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At the Cutting Edge

SP1 transcription factors in male germ cell development and differentiation

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Abstract

Transcription factor SP1 is a zinc finger protein that has been implicated in regulating the expression of several genes involved in cellular differentiation and embryonic development. The zinc finger region of SP1 transcription factors binds to GC or GT-box elements present in the promoters of a number of male germ cell target genes that are developmentally expressed during spermatogenesis. The glutamine and serine/threonine-rich regions of the SP1 proteins recruit co-regulatory factors to the multi-protein preinitiation complex that are important for mediating transcriptional activation in male germ cells. Studies in our laboratory have identified several alternatively spliced transcripts encoding SP1 isoforms that display stage and cell-type-specific expression profiles in differentiating germ cells in the seminiferous epithelium of the testis. This review summarizes the expression patterns and functional significance of these SP1 transcription factor variants during spermatogenesis.

Keywords: Gene regulation; Transcriptional activation; SP1 transcription factors

Contents

1. Introduction

The program of testicular male germ cell differentiation, represents an ideal biological paradigm to elucidate the mechanisms involved in mediating and regulating developmental gene expression. Stem cell populations present within the seminiferous tubules of the testis continuously supply spermatogonia that undergo a series of mitotic divisions and eventually differentiate into type B spermatogonia that are committed to a terminal developmental program. The type B spermatogonia divide to become spermatocytes that progress through meiotic and post-meiotic developmental stages to produce haploid spermatids that proceed through a series of complex morphological changes resulting in spermatozoa (Fig. 1). Differential gene expression, SAGE and microarray analysis studies from our laboratory and

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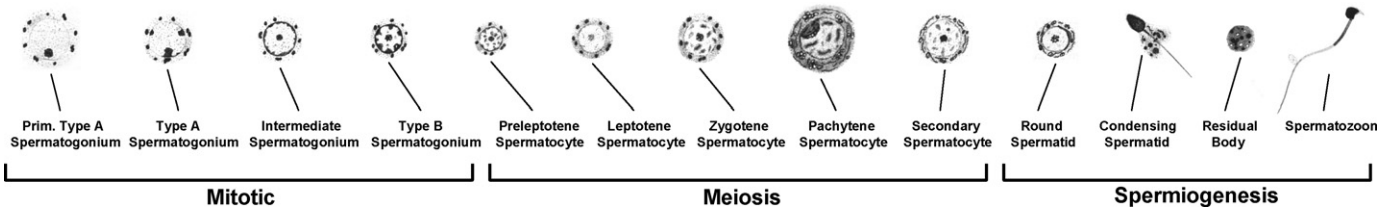

Fig. 1. Profile of male germ cells in the respective mitotic, meiotic and post-meiotic stages that represent the progression of male germ cell differentiation from spermatogonia to spermatozoa in the seminiferous tubules of the mouse testis.

others have shown that this unique cellular differential gene expression program required for sperm production has at least four major transcriptional phases (Thomas et al., 1989; Chan et al., 2006; Schlecht et al., 2004; Yu et al., 2003; Shima et al., 2004) that are mediated by a number of transcription factors including SP1. In addition, alternative splicing events occur in male germ cells that are important for producing the extensive diversity of proteins required for the precise progression of this cellular differentiation process (Eddy, 2002; Walker et al., 1999; Yu et al., 2003; Huang et al., 2005).

Although SP1 transcription factors are ubiquitously expressed in most cell types, they have been implicated in mediating developmental-specific gene expression by mechanisms that still remain largely unknown. Our laboratory has identified three SP1 transcript variants that encode functional proteins that play distinctive roles in regulating the male germ cell developmental program occurring in the seminiferous epithelium of the testis. These include 4.1, 3.7 and 3.2 kb transcripts that encode 90 and 60 kDa SP1 isoforms (Thomas et al., 2005). In addition, a novel 1.4 kb alternatively spliced SP1 transcript that represents the 3′ untranslated region (3′ UTR) is also expressed mainly during the meiotic phase of male germ cell differentiation. Our hypothesis is that this 5′ truncated SP1 RNA transcript subserves a unique regulatory function during this stage of germ cell development. In support of this hypothesis, a number of recent studies have provided strong evidence for post-transcriptional regulation by small RNA and 5′ and 3′ truncated RNA transcripts (Lai et al., 1989; Pestra et al., 1984; Abdelrahin et al., 2002).

