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Review

Transcriptional control of spermatogonial maintenance and differentiation

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ABSTRACT

Spermatogenesis is a multistep process that generates millions of spermatozoa per day in mammals. A key to this process is the spermatogonial stem cell (SSC), which has the dual property of continually renewing and undergoing differentiation into a spermatogonial progenitor that expands and further differentiates. In this review, we will focus on how these proliferative and early differentiation steps in mammalian male germ cells are controlled by transcription factors. Most of the transcription factors that have so far been identified as promoting SSC self-renewal (BCL6B, BRACHYURY, ETV5, ID4, LHX1, and POU3F1) are upregulated by glial cell line-derived neurotrophic factor (GDNF). Since GDNF is crucial for promoting SSC self-renewal, this suggests that these transcription factors are responsible for coordinating the action of GDNF in SSCs. Other transcription factors that promote SSC self-renewal are expressed independently of GDNF (FOXO1, PLZF, POU5F1, and TAF4B) and thus may act in non-GDNF pathways to promote SSC cell growth or survival. Several transcription factors have been identified that promote spermatogonial differentiation (DMRT1, NGN3, SOHLH1, SOHLH2, SOX3, and STAT3); some of these may influence the decision of an SSC to commit to differentiate while others may promote later spermatogonial differentiation steps. Many of these transcription factors regulate each other and act on common targets, suggesting they integrate to form complex transcriptional networks in self-renewing and differentiating spermatogonia.

Contents

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1. Introduction

Spermatogenesis is a highly coordinated process requiring an orchestrated program of gene expression controlled by extrinsic and intrinsic factors. The extrinsic factors are derived from non-germ cells, including Sertoli cells, Leydig cells, and peritubular myoid cells within the testes; they trigger specific events in germ cells that dictate or influence spermatogenesis. The intrinsic factors are generated in germ cells and act within them. Among the intrinsic factors are transcription factors, which is the subject of this review.

Spermatogenesis is one of the few developmental processes that occur in adults. In order for spermatogenesis to be maintained, it is essential for there to be self-renewing cells in the testis. Specialized cells called spermatogonial stem cells (SSCs) serve in this capacity. In mammals, SSCs are located in a so-called "stem cell niche" in the basal compartment of the seminiferous epithelium, where SSCs proliferate to generate a clone of cells. The mitotic expansion of these clones is the foundation that sustains spermatogenesis. In mice, SSCs give rise to another type of undifferentiated spermatogonia called spermatogonial progenitors, which have a large proliferative capacity but are ultimately committed to differentiate. SSCs and spermatogonial progenitors are collectively called undifferentiated A-spermatogonia. Rather than being distinct cell types, SSCs and spermatogonial progenitors may represent distinct cellular states of a single cell type. After a series of cell divisions, these undifferentiated A-spermatogonia become differentiating A-spermatogonia, which, in turn, give rise to B-spermatogonia that further proliferate and differentiate into meiotic spermatocytes (Fig. 1). Upon completion of meiosis, spermatocytes convert into round spermatids and undergo a series of differentiation steps in the seminiferous epithelial tubule to become what is nearly a functional sperm: the elongated spermatid.

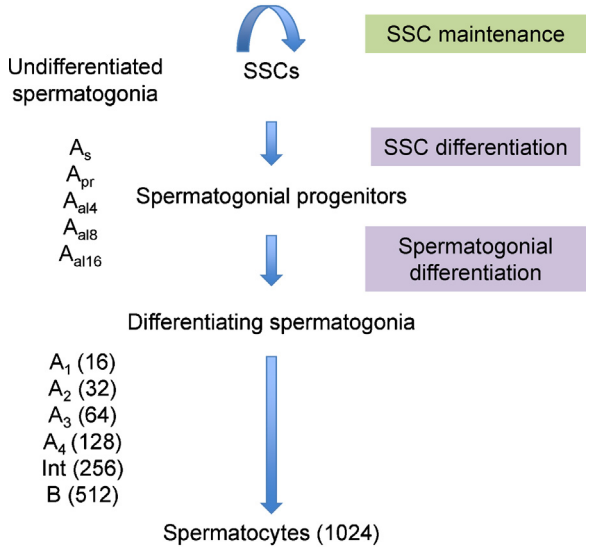

Fig. 1. The three regulatory steps of spermatogonial development. Not pictured is the fact that SSCs are a subset of undifferentiated A_s spermatogonia (and probably also A_{pr} and A_{al4} spermatogonia).

In this review, we will focus on mammalian transcription factors that are critical for SSC maintenance and spermatogonial differentiation (Fig. 1). Other recent reviews cover transcriptional regulators critical for other phases of mammalian spermatogenesis, including both meiotic and post-meiotic events [1–3].

2. SSCs

SSCs first arise a few days after birth in mice from non-dividing germ cells called gonocytes. SSCs are a subset of undifferentiated spermatogonia that are typically classified into being either A-single (A_s), A-paired (A_{pr}), or A-aligned (A_{al4}, A_{al8}, A_{al16}, and in rare case, A_{al32}) spermatogonia (Fig. 1). This morphological classification is based on the unusual ability of A-spermatogonia to remain connected after mitosis by intercellular bridges created as a result of incomplete cytokinesis. The classical view has been that only A_s spermatogonia are SSCs, but it now appears that many A_{pr} and even some of the shorter A_{al} spermatogonia have the potential to be SSCs [4,5].

Several protein markers, including PLZF, GFRα1, and ID4, have been shown to mark SSCs and other undifferentiated spermatogonia. Each differs in their specificity. Thus, while PLZF is expressed in all stages of undifferentiated spermatogonia [6,7], GFRα1 is mostly in A_s, A_{pr} and A_{al4} spermatogonia, and ID4 is specific for A_s spermatogonia [8]. To date, no marker has been identified as being exclusively expressed in SSCs [9]. Thus, the only reliable current means to unambiguously identify SSCs is to use an *in vivo* method: the germ cell transplantation assay [10]. The basis for this assay is that, by definition, SSCs are the only testicular germ cells that can colonize and initiate spermatogenesis. Thus, transplantation of a SSC (but not other cells) into a germ cell-free seminiferous tubule leads to the formation of a colony of descendent cells (after 2–3 months) that can be easily visualized. While it is not an entirely efficient assay (only ~10% of SSCs typically form a colony), *in vivo* transplantation allows one to compare the number of SSCs in different scenarios.

As an alternative to studying SSCs *in vivo*, *in vitro* SSC culture systems have been established. Two different SSC culturing methods that were established around the same time have been widely used. One method involves culturing so-called germline stem (GS) cells from neonatal (postnatal day-0 [P0] to P2) mouse testis [11]. In the other method, undifferentiated spermatogonia isolated from P6 to adult mice testes are enriched using the cell-surface marker, THY1, and then cultured [12]. Essential for the growth and maintenance of the SSCs in both GS and Thy1$^+$ spermatogonial cell cultures is glial cell line-derived neurotrophic factor (GDNF). By using GDNF in combination with basic fibroblast growth factor (bFGF; also known as FGF2), both methods have successfully been used to culture and expand SSCs for >3 months without losing their stem cell activity, as assayed by the germ cell-transplantation assay [11,12]. Of note, these cultures harbor not only SSCs but also other spermatogonia, including spermatogonial progenitors. Therefore, it appears that these *in vitro* culture systems recapitulate what normally occurs in the stem cell niche in the testis *in vivo*: both the self-renewal and the differentiation of SSCs.

In vitro SSC culture systems afford considerable advantages over generating and characterizing SSC-mutant mice, both in terms of

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