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Perspective

Mechanistic Insights into the Regulation of the Spermatogonial Stem Cell Niche

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ABSTRACT

Potential therapeutic use of stem cells in the treatment of human diseases depends on our ability to control the balance of their differentiation and self-renewal in vitro and in vivo. The stem cell "niche," or specialized microenvironment, is now recognized as one of the major contributors to this regulation in many species. Our recent study, which was reported in Nature, was the first to demonstrate that expression of a vertebrate animal transcription factor is essential for the maintenance of a stem cell niche. In that letter, targeted disruption of ERM (Ets-related molecule), which was localized only in the somatic support cell of the testis, the Sertoli cell, resulted in failure of self-renewal by spermatogonial stem cells, following the first wave of spermatogenesis. One of the more important conclusions drawn was the realization that regulation of the stem cell niche during the perinatal period, a phase characterized by rapid mitosis of both spermatogonial stem cells and Sertoli cells, differed from that in the adult. It appears that the ERM-regulated pathways are coincident with the termination of Sertoli cell proliferation and commencement of the cycle of spermatogenesis, which is sustained by the same cell that regulates the stem cell niche. Several likely targets for ERM regulation are discussed, as well as their potential implications for increasing our understanding of spermatogonial stem cell activity and the uniqueness of the Sertoli cell's immune privilege and possible utility for the protection of transplanted adult stem cells.

INTRODUCTION

In a recent study of spermatogonial stem cells, we demonstrated that Ets-related molecule (ERM) is required for their self-renewal and maintenance of spermatogenesis in the adult mouse.[1] This was the first evidence that a transcription factor regulates a stem cell niche in a vertebrate animal. The study found that in testis ERM was localized in the Sertoli cell, the only somatic cell of the seminiferous epithelium, and firmly established that in adult testes Sertoli cells maintain the spermatogonial stem cell niche. However, the molecular mechanisms involved and signaling pathways responsible for this activity have only begun to be answered, despite the critical nature of this Sertoli-germ cell interaction. Spermatogonia are the consummate stem cells because they not only self-renew throughout life, but they have the capacity for immortality by serving as genomic vectors capable of perpetuating the genome from one generation to another. Thus, the spermatogonial stem cell niche provides an excellent model for the study of mechanisms that may be common for the regulation of adult stem cell proliferation and differentiation. In this brief review, we will highlight the significance of data obtained from targeted disruption of the ERM gene and discuss potential implications for future research.

The stem cell niche is a specialized microenvironment provided by supporting cells, which promotes self-renewal and retention of stem cells in their undifferentiated state. The current scientific attention that is given to this biological compartment came about in part because the adult stem cell, although often being pluripotential in its ability to differentiate into multiple cell types, has unique requirements for its maintenance within specific organs and tissues. These requirements include both intrinsic as well as extrinsic signals. The Sertoli cell is recognized as one of those unique support cells, as it provides extrinsic signals for establishment and coordination of the complicated steps associated with spermatogenesis. As early as 1865, this tree-like cell was identified as being "sustentacular", or the "mother" cell,[2] because it formed an intimate physical relationship with germ cells (Fig. 1). However, until recently this label was not completely understood, especially regarding maintenance of the spermatogonial stem cell niche.

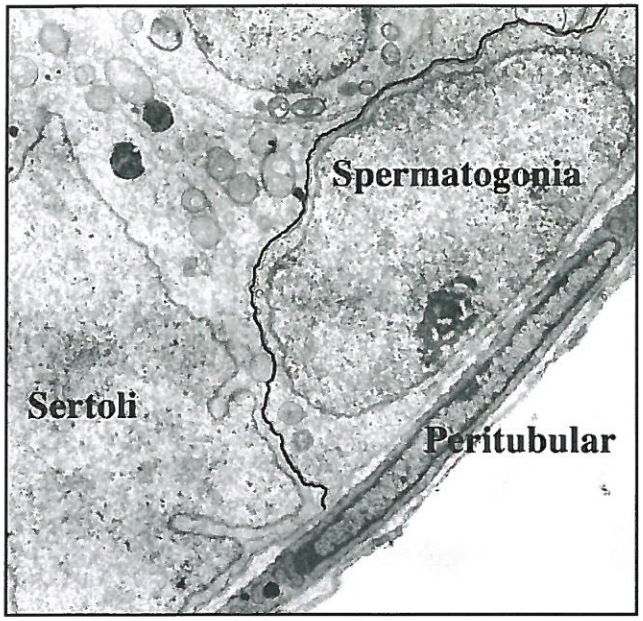

Figure 1. The spermatogonial stem cell niche. This electron micrograph shows the physical attachment of a Sertoli cell to a type A spermatogonium in situ, with both cell types lying on a basement membrane that is common with the surrounding peritubular cell. Plasmalemma of the spermatogonium touches the Sertoli cell on three sides (illustrated with a black line).

