Glial cell line-derived neurotrophic factor maintains a POZ-itive influence on stem cells

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In adult males, germ-line stem cells have the remarkable ability to both self-renew and differentiate, ensuring that a continuous population of mature spermatozoa is produced throughout the lifetime of the animal. This balance between self-renewal and differentiation is thought to depend on the proper cellular environment, or stem cell "niche," that provides the appropriate signals at the right time for these processes. One growth factor shown to be essential for mammalian spermatogonial stem cell (SSC) self-renewal is the glial cell line-derived neurotrophic factor (GDNF) (1). GDNF signals through a receptor complex containing the RET receptor tyrosine kinase and the GDNF family receptor α1 (GFRα1). *Gdnf*$^{-/-}$ mouse testes transplanted into WT recipients exhibit a rapid and dramatic loss of SSCs, resulting in testes that contain only the supporting Sertoli cells (2). The development of a culture method and transplantation assay for mouse SSCs has shown that when GDNF is removed from the culture media, the agametic recipient testes into which these cells are transplanted fail to be repopulated, indicating a loss of SSCs (3). Clearly, GDNF is necessary for SSC maintenance, but until now the downstream target genes activated by GDNF and demonstrated as important for SSC self-renewal were unknown. The work of Oatley *et al.* (4) in a recent issue of PNAS identifies genes regulated by GDNF by using the SSC culture system and microarray analysis. One of these genes, *Bcl6b*, is a member of the POZ (poxvirus and zinc finger) family of transcriptional repressors that also includes *Plzf*, previously shown to be required for SSC maintenance (5, 6). These results underscore the importance of the niche in the mammalian testis and extend its molecular characterization by identifying downstream targets of GDNF.

The concept of the niche was first proposed by Schofield (7) to describe the relationship between the hematopoietic stem cell and its local microenvironment. For male germ-line stem cells, the best-characterized niche is that of the *Drosophila* testis. Localizing to the apical somatic hub cells are seven to nine germ-line stem cells that divide asymmetrically. Each stem cell divides to produce one daughter cell that remains attached to the hub and one gonialblast that migrates away and differentiates. The hub generates and releases signals to control this stem cell self-renewal, including two members of the bone morphogenic protein (BMP) growth factor family, *glass bottom boat* (*gbb*) and *decapentaplegic* (*dpp*) (8). One of the downstream targets of *gbb* is a *dpp*-responsive gene, *Dad*, which exhibits transcriptional activation in the stem cells to maintain self-renewal. Through *gbb* and *dpp*, BMP signaling represses the expression of *bag of marbles* (*bam*), a gene that is transcriptionally active in differentiating spermatocytes but repressed in the mother stem cells and daughter gonialblasts (8). A family of conserved RNA-binding proteins encoded by *piwi* also represses *bam* through both BMP-dependent and BMP-independent signaling pathways (9, 10). Thus, through the expression of *gbb*, *dpp*, and *piwi*, and the repression of *bam*, the BMP growth factor family maintains the stem cell niche in the testis.

Another signal generated from the *Drosophila* hub is Unpaired/Outstretched (Os), which activates the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) signaling pathway to maintain stem cell self-renewal (11, 12). Os is a secreted ligand that is specifically expressed in the hub and upon its release binds to the receptor Domeless on the surface of adjacent stem cells. Receptor binding activates the JAK homologue Hopscotch and the STAT homologue Stat92E, which then translocates to the nucleus where it activates downstream target genes important for stem cell self-renewal (11). When the JAK–STAT signaling pathway is inhibited, germ cells are depleted from the testis after the complete differentiation of all of the stem cells. Recent evidence has also shown that the centrosome protein centrosomin, together with the adenomatous polyposis coli tumor suppressor, regulates the mitotic spindle orientation in stem cells to ensure that asymmetric cell division generates a daughter gonialblast oriented away from the hub (13). *Drosophila* E-cadherin and β-catenin homologue Armadillo colocalize at the hub–stem cell interface, implicating their role in this orientation process.

Given these defined pathways that maintain the *Drosophila* germ-line stem cell niche, one may ask whether there is a similar niche in mammalian testes, and if so, what are the required signaling pathways and downstream target genes? Performing some of the functions of *Drosophila* hub cells in mammals are the Sertoli cells, which physically interact with SSCs and release growth factors that are proposed to maintain stem cell self-renewal. One growth factor identified for this role is GDNF. As a distant member of the TGF-β family, GDNF was shown to be required for enteric innervation and embryonic kidney development (14, 15). *Gdnf*$^{-/-}$, *Gfrα1*$^{-/-}$, and *Ret*$^{-/-}$ mouse pups all die on postnatal day 1 (14, 16, 17). However, *Gdnf*$^{+/-}$ heterozygous mutant males show extensive germ cell depletion, with some seminiferous tubules displaying a progressive Sertoli cell-only phenotype (1). To overcome neonatal lethality and conclusively test whether *Gdnf*-null mouse testes can support spermatogenesis, *Gdnf*-deficient testes were transplanted from postnatal day 0 mutants into nude recipients and allowed to develop (2). The resulting testes exhibited severe SSC depletion by postnatal day 7, with an ability of the stem cells to maintain their undifferentiated state. Thus, the GDNF pathway in mammals appears to be as essential as the JAK–STAT pathway in *Drosophila* in maintaining the niche.

