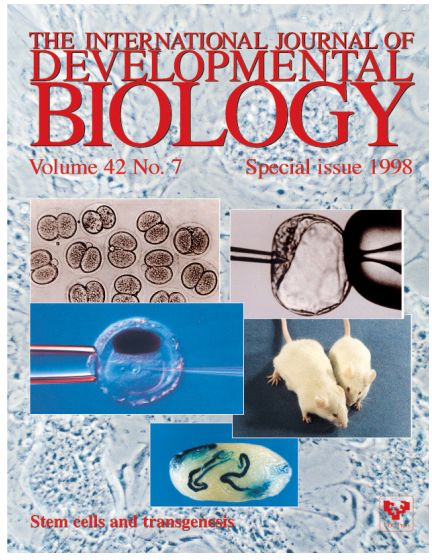

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