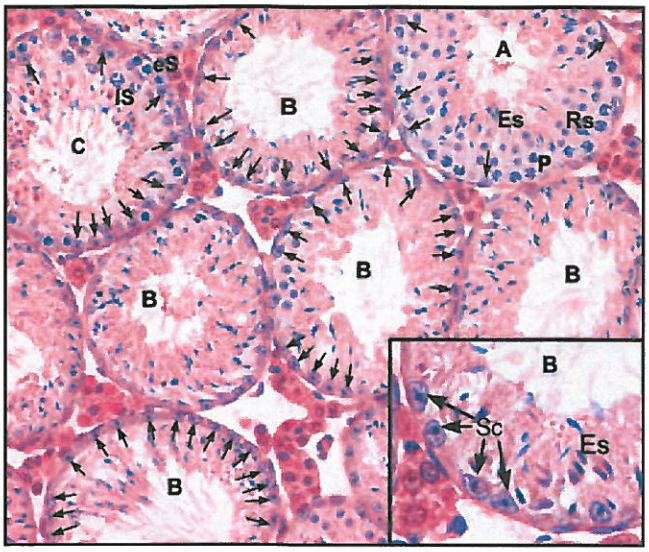

Figure 2. *ERM* -/- testis at 6 weeks of age. One tubule lacks spermatogonia but otherwise appears normal (A); Sertoli cells line the basement membrane (arrows) and other germ cell types fill the epithelium, including pachytene spermatocytes (P), round spermatids (Rs), elongated spermatids (Es). Most of the other tubules (B) contain only Sertoli cells (arrows) along the basement membrane, with attached elongated spermatids. The inset photo is a higher magnification of Sertoli cells (Sc) with attached elongate spermatids (Es), but other germinal layers are missing. The tubule labeled C shows a mixed response, with Sertoli cells (arrows), early (eS) and late spermatocytes (lS), and elongated spermatids, but round spermatids and spermatogonia are missing.

ERM IS ESSENTIAL FOR MAINTENANCE OF THE ADULT SPERMATOGONIAL STEM CELL

In mice with a targeted deletion of ERM (*ERM*$^{-/-}$), spermatogonial stem cells eventually disappear, but this only occurs after completion of the first wave of spermatogenesis.[1] By six weeks of age, the germinal epithelium is lost one layer at a time, beginning with spermatogonia and ending with elongated spermatids (Fig. 2). Thus, with maturation of the epithelium more advanced germ cells appear normal, but the underlying new generation of spermatogonia, spermatocytes and eventually spermatids fail to appear, which leads to seminiferous tubules surrounded only by Sertoli cells (Fig. 3). In the normal testis, Sertoli cells maintain stem cell self-renewal and differentiation, but in *ERM*$^{-/-}$ mice spermatogonial stem cells fail to renew, and these cells are lost through differentiation (Fig. 4). In this first study of ERM inactivation, considerable effort was made to determine in which cell types it was expressed, and the conclusion, based upon at least six different types of data, was that ERM expression is exclusive to Sertoli cells in testis.

ERM, along with PEA3 and ER81, comprises the PEA3 group of transcription factors that are members of the large Ets protein family. Although the mouse testis expresses high levels of ERM mRNA,[3,4] and this organ, along with brain, colon and lung, has the highest mRNA levels, its function is not known for any organ. Thus, its role in the Sertoli cell remains unanswered. However, by comparing isolated Sertoli cells from wild-type (*ERM*$^{+/+}$) and *ERM*$^{-/-}$ testes,[1] we were able to demonstrate that this transcription factor is unique, not only in its regulation of Sertoli cell factors, but also of spermatogonial cell markers, which coincided with the sequential depletion of germ cell layers (Tables 1 and 2).