2. Alternative splicing events produce SP1 transcript variants in male germ cells

DNA sequence analysis of cDNAs corresponding to mRNA transcripts for SP1 variants isolated from male germ cells indicate that they were produced by alternative splicing mechanisms. The 4.1 and 3.7 kb transcripts are produced by alternative 5′ and 3′ splicing events (see Fig. 2) whereas the 3.2 kb transcript utilizes an internal splice site in Exon 3 that results in the elimination of one of the glutamine-rich transactivation domains (domain A). The 1.4 kb transcript is produced by utilization of a 3′ splice site that results in 5′ truncated RNA transcripts that contain only the 3′ untranslated region. In addition to our studies, Persengiev et al. (1996) demonstrated that different SP1 transcripts (8.8, 8.2 and 2.5 kb) are expressed in a stage and cell-type-specific manner during spermatogenesis. Alternative splicing is a major mechanism responsible for generating isoforms of transcription factors that are frequently associated with tissue or cell-type-specific gene expression (Lopez, 1995; Xu et al., 2002; Veaubles, 2002). The importance and biological significance of alternatively spliced transcription factors involved in regulating the program of male germ cells differentiation and development was clearly demonstrated for the CREB and CREM transcription factors (Walker et al., 1999; Meyer and Habener, 1993; Sassone-Corsi, 1997, 2000; Beissbath et al., 2003).

3. Expression patterns and functions of the SP1 variants

Reverse Northern Blot and quantitative real-time PCR studies have indicated that the 4.1, 3.7 and 3.2 kb SP1 transcripts are expressed in a developmentally specific manner during spermatogenesis. Reverse Northern Blot studies using a probe specific for the 5′ end of the 4.1 kb SP1 transcript identified four alternatively spliced SP1 transcripts 4.1, 3.7, 3.2 and 2.5 kb that showed developmentally specific expression profiles in the differentiating male germ cell populations (Thomas et al., 2005). A 3′-specific probe showed slightly different patterns of expression for the 4.1, 3.7, 3.2 and 2.5 kb transcripts and identified an additional 1.4 kb alternatively spliced SP1 transcript that was expressed mainly during the meiotic stages of spermatogenesis (Fig. 3). Since slightly different patterns of expression for the SP1 transcript variants were observed with the 5′ and 3′-specific hybridization probes, quantitative real-time RT PCR studies were performed to determine their steady state levels of expression using $\Delta\Delta C_t$ comparative quanitation methods as described by Livak and Schmittgen (2001). These studies indicated that the 3.7 kb SP1 transcript was the most highly expressed SP1 transcript during the meiotic phase in the preleptotene, leptotene/zygotene and pachytene spermatocytes. However, the 4.1 and 3.2 kb SP1 transcripts also showed increased expression levels during the meiotic phase of spermatogenesis (Thomas et al., 2005). These and other studies suggest that there are functionally distinct populations of SP1 transcripts that are expressed during the mitotic, meiotic and post-meiotic stages of spermatogenesis. The 8.8 and 8.2 kb transcripts encoding 110 kDa SP1 isoforms are expressed mainly during the mitotic phase in the type A and B spermatogina (Persengiev et al., 1995, 1996). The 4.1 and 3.7 kb SP1 transcripts encoding the 90 kDa SP1 isoforms are expressed in overlapping patterns during the meiotic phase, whereas, the 3.2 and 2.5 kb SP1 transcript encoding the 60 kDa SP1 isoforms although expressed at lower levels throughout spermatogenesis showed increased levels of expression during meiosis. Functional studies demonstrating that these SP1