Although the activation of JAK–STAT is considered to be a local signaling event in the *Drosophila* niche, recent experimentation has shown that differentiating cells that are physically detached from the hub can revert to their stem cell identity and migrate back to the hub cells upon receiving JAK–STAT signals (18). It is possible, then, that these signals have a much longer range than previously thought. In the same

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way, GDNF signals could act either locally as short-range modulators or at a distance as long-range effectors. It is unknown whether the sites of GDNF secretion on the surface of Sertoli cells are polarized or uniform. Likewise, it is unclear whether GDNF release is pulsatile or constant during the seminiferous epithelium growth cycle. These lingering questions warrant further investigation.

With signaling molecules that promote SSC self-renewal in *Drosophila* and mice now identified, the search for their target genes is an important next step in defining the stem cell niche. To this end, Oatley *et al.* (4) examine the effects of withdrawing GDNF from the culture medium on global gene expression levels in SSCs and the effects of replacing GDNF after this withdrawal on the SSC transcriptome. Six transcripts show significant changes in expression levels in response to GDNF withdrawal and replacement, including *Bcl6b*. This use of transcriptional profiling was also recently implemented in the identification of JAK–STAT target genes in *Drosophila* testes (19) and the discovery that Ets-related molecule is expressed in Sertoli cells and required for SSC self-renewal (20).

The identification of a GDNF-responsive POZ-domain protein important for SSC self-renewal is of great interest (Fig. 1). *Bcl6b* is the mouse orthologue of human *BCL6*, which was first discovered through its involvement in chromosomal translocations associated with non-Hodgkin's lymphoma, and which represses IL-2-induced B cell differentiation (21). *Bcl6b* is in the same family as *Plzf*, which functions cell autonomously in SSCs to promote self-renewal (5, 6). Like PLZF, BCL6b has a POZ domain through which it interacts with corepressors N-CoR and SMRT and associates with a large transcriptional regulatory complex that includes mSIN3 and the histone deacetylase HDAC1 in other cell types (22). It is unclear whether these same interactions occur in SSCs. *Bcl6b* siRNA knockdown in cultured SSCs, followed by transplantation into agametic mouse testes, results in dramatically reduced colony formation, a clear sign of impaired repopulating ability (4). The additional observation of impaired spermatogenesis in *Bcl6b*$^{-/-}$ testes suggests that transcriptional silencing as a downstream effector of GDNF signaling may be an important regulator of the stem cell niche in mammals. Just how this signal transduction event translates into increased expression of POZ-domain proteins in SSCs will be an important question to answer as the examination of the stem cell niche in the mammalian testis continues.

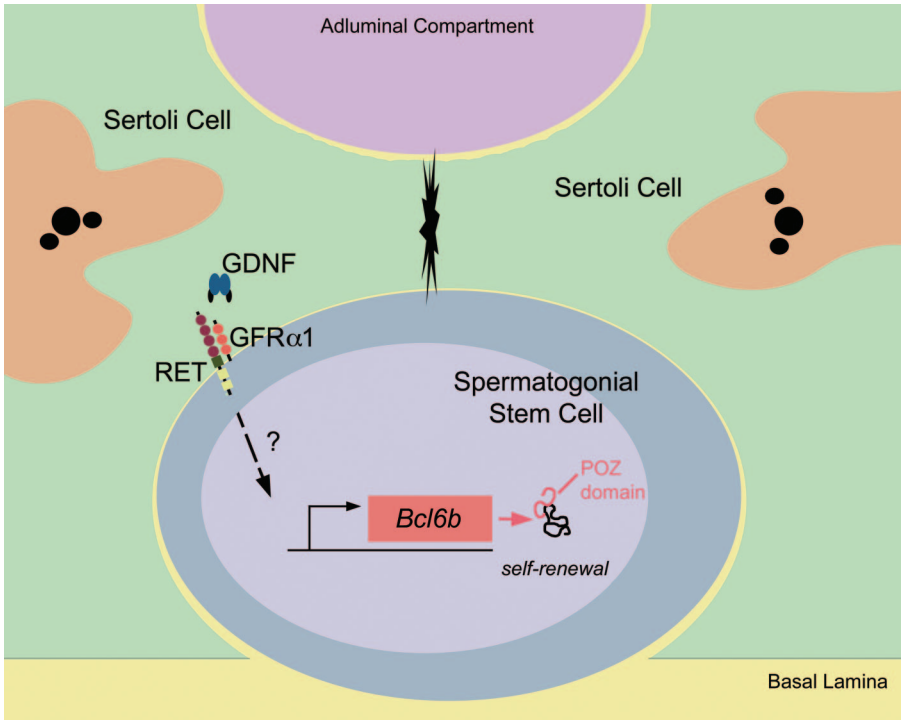

Fig. 1. GDNF secretion by Sertoli cells induces transcription of *Bcl6b* in spermatogonial stem cells. Model identifying a target gene of GDNF signaling. Sertoli cells secrete GDNF, which binds to the RET/GFRα1 receptor complex on the surface of spermatogonial stem cells, thereby transducing signals to the nucleus. *Bcl6b* becomes transcriptionally active and translates into a POZ-domain-containing nuclear transcriptional repressor protein. *Bcl6b* expression is important for stem cell self-renewal, but the signal transduction mechanism regulating its activity downstream of RET/GFRα1 is not clear.

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