What is regulated by ERM? ERM is a Sertoli cell gene, but it has an impact on germ cells as well. Using selective microarray and RT-PCR analyses,[1] it was demonstrated that spermatogonia-specific genes had the greatest reduction (3.5- to 14-fold) in expression (Table 1), while genes associated with mature or more differentiated germ cells were unchanged at four weeks of age. However, over time all germ cells are lost in the *ERM*$^{-/-}$ mice, but the first wave of spermatogenesis appears normal until the spermatogonia begin to disappear. Several genes were also found to be regulated by ERM in Sertoli cells (see appendix Table 4[1]). One of the more significant findings detected by microarray analysis of *ERM*$^{-/-}$ Sertoli cells was a 9- to 25-fold reduction in several chemokines, including SDF-1 or CXCL-12 (Stromal cell-derived factor), and a tenfold reduction in matrix metalloproteinase-12 (MMP-12). Chemokines are well

Table 1 **Effect of targeted disruption of ERM on the expression of germ cell genes**

	Gene[1]	Mouse	
Germ cell		**WT**	***ERM*$^{-/-}$**
Spermatogonial	Stra8	Normal	Decreased
	Dazl	Normal	Decreased
	Crabp	Normal	Decreased
	Lsh	Normal	Decreased
	Rbm	Normal	Decreased
	PLZF	Normal	Decreased
More differentiated	Prm1	Normal	Normal
	HPRT	Normal	Normal

[1]Stra8, stimulated by retinoic acid gene 8; Dazl, deleted in azoospermia homolog; Crabp, cellular retinoic-acid-binding protein; Lsh, lymphoid-specific helicase; Rbm, RNA-binding-motif; PLZF, promyelocytic leukemia zinc finger; Prm1, protamine 1; HPRT, hypxanthine-guanine phophoribosyltransferase.

Table 2 **Effect of targeted disruption of ERM on the expression of Sertoli cell genes**

Gene[1]	Sertoli cell	
	WT	*ERM -/-*
GDNF	Normal	Normal
SDF-1	Normal	Decreased
CXCL5	Normal	Decreased
CCL7	Normal	Decreased
MMP-12	Normal	Decreased

[1]GDNF, glial cell derived neurotrophic factor; SDF-1, stromal cell-derived factor; CXCL5, chemokine (C-X-C motif) ligand 5; CCL7, chemokine (CC motif) ligand 7; MMP-12, matrix metalloproteinase 12.

known for their involvement in cell migration and in particular, hematopoietic stem cell attraction and self-renewal.[5-11] Furthermore, new data suggest that chemokines and MMPs may be working together in the regulation of stem cell recruitment and migration.[12,13] Thus, it appears that in Sertoli cells, ERM is responsible for maintaining the production of factors common to other organs and tissues, which may help to retain stem cells inside their niches and balance their self-renewal and differentiation pathways. With ERM inactivation, spermatogonial stem cells are not maintained and differentiation is facilitated (Fig. 5), until the germinal epithelium is depleted (Fig. 2).

THE SPERMATOGONIAL STEM CELL NICHE CHANGES WITH AGE

The niche has been shown to be essential for maintenance of stem cell populations in the gut, skin, bone marrow and other organs.[14-16] Regulation of stem cell development typically depends on supporting cells that not only physically establish the niche but also communicate with stem cells to regulate their proliferation and differentiation. For example, in bone marrow, the stromal fibroblast and osteoblast are the essential supporting cells that regulate development of hematopoietic stem cells.[17] Much of our understanding of the stem cell niche came from the study of *Drosophila*, whose maintenance of germline stem cells is also dependent on signals from a somatic cell, which ensures asymmetric division of stem cells, producing one cell for self-renewal and one for differentiation.[15,18,19] In mammals, the somatic Sertoli cell is responsible for maintaining the germinal stem cell.

Until recently, most data suggested that a single Sertoli cell factor, GDNF, was most likely responsible for maintaining the spermatogonial stem cells[20-28] and that this activity extended throughout the life of the animal. GDNF is protein member of the TGF-β superfamily that is secreted by Sertoli cells (Table 2). The GDNF knockout is neonatally lethal. Therefore, evidence for its essential role in stem cell maintenance was unconvincing until fetal testes from GDNF knockout mice were transplanted under the back skin of a nude mouse host, thus circumventing neonatal lethality.[20] In the transplanted testes, spermatogonial stem cells quickly become depleted, as the germ cells differentiated but also experienced reduced proliferation. Others have shown that mice heterozygous for the GDNF knockout are viable but also have a progressive depletion of testicular stem cells.[28] Conversely, mice that over express GDNF have an increased number of undifferentiated spermatogonia.[29] Extensive in vitro data also indicate that GDNF stimulates spermatogonial stem cell proliferation without causing differentiation[22,23,26,30] and its coreceptor GFRα1 is now used as a marker for their isolation and identification.[22,23,31,32]

Based on the study of GDNF, one might assume that regulation of spermatogonial stem cells is straightforward and dependent only on the secretion of this Sertoli cell protein. However, two sets of data now suggest that spermatogonia stem cell regulation changes as the testis develops from perinatal to the pubertal age:

(1) The perinatal period is regulated by GDNF. The recent study by Naughton's laboratory[20] demonstrated that transplanted fetal *GDNF*$^{-/-}$ testes do not maintain spermatogonial stem cells nor support proliferation, but rather allow differentiation. The transplanted seminiferous tubules do not enter meiosis and thus never establish spermatogenesis. Sertoli cells do not express GDNF receptor; thus, ERM must be regulated by another pathway.

(2) The pubertal period is dependent on ERM. In *ERM*$^{-/-}$ mice, spermatogenesis is normal during the perinatal period, with completion of the first wave of spermatogenesis and formation of spermatozoa.[1] However, GDNF expression is normal in *ERM*$^{-/-}$ testes, even during the period when germ cells become depleted. Thus, GDNF is not regulated by ERM and is sufficient to maintain spermatogonial stem cells in the perinatal period, but ERM is essential for stem cell renewal in pubertal and adult testes.

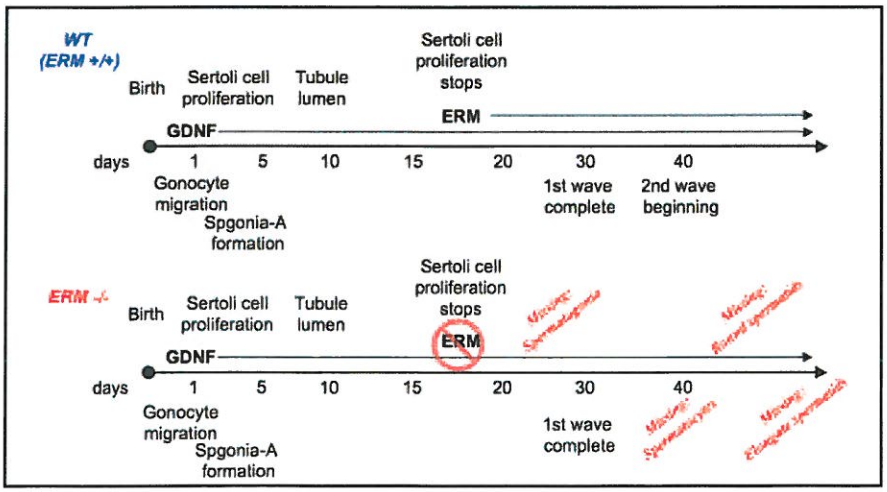

Figure 3. A timeline for the spermatogonial stem cell niche. In the wild-type (WT), GDNF is expressed from birth through adult, while ERM is expressed approximately during the period when Sertoli cells cease to divide. In the *ERM*$^{-/-}$ testis, GDNF continues to be expressed after ERM is no longer functional. Thus, GDNF permits the first wave of spermatogenesis; however, since ERM is not turned on in the perinatal period, the spermatogonial stem cells begin to disappear, while the more mature type-A spermatogonia continue along the normal path of spermatogenesis. Soon thereafter, layer after layer of germ cells disappears, eventually leaving elongated spermatids attached to Sertoli cells with no other germ cells beneath. Finally, by approximately 10 weeks of age, only Sertoli cells remain in the seminiferous tubules.

IMPORTANT QUESTIONS RAISED BY THESE NEW FINDINGS

When are ERM-regulated factors expressed? It now appears that multiple factors are involved in the regulation of spermatogonial stem cells, but the critical windows of time during which these factors are expressed must be determined. From birth to puberty, the seminiferous epithelium shows tremendous change and the testis grows over 80-fold in the mouse. Rapid growth occurs during the perinatal period, as Sertoli cells proliferate quickly after birth and continue to divide until about day 12–16, which establishes the seminiferous epithelial foundation. The number of Sertoli cells determines the ultimate adult testis size and sperm production, because the number of germ cells supported by the Sertoli cell is finite.[33] During this period of Sertoli cell mitosis, gonocytes migrate to the basement membrane, become spermatogonial stem cells and also begin rapid proliferation and differentiation. Thus, it is possible that this early time period, during which there is simultaneous proliferation of both Sertoli cells and spermatogonia, may have regulatory requirements that are unique. With each Sertoli cell division a new stem cell niche is created during this period of rapid growth, which would be filled by the continued proliferation of the new spermatogonial stem cells. Stem cells during this period of growth are likely to favor self-renewal (Fig. 4), similar to spermatogonia after irradiation of the testis.[34-37] Thus, GDNF is sufficient for stem cell maintenance during the perinatal period because it enhances self-renewal,[20-22,32] as would be required in order to fill expanding niches provided by new Sertoli cells. However, ERM is required for the continuation of stem cell maintenance beyond the first wave of spermatogenesis. The reason for this change in niche regulation remains to be determined.

Is ERM expression dependent upon Sertoli cell maturation and cessation of its proliferation? Sertoli cells cease to divide as they mature. They establish Sertoli-Sertoli junctional complexes and begin physiological support of germ cell differentiation (Fig. 3), which is vital for spermatogonial entrance into meiosis and subsequent spermiogenesis.[38,39] This sudden change in both Sertoli cell structure and function occurs around the time that ERM begins to be expressed and may signal the requirement for establishing a balance between spermatogonial stem cell differentiation (an adult need) and self-renewal (required developmentally and in the adult) (Fig. 5). Mature Sertoli cells regulate the simultaneous maintenance of several different types of developing germ cells within a carefully timed cycle of spermatogenesis.[40,41] The cycle has been divided into numerous epithelial stages or cellular associations,[42,43] with each stage exhibiting specific molecular and hormonal responsive patterns that are unique to the Sertoli and germ cells.[44-46] Thus, stage specificity of Sertoli cell function may be a major reason that a factor/s other than GDNF is required for regulation of the stem cell niche in adult testes, compared to the perinatal period.

What are the potential targets for ERM regulation? As a transcription factor in Sertoli cells, ERM could act through several different pathways, not exclusive to but including the following: (a) enhancing spermatogonia stem cell proliferation through production by the Sertoli cells of growth factors or other molecules that alter the cell cycle in spermatogonial stem cells; (b) balancing asymmetric and symmetric division of the stem cells; (c) modulating the Sertoli-spermatogonial cell junction; (d) altering basement membrane factors or stem cell membrane receptors that interact with basement membrane; and (e) recruiting or attracting stem cells to remain in the niche by increasing Sertoli cell secretion of chemokines. In the next few paragraphs, these ideas will be examined for their potential to explain downstream actions of ERM.

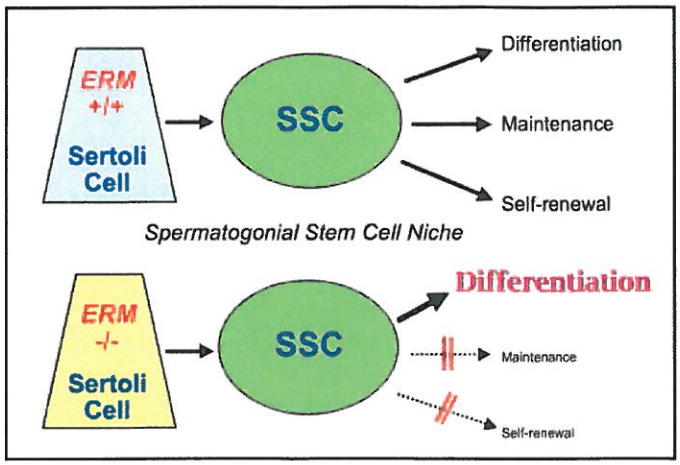

Figure 4. Sertoli cell expression of ERM is essential for maintenance of the spermatogonial stem cell (SSC) niche. In the *ERM*$^{-/-}$ mice, Sertoli cells do not maintain SSC self-renewal, but rather show a continuation of stem cell differentiation.

The phenotypic similarities between *ERM* and *GDNF* knockouts and the fact that GDNF continues to be expressed in the *ERM*$^{-/-}$ *testis* suggests that an overlapping pathway/s may involve these two factors. However, current data suggest that neither factor would be capable of directly regulating the other, but there is the potential for ERM to indirectly alter GDNF activity, especially if such regulation is required in the adult testis to reach equilibrium between stem cell self-renewal and differentiation (Fig. 5). Spermatogonia stem cells express both GFRα1 and RET, which are coreceptors for GDNF. GFRα1 (GDNF Family Receptor α1) contains the ligand-binding region and is bound extracellularly to the germ cell plasmalemma. Since GFRα1 lacks a transmembrane or intracellular region, signaling transduction requires the transmembrane tyrosine kinase receptor, RET. Like the GDNF knockout, both GFRα1 and RET knockouts are neonatally lethal, but involvement of GFRα1 and RET in stem cell maintenance was demonstrated by grafting neonatal testes from these mice into nude mice hosts. These studies showed that both GFRα1 and RET are necessary for maintaining undifferentiated spermatogonia stem cells, and knockout of either results in loss of the stem cells and failure of spermatogenesis.[20] These data from knockout mice confirm earlier studies with the RET hypomorphic mice, which showed abnormalities in germ cell maturation.[47] In addition, these findings are consistent with in vitro studies indicating that treatment of spermatogonial stem cell cultures with soluble GFRα1 promoted cell proliferation.[26] Thus, ERM expression with the onset of puberty may be involved in an indirect regulation of the GDNF pathway, which could occur by altering expression of either receptor or other factors that influence their expression.

Taf4b and PLZF (Promyelocytic leukemia zinc-finger) are also essential for spermatogonia stem cell maintenance, but these factors are only found in germ cells. TAF4b is a germ cell specific component of the RNA polymerase complex and plays an important role in transcriptional regulation. Testicular phenotypes of *TAF4b* and *ERM*$^{-/-}$ knockouts are quite similar, as they both experience completion of the first wave of spermatogenesis, but undergo progressive losses of subsequent germ cell progenitor cells, leading to Sertoli cell only

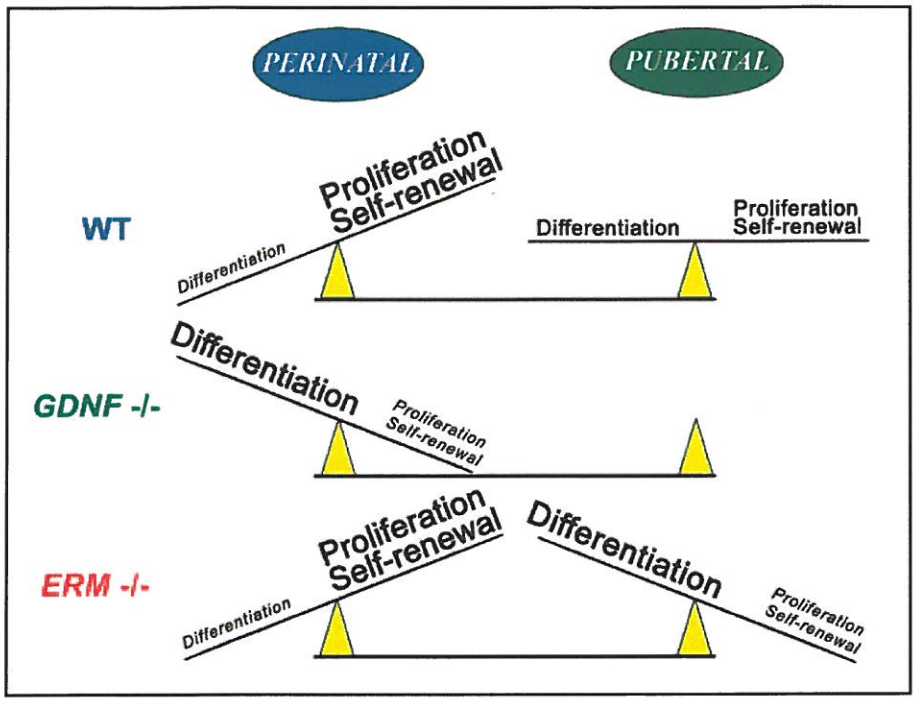

Figure 5. The stem cell population requires a balance between differentiation, proliferation and self-renewal. During the perinatal period of development, the balance is higher for self-renewal, as the testis size becomes established through Sertoli cell proliferation. In the pubertal period and adult, testis size and sperm production reach a plateau, with a balance between stem cell differentiation and self-renewal, as Sertoli cells no longer divide and they support a fixed number of germ cells. In the *GDNF*$^{-/-}$ mouse, stem cell self-renewal is inhibited and differentiation is enhanced, without spermatogenesis becoming established. In the *ERM*$^{-/-}$ mouse, the perinatal period appears to be normal, but stem cell self-renewal is inhibited during the pubertal period, while differentiation is enhanced (as apoptosis was not changed), resulting in the depletion of successive layers of new germ cells.

seminiferous tubules.[1,48] In both cases, the loss of stem cells is hormone independent. The difference is found in their exclusive cell type expression; ERM is in Sertoli cells, while TAF4b is only found in germ cells. Impairment produced in the *TAF4b* knockout testis reflects germ cell deficits exclusively, as the Sertoli cells support spermatogenesis of transplanted WT spermatogonia.[48] Based on the depletion of germ cells in the *TAF4b* knockout mouse, it is not surprising to find that the expression of RET, GDNF's coreceptor, is decreased significantly in the *TAF4b*$^{-/-}$ germ cells,[48] which provides a potential link between ERM, TAF4b and the GDNF pathway.

PLZF is a transcriptional repressor protein that inhibits stem cell differentiation and helps to maintain their presence in the testis and other organs. In hematopoietic cells there is high expression of PLZF in undifferentiated multipotential precursor cells, and low expression in differentiated cells[49] PLZF also exerts growth suppressive activities and induces accumulation of cells in G_0/G_1 of the cell cycle,[50,51] possibly through its repression of the protooncogene c-myc.[52] Data reporting that PLZF is important for stem cell differentiation are consistent with two recent papers suggesting that PLZF is vital in the regulation of spermatogonia stem cells.[53,54] Both the naturally occurring mutant lacking *PLZF* (Luxoid) and the *PLZF* knockout mice[53,54] have progressive losses of spermatogonia with age. The phenotypic similarities between *PLZF* and *ERM* knockouts suggest that PLZF could be downstream of ERM or at the very least that PLZF and ERM deficiencies act through common pathways, which produces in both knockout mice a delayed loss of stem cells.

Finally, *ERM*$^{-/-}$ mice survive into adulthood without obvious phenotypic abnormalities,[1] suggesting there are no major problems with hematopoietic, skin or intestinal stem cells, and these animals have no other obvious structural/functional deficits. Thus, ERM is obligatory for stem cell maintenance only in the testis, as far as we presently know. However, it is now recognized that many of the pathways involved in stem cell maintenance are conserved across species. Therefore, the challenge will be to determine how and for what reasons ERM provides a unique regulation of adult spermatogonial stem cell maintenance. Two important areas of future study that are common to other stem cell niches are the cell-to-cell adhesion junctions that are formed[55-57] and the role of basement membrane interactions with both spermatogonia and Sertoli cells.[58-61] It is possible that ERM will participate in the regulation of both these components. For example, the formation of adherens junctions[62] and cell cycle regulation[63] have been associated with Jagged-1 and its receptors Notch 1, 2 and 3. ERM could regulate the Sertoli cell expression of Jagged-1, as its receptors Notch-1, 2 and 3 are found on the spermatogonial membrane.

In conclusion, the Sertoli cell transcription factor ERM is unique in that its expression is required after the first wave of spermatogenesis has been established. A known secretory protein of Sertoli cells, GDNF, is sufficient for maintaining spermatogonial stem cell self-renewal during the perinatal period, but other factors are needed in the adult testis, possibly associated with the cessation of Sertoli cell proliferation and the onset of stage-specific activities that are required for continual output of sperm. Disruption of the ERM pathways may contribute to several unresolved testicular problems. For example, recent work by the Brinster laboratory indicates that the decrease in spermatogenesis with aging reflects a degradation of the stem cell niche, rather than stem cell abnormalities.[64] Thus, experimental modulation of ERM expression may help to uncover new methods for the treatment of male infertility or possibly lead to the development of a novel male contraceptive. The discovery of ERM expression in Sertoli cells is also important for a less obvious reason-this cell type exhibits immune privilege and is being cotransplanted with other cell types, such as pancreatic islet beta cells.[65-69] Thus, it may be possible to use the Sertoli cell also for the protection of transplanted adult stem cells in specific organs or tissues.

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