Novel role for a sterol response element binding protein in directing spermatogenic cell-specific gene expression

Hong Wang

University of Massachusetts Medical School

Let us know how access to this document benefits you.
Follow this and additional works at: https://escholarship.umassmed.edu/oapubs

Part of the Life Sciences Commons, and the Medicine and Health Sciences Commons

Repository Citation

This material is brought to you by eScholarship@UMassChan. It has been accepted for inclusion in Open Access Publications by UMass Chan Authors by an authorized administrator of eScholarship@UMassChan. For more information, please contact Lisa.Palmer@umassmed.edu.
Novel Role for a Sterol Response Element Binding Protein in Directing Spermatogenic Cell-Specific Gene Expression

Hang Wang,1 Jovena T. San Agustin,2 George B. Witman,2 and Daniel L. Kilpatrick1*
Department of Physiology1 and Department of Cell Biology,2 University of Massachusetts Medical School, Worcester, Massachusetts

Received 1 July 2004/Returned for modification 1 August 2004/Accepted 26 September 2004

Sperm are highly specialized cells, and their formation requires the synthesis of a large number of unique mRNAs. However, little is known about the transcriptional mechanisms that direct male germ cell differentiation. Sterol response element binding protein 2gc (SREBP2gc) is a spermatogenic cell-enriched isoform of the ubiquitous transcription factor SREBP2, which in somatic cells is required for homeostatic regulation of cholesterol. SREBP2gc is selectively enriched in spermatocytes and spermatids, and, due to its novel structure, its synthesis is not subject to cholesterol feedback control. This suggested that SREBP2gc has unique cell- and stage-specific functions during spermatogenesis. Here, we demonstrate that this factor activates the promoter for the spermatogenesis-related gene proacrosin in a cell-specific manner. Multiple SREBP2gc response elements were identified within the 5' flanking and proximal promoter regions of the proacrosin promoter. Mutating these elements greatly diminished in vivo expression of this promoter in spermatogenic cells of transgenic mice. These studies define a totally new function for an SREBP as a transactivator of male germ cell-specific gene expression. We propose that SREBP2gc is part of a cadre of spermatogenic cell-enriched isoforms of ubiquitously expressed transcriptional coregulators that were specifically adapted in concert to direct differentiation of the male germ cell lineage.

Sperm are highly differentiated cells that are uniquely adapted to their function as motile cells mediating fertilization. As such, they serve as an important model for exploring regulatory programs responsible for cellular differentiation (17). Spermatogenesis consists of a complex interplay between cell-specific gene transcription, RNA processing, and translational regulation (8, 17). It occurs in a series of proliferation and differentiation stages, which can be subdivided into mitotic, meiotic, and spermiogenic phases. Each phase is characterized by distinct cell types, namely, spermatogonia, spermatocytes, and spermatids, respectively. The highly specialized nature of sperm is reflected in the large number of cell-specific transcripts and proteins they express (8), many of which are associated with unique sperm structures such as the acrosome, sperm tail, and the highly compacted sperm chromosomal DNA. Unique proteins also are required to meet specialized requirements for energy metabolism, meiosis, and the maturation of haploid cells, including cell-specific proteins that compensate for X chromosome inactivation (e.g., phosphoglycerate kinase 2 [pgk-2]) (9). These various gene products also must be expressed at the appropriate time to ensure normal development. Thus, sperm formation requires both the generation of a large number of cell-specific gene products and the coordination of this differentiation program in a stepwise, stage-appropriate manner. A key question is the nature of the transcriptional network that controls the elaboration of this program.

Cell-specific transcription from alternative promoters or unique genes plays a predominant role in directing male germ cell differentiation (8). Numerous spermatogenic cell-enriched transcription factors have been identified, many of which are selectively expressed during meiotic and/or early haploid stages (4, 6, 25, 33). For example, the spermatogenic cell-specific factor CREMγ is an activator of several genes expressed in haploid spermatids and is required for completion of spermogenesis (5, 28). CREMγ also interacts with a germ cell-specific coactivator termed ACT (11), and unique germ cell isoforms of basal transcription factors have been identified (15, 27). All this indicates that spermatogenic cells have evolved a highly specialized transcriptional program. However, functional identification of transcription factors responsible for controlling spermatogenic cell differentiation has been elusive. In particular, CREMγ is the only spermatogenic cell-enriched transcription factor for which a physiological role and specific germ cell-specific target genes have been determined (7). Moreover, nothing is currently known about the cell-specific regulators of gene promoters expressed in spermatocytes.

Sterol response element binding protein 2gc (SREBP2gc) is a 55-kDa, germ cell-enriched form of the basic helix-loop-helix leucine zipper (bHLHZip) transcription factor SREBP2 (50). Its expression is highly up-regulated during late meiosis and in early-round spermatids, suggesting stage-specific functions. In somatic cells, SREBP2 regulates genes involved mainly in cholesterol synthesis (19), and its transcriptional activity is highly dependent on the function of coregulatory factors, such as CREB/CREM, NF-Y, Sp1, and the SREBP antagonist YY1 (10). SREBPs are synthesized as membrane-bound precursor proteins that are proteolytically processed in the Golgi apparatus to generate a cytoplasmic, transcriptionally active mature SREBP. Sterols regulate this processing step as part of a homeostatic, inhibitory feedback mechanism by blocking the
transport of the SREBP precursor from the endoplasmic reticulum to the Golgi apparatus (19). In contrast to this, translation of the alternatively spliced SREBP2gc mRNA generates a soluble, constitutively active transcription factor that consequently is insensitive to cholesterol feedback control (50). These observations suggested that SREBP2gc performs novel functions during spermatogenesis, not restricted to cholesterol metabolism alone. The present studies demonstrate that SREBP2gc regulates the transcription of a spermatogenic cell-specific gene proacrosin, which is expressed in both spermatocytes and round spermatids. This factor likely regulates multiple gene targets as part of a global transcriptional program directing meiotic and postmeiotic stages of spermatogenic cell differentiation.

MATERIALS AND METHODS

Plasmid DNA constructs. An ~1-kb genomic fragment containing 5'-flanking, exon 1, intron 1, and partial exon 2 sequences for the rat proacrosin gene (GenBank accession number X85850) was generated by PCR (primer sequences are available upon request). This was inserted into pGEM-T Easy vector (Promega, Madison, Wis.) and then released with SacI and SacII and subcloned into the pGL3-Basic vector by using SacI and Smal sites. This step eliminates a polylinker region within the pGL3-Basic plasmid that contains an E box responsive to SREBPs (3). Additional proacrosin promoter constructs containing mutations in SREBP2gc binding sites were generated by PCR. Detailed procedures and conditions and various primer sequences are available upon request. The wild-type and SRE-1 site mutant squalone synthase (SOS) gene promoter constructs were previously described (14).

RNA and protein analyses. Total RNAs were prepared and analyzed by Northern analysis and reverse transcription-PCR (RT-PCR) as previously described (50). A 1.8-kb rat SREBP2gc cDNA was used as the probe for Northern analysis. Nuclear extracts were prepared from cell lines and enriched mouse spermatogenic cells by high salt extraction (26). Western blotting was performed as described previously (50) using antiserum raised against mouse SREBP2. The genic cells by high salt extraction (26). Western blotting was performed as described previously (50) using antiserum raised against mouse SREBP2. The oligodeoxynucleotides used for generating various DNA probes and competitors for electrophoretic mobility shift assays (EMSA) as well as primers for RT-PCR are available upon request. EMSAs were performed using nuclear extracts and an SRE-1 probe, as in previous studies of SREBP2gc (50).

Cell cultures and transfections. Cell lines were cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 100 U of penicillin-streptomycin (PS)/ml and 10% fetal bovine serum (FBS), except for GC-4spc cells, which were identified Eagle’s medium (DMEM) containing 100 U of penicillin-streptomycin and 10% serum. The oligodeoxynucleotides used for generating various DNA probes and competitors for electrophoretic mobility shift assays (EMSA) as well as primers for RT-PCR are available upon request. EMSAs were performed using nuclear extracts and an SRE-1 probe, as in previous studies of SREBP2gc (50).

RESULTS

SREBP2gc is expressed in a spermatogenic cell line. To test the hypothesis that SREBP2gc mediates spermatogenic cell-specific gene expression, we first examined whether it was expressed in cell lines derived from male germ cells. GC-4spc cells were originally selected with a neomycin resistance expression vector driven by the human pgk-2 promoter (47). They express several spermatocyte-related genes, including proacrosin and pgk-2, but not various markers for testicular somatic cells or a spermatogonial-associated gene promoter. Northern and RT-PCR analyses of GC-4spc cells detected an SREBP2gc mRNA that was identical in size (~2.5 kb) and similar in amount to that for the adult mouse germ cell transcript (Fig. 1A and B). They also detected an abundant SREBP2 transcript (~5 kb) corresponding to the precursor mRNA (Fig. 1B). Further, GC-4spc cells also contain substantial amounts of sequence-specific SRE binding activity based on EMSAs (Fig. 1C). Western blotting of nuclear extracts confirmed the presence of a 55-kDa SREBP2 protein corresponding in size to SREBP2gc (Fig. 1D). SREBP2 precursor protein (~125 kDa) was also detected in GC-4spc cells (data not shown), consistent with the presence of its mRNA in this cell line.

Due to SREBP2gc’s unique structure, SREBP2gc protein levels in spermatogenic cells are unaffected by sterol concentrations that suppress formation of transcriptionally active SREBPs in somatic cells by feedback inhibition of precursor processing (50). Thus, we examined whether SREBP2 precursor levels were affected by sterols in GC-4spc cells. In Western blots, amounts of the 55-kDa SREBP2gc protein under sterol-loaded and sterol-depleted culture conditions were the same (Fig. 2A). Similarly, SRE DNA binding activity in GC-4spc cells was unaltered by sterol load (Fig. 2B). Thus, SREBP2 precursor and DNA binding activity in GC-4spc cells are insensitive to sterols, consistent with the properties of SREBP2gc from spermatogenic cells.

Interestingly, we did not detect significant formation of the mature, 66-kDa protein derived from the SREBP2 precursor upon sterol depletion (Fig. 2A). This suggests that SREBP2 precursor processing is defective or suppressed in GC-4spc cells. This is reminiscent of the sterol-resistant SRD-3 mutant cell line that expresses a constitutively active SREBP2 protein analogous to SREBP2gc (54). This novel isoform represses proteolytic processing of endogenous SREBP precursors. SREBP2gc may have similar effects on the generation of ma-
ture SREBP2 protein in GC-4spc cells, as well as in pachytene spermatocytes and round spermatids which express small amounts of the SREBP2 precursor mRNA (50).

To examine whether GC-4spc cells express endogenous SRE-dependent transcriptional activity, they were transfected with SQS gene promoter-luciferase plasmids (13). The SQS gene is responsive to SREBPs and is expressed in spermatoocytes as well as spermatids (44). We observed much higher basal SQS gene promoter activity in GC-4spc cells than in somatic cell lines such as 3T3L1 (Fig. 3A), which lack detectable SREBPs under serum-containing conditions (50). Importantly, basal promoter activity in GC-4spc cells was highly dependent on a functional SRE site (50-fold difference between wild-type and mutated (MSQS) SRE sites, and luciferase activity was determined. (B) Cotransfection of GC-4spc cells with wild-type or mutant SQS gene promoter plasmids together with either an expression vector for SREBP2gc (BP2GC) or the empty parent plasmid (CMV7). (C) Cotransfection of the rat proacrosin promoter together with SREBP2gc or pCMV7 expression plasmids in different cell lines. (D) Cell lines were cotransfected with either SQS (NIH 3T3 and GC-1spg) or CY51 (JEG3 and GC-4spc) gene promoter plasmids and expression vectors. Data are shown as the increases in activity in the presence of SREBP2gc relative to that for pCMV7.

FIG. 3. Expression of SREBP transcriptional activity in GC-4spc cells. (A) GC-4spc and 3T3L1 cells were transfected with human SQS gene promoter constructs containing either wild-type or mutated (MSQS) SRE sites, and luciferase activity was determined. (B) Cotransfection of GC-4spc cells with wild-type or mutant SQS gene promoter plasmids together with either an expression vector for SREBP2gc (BP2GC) or the empty parent plasmid (CMV7). (C) Cotransfection of the rat proacrosin promoter together with SREBP2gc or pCMV7 expression plasmids in different cell lines. (D) Cell lines were cotransfected with either SQS (NIH 3T3 and GC-1spg) or CY51 (JEG3 and GC-4spc) gene promoter plasmids and expression vectors. Data are shown as the increases in activity in the presence of SREBP2gc relative to that for pCMV7.
contrast, this promoter was poorly expressed or undetectable in somatic cell lines, and no significant stimulation by SREBP2gc was observed in any of these (Fig. 3C and data not shown). SREBP2gc also did not activate the proacrosin promoter in a different spermatogenic cell-derived cell line, GC-1spg (data not shown), which resembles late spermatogonial stages and does not express the proacrosin promoter (18, 47). More generally expressed SREBP target promoters (CYP51 [37] and SQS gene promoters) were strongly activated by SREBP2gc in all cell lines tested (Fig. 3D), demonstrating that the cotransfected factor is transcriptionally active in each case. Thus, SREBP2gc potently activates the proacrosin promoter in a cell-specific manner.

The proacrosin promoter contains SREBP2gc response elements. The rat and mouse proacrosin promoters are highly homologous and contain a number of conserved trans-factor consensus elements (24, 39) (Fig. 4A). These include sites for known SREBP coregulators: Y boxes, cyclic AMP response elements (CREs), YY1 sites, and GC boxes. A search for SRE-like sequences identified five potential SREBP2gc response elements within the rat and mouse proacrosin promoters that were conserved in their locations and general sequence

**TABLE 1. Sequences of putative SREs in the mouse, rat, and human proacrosin promoters**

<table>
<thead>
<tr>
<th>SRE</th>
<th>Source</th>
<th>Sequence</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>SREpa1</td>
<td>M</td>
<td>GCACCTCAGCACAGATCAG (-123, -141)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>TGGCACCTCAGCG (-133, -145)</td>
<td></td>
</tr>
<tr>
<td>SREpa2</td>
<td>M</td>
<td>ATGGGGTTGGTTGCACATGATACCTTCACCACCCTGAGGTCAG (-168, -209)</td>
<td>GATAAGATATA--GATACCGATA</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>CTCATGAGTACCTCACCACCGCTGAGGCGG (-170, -198)</td>
<td></td>
</tr>
<tr>
<td>SREpa3</td>
<td>M</td>
<td>GGCTGGCCAA (-240, -249)</td>
<td>GGATAAGATA</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>GGCTGGCCAA (-239, -248)</td>
<td></td>
</tr>
<tr>
<td>SREpa4</td>
<td>M</td>
<td>ACCTTTCCATATCT (-782, -796)</td>
<td>GGATAAGATAG-----AAGATA</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>GCCCTTCCATATGTAAGAGG (-763, -784)</td>
<td></td>
</tr>
<tr>
<td>SREpa5</td>
<td>M</td>
<td>CTGGGATGGTTAGGA (-844, -857)</td>
<td>GATAAGATAAG--A</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>CTGGGATGGTTAGGA (-822, -837)</td>
<td></td>
</tr>
</tbody>
</table>

**a Human SREs TTAGGAGGCACAGGC (-13, -24), ACCTGGCCCTGACT (-97, -109), GGCTGGGCTGGG (-262, -274), and GTCTGGCAGTGGAC (-333, -345). The significance of underlining and italics is indicated in footnote c.**

**b M, mouse; R, rat.**

**c SRE half-sites are underlined, and NNCNNCNAN motifs are italicized. Numbers in parentheses indicate the positions of the first and last bases within respective proacrosin promoter nucleotide sequences relative to the translational start site (+1).**

**d Substitution or deletion mutations of rat sites.**
features (SREpa1 to -5; Fig. 4A and Table 1). In most instances, two or more previously identified SRE half-sites were present, and several contained an NNCNNNCON motif found in several SREs (45). The presence of multiple SREs within a target promoter is not uncommon (20). Interestingly, the SREpas for the rat and mouse were segregated into upstream (SREpa4 and -5) and downstream (SREpa1, -2, and -3) groups that were closely adjacent to consensus sequences for known SREBP coregulators (Fig. 4A). Such close proximity of SREs and coregulator sites is typical for SREBP-responsive promoters (40). Multiple SRE-like sequences along with neighboring coregulator sites also were identified in the human proacrosin promoter (Fig. 4A; Table 1), suggesting conservation of promoter organization in humans.

Competition EMSAs were performed on candidate SREs for the rat proacrosin promoter using mouse germ cell extracts. All but one (SREpa1; data not shown) exhibited good binding to native SREBP2gc (Fig. 4B to D). Mutated versions of these four rat SREpas showed greatly diminished binding. Southwestern analysis previously demonstrated that the 55-kDa SREBP2gc protein in spermatogenic cells bound to SRE sequences (50). This assay confirmed the binding of rat proacrosin SRE sites by endogenous SREBP2gc in mouse germ cell extracts (Fig. 4E).

We next examined the functional importance of the SREpa sites by promoter mutation analysis. Three different rat proacrosin promoter constructs were generated, two in which either the upstream sites (SREpa4,5mut) or the downstream sites (SREpa2,3mut) were mutated and a third containing mutations of all four sites (SREpa2-5mut). These promoters were then tested in GC-4spc cells for basal and SREBP2gc-stimulated activities (Fig. 5A). Mutation of the two upstream SREpas reduced basal activity approximately threefold, while activation by SREBP2gc was only modestly affected. In contrast, mutation of the downstream SREpa2 and -3 sites resulted in complete loss of SREBP2gc-induced activation (Fig. 5A). The combined upstream and downstream mutant promoter also showed no SREBP2gc-dependent stimulation. Thus, SREpa2 and -3 are critical for SREBP2gc induction of the proacrosin promoter in GC-4spc cells. SREpa4 and -5 have only a modest role in this but appear to be required for optimal basal promoter activation by endogenous SREBP2gc. Mutation of either SREpa2 and -3 alone or of all four sites caused a small increase in basal activity (Fig. 5A).

In vivo expression of the proacrosin promoter depends on SREBP2gc response elements. To test the importance of promoter (P < 0.01); **, significantly different from basal activity for the wild-type (WT) promoter (P < 0.005). (B) Luciferase activities for wild-type and SREpa2-5mut (MUT) rat proacrosin-luciferase constructs in testicular extracts from male transgenic mice. Numbers along the x-axis indicate independent transgenic lines. Mean activity for the mutant construct (1.6 × 10^5 RLU) was significantly different from that for the wild-type promoter (6.4 × 10^5 RLU) (P = 0.014). (C) Staining for luciferase protein in testes of adult transgenic mice expressing wild-type (line 46; WT46) or SREpa2-5mut (line 11; MUT11) proacrosin promoter constructs. Luciferase staining is distinguishable in spermatids by its cytoplasmic localization. Scales are shown for photomicrographs in the upper row as well as the lower two panels, respectively. NO AB, no primary antibody control.

FIG. 5. SREBP2gc binding sites are required for proacrosin promoter activation in vitro and in vivo. (A) Activities of different proacrosin promoter plasmids in GC-4spc cells cotransfected with either empty pCMV7 (blue bars) or SREBP2gc (red bars) expression vectors. *, significantly different from basal activity for the wild-type (WT) promoter.
SREBP2gc-induced activation in proacrosin promoter expression during spermatogenesis, we generated transgenic mice harboring wild-type or SREpa mutant proacrosin-luciferase fusion genes. The SREpa2-5mut promoter was examined to test the cumulative role of all SREBP2gc response elements. Previous studies showed that the ~1-kb rat proacrosin promoter used here directed faithful cell-specific gene expression in spermatocytes and spermatids of transgenic mice (30). As observed in numerous earlier studies (22, 34, 57), there was no correlation between transgene copy number and luciferase activity for either promoter construct (data not shown). Out of six transgenic males containing wild-type rat proacrosin promoter sequences, four expressed moderate-to-high levels of luciferase activity in the testis, while two exhibited low activity (Fig. 5B). This expression frequency (67%) is typical for active transgene promoters (26, 34). No activity was detected in somatic tissues from any transgenic mice (data not shown). In contrast, the SREpa2-5 mutant promoter was expressed at much lower levels in testes of founder males, with only 18% (2 of 11) having moderate testicular expression and none showing high expression.

Immunohistochemical staining confirmed the presence of luciferase protein in the cytoplasm of round spermatids expressing the wild-type proacrosin promoter, with the strongest staining occurring in spermatid stages VI and VII (Fig. 5C). Weaker cytoplasmic staining was observed in tubules containing spermatids at other phases of development, including late, condensing spermatids. No obvious staining was discernible in spermatocytes, consistent with stage-dependent translational regulation of endogenous proacrosin mRNA and proacrosin transgene-derived transcripts (30, 31). Transgene expression was undetectable in testicular somatic cell types (peritubular, Sertoli, and interstitial cells). In contrast, expression of the SREpa2-5mut proacrosin promoter was reduced in the cytoplasm of all spermatid stages of mouse line 11 (Fig. 5C), which exhibits lower but detectable luciferase activity (Fig. 5B). Thus, SREBP2gc response elements are critical for proacrosin promoter expression during spermatogenesis.

DISCUSSION

The importance of SREBPs in the homeostatic control of cholesterol and fatty acid synthesis in somatic cells is well established (19). However, the finding of a constitutively active, sterol-insensitive form of SREBP2 that is expressed in a developmentally regulated manner during spermatogenesis indicated a broader role for this factor not limited to lipid metabolism alone (50). The present findings directly implicate SREBP2gc in the stage-dependent expression of the spermatogenic cell-specific gene proacrosin, which is expressed in both spermatocytes and spermatids. SREBP2gc is only the second spermatogenic cell-enriched transcription factor (CREM* is the first) shown to regulate a germ cell-specific promoter, and it is the first such factor shown to activate a gene expressed during male meiosis. Further, it is likely that SREBP2gc regulates multiple spermatogenic-cell-specific genes, not proacrosin alone. Thus, this factor may be an integral part of a more global differentiation program, and defining additional target promoters for SREBP2gc in male germ cells is an important future goal. In particular, disruption of SREBP2gc function during spermatogenesis will establish the extent to which this factor is involved in directing spermatogenic differentiation as well as the nature of its gene targets. It also should provide the first insight into the cell-specific transcriptional mechanisms operating in meiotic spermatocytes.

Based on the present results, it appears that a ubiquitous somatic factor (SREBP2) was adapted by spermatogenic cells to function in an entirely new manner as a trans regulator of germ cell-specific genes. In fact, precedent for this notion already exists in the form of CREM*: analogous to SREBP2gc, it is a spermatogenic cell-specific variant of a generally expressed transcription factor family generated by alternative splicing. Both factors also possess unique properties that circumvent regulatory mechanisms operating in somatic cells and that are critical for their function as spermatogenic cell trans regulators. For CREM*, alternative splicing converts the CREM repressor into a germ cell-specific activator of CREs (12). Further, phosphorylation mechanisms normally required for interactions with the CREB coactivator CBP do not apparently operate in spermatids. Instead, CREM* interacts with the phosphorylation-independent coactivator ACT, which is expressed only in haploid spermatogenic cells along with CREM* (11). This alternative pathway apparently evolved to provide for both stage- and cell-specific activation of CRE-dependent promoters in germ cells. Similarly, alternative RNA processing in spermatogenic cells generates an SREBP2 isoform that bypasses sterol-dependent inhibitory mechanisms, permitting stage-dependent up-regulation of a constitutively active factor and its target promoters in late spermatocytes and early spermatids.

It is of interest that SRE- and CRE-binding proteins act together to regulate numerous promoters in somatic cells (40). It therefore seems likely that SREBP2gc and CREM* coordinately regulate common spermatogenic cell-specific promoters in spermatids. This may reflect coevolution of functionally related transcription factors, in which interacting partners take on cell-specific functions in parallel. In fact, these two proteins may be members of a larger group of factors, including YCAAT- and GC box binding factors, as well as YY1-like proteins, specifically arising from more generally expressed trans-regulator families to control gene expression in the male germ line. Such adaptation may be an efficient means for generating germ cell-specific transcription factors since it utilizes generally expressed, and perhaps ancient (52), trans factors as well as response elements commonly found in RNA polymerase II promoters. Notably, many germ cell-specific promoters expressed in late spermatocytes and/or round spermatids contain CRE, YY1, and Y- and GC-box elements (23, 38, 55, 56), and unique, spermatogenic-cell- or testis-enriched nuclear factors that bind these sites have been previously identified (16, 32, 35, 39, 42, 43, 49). Additional, novel coregulator isoforms also may function in late spermatogenesis.

Analysis of the proacrosin gene, which contains binding sites for all major SREBP coregulators and which is expressed in both of these stages, provides an excellent opportunity to explore the role of coregulators in both cell- and stage-dependent activation by SREBP2gc. Such analyses ultimately will expand our understanding of the transcriptional network regulating spermatogenesis and the unique placement of SREBP2gc within it. GC-4spc cells should prove useful in this regard due
to their expression of SREBP2gc as well as the cell-specific regulation of proacrosin promