PSM2, a novel protein similar to MCAF2, is involved in the mouse embryonic and adult male gonad development

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Accepted 23 December 2005

Key words: gene expression, gonad, in situ hybridization, MCAF, psm2

Abstract

By screening RIKEN database, we obtained a mouse ovary and uterus derived clone (RIKEN clone ID: 5031403I04). Multiple tissues expression analysis revealed that the clone was specifically expressed in ovary and to a higher extent in testis. It is expressed in early mouse embryo, especially in the embryonic gonad from 11.5 dpc (days post coitus). Furthermore, it is also expressed continuously from newborn testis to adult. Using testis sections in situ hybridization, we found the mRNA was localized to spermatogonia, round spermatids and mainly to spermatocytes. We cloned the cDNA from mouse testis. The gene consists of 10 exons spanning approximately 48 kb on mouse chromosome 16. The cDNA encodes a putative nuclear protein of 319 amino acids containing a coiled-coil motif. The deduced protein has high similarity with human MCAF2 (MBD1-containing chromatin-associated factor 2), so we termed it as PSM2 (protein similar to MCAF2) in this article. We therefore hypothesized that PSM2 might interact with some important partners by the conserved domain and be involved in the transcription modulation during gonad development.

Introduction

Germ cells which represent a unique cell lineage in multicellular organisms, transmit genetic material from one generation to the next. The mechanisms of germ cell specification in mammals are markedly different from lower organisms [1]. Germ cells originating as a cluster of cells in the extraembryonic mesoderm emerge at midway through gestation, and then migrate into the site of the developing gonad by approximately 10–11 days post coitus (dpc). At around 12.5 dpc, male gonad becomes distinguishable by appearance of testis cords. Then the germ cells in testis go into mitotic arrest beginning at 13.5 dpc, whereas they enter into meiosis in the fetal ovary [2]. The PGCs give rise to prospermatogonia shortly after the initial differentiation of the fetal testis. During prepuberal development, mitotic spermatogonia give birth to meiotic spermatocytes, postmeiotic spermatids and finally spermatozoa [3].

MCAF/AM family proteins which are characterized by the presence of two evolutionarily conserved domains involving in protein–protein interaction, have the role of epigenetic modulation [4]. MCAF (MBD1-containing chromatin-associated factor), also known as the human homologue of murine ATFa-associated modulator (mAM), mediates MBD1-dependent transcription repression through interaction with MBD1 [5]. MBD1 which is a methyl-CpG binding domain protein, recognizes methylated CpG dinucleotide and recruits H3-K9 methyltransferases such as SETDB1 via MCAF1 to methylated DNA

Molecular Biology Reports (2006) 33:159–166
regions, and MBD1-MCAF1-SETDB1 complex induce heterochromatin formation and repress transcription in methylated DNA region [6]. On the other hand, MCAF proteins act as a co-activator-like factor for Sp1 which can induce expression of a variety of genes through binding to G-rich elements such as the GC box in the promoter and enhancer. mAM (mouse ATFα-associated modulator), also named activating transcription factor 7 interacting protein, is able to down-regulate transcriptional activity in an ATPase-independent manner. It can interact with the basal transcription machinery (TF II E, TF II H) and RNA polymerase II itself and mAM exerts its effect through protein–protein interaction [7]. Thus, MCAF proteins are able to interact with a wide range of molecules and likely to function as a transcription modulator, depending on the interaction partners or on the cell type [4].

Coiled-coil which is one of the most common folding motifs, has been found in a wide range of proteins including structural proteins as well as transcription factors [8]. Coiled-coil-containing proteins can participate in a broad range of different biological activities, such as muscle contraction, transcription, metabolism, membrane channels, molecular chaperons, etc. [9]. Several proteins which harbor coiled-coil domain are expressed uniquely in testes, and play a significant part in spermatogenesis, for example Nurit [10], MORC [11] and so on.

In the present study, we cloned and characterized a mouse adult gonad predominantly expressed gene, psm2. psm2 shows an interesting expression pattern during gonadogenesis. Sequence analyses revealed that psm2 encoded a protein similar to MBD1-containing chromatin-associated factor2. It is likely that the gene may play an important role in gonad development.

Materials and methods

RNA isolation

Total RNA was prepared from various tissues of Bal B/C mouse. Bal B/C mice used in this study were provided by Animal Experiment Center of Disease Prevention & Control Centers of Hubei Province, China. Prenatal days were counted as the number of days following the identification of a vaginal plug (E0.5). Postnatal days were counted as number of days following birth.

RACE analysis

According to the method described by Frohman et al. [12], 3′RACE was performed using a common primer 3AVAP and gene specific primer F1, which was designed according to the sequences from RIKEN; 5′RACE was performed with 5AAP and R1. A touchdown RACE-PCR was accomplished and the products were cloned into pGEM-T vector (Promega) and sequenced according to the protocol described previously [13]. All the primer sequences are listed in Table 1. All PCR results were repeated three times at least.

Reverse transcription PCR (RT-PCR)

Proper quantity of total RNA was reverse transcribed to first strand cDNA using 3′AP-olig-dT(12–18)/olig-dT18 and M-MLV reverse transcriptase (Promega). Touchdown PCR was performed according to the protocol described previously [14]. Primers and reaction conditions were shown in Table 1: RTF and RTR for psm2; BAF and BAR for β-actin; SCP3F and SCP3R for scp3.

Bioinformatics analysis of PSM2

The nucleotide sequence and deduced amino acid sequence analysis were made online using the NCBI blastN server and ensembl blastX server, respectively. Multiple alignments were performed with CLUSTAL W [15].

In situ hybridization

The cDNA fragment (343–971 bp) was amplified and subcloned into pGEM-T vector (Promega). The primers for the cDNA fragment are: pF, 5′-GGG CTC TGG AAA GAC-3′; pR, 5′-CTG TAG TTG GAC GG-3′. Sense and antisense digoxigenin-labeled RNA probes were prepared in vitro transcription using SP6 or T7 RNA polymerase, respectively. Testis was cryosectioned and the sections were cut at 12 μm, section in situ hybridization was carried out according to the protocol described previously with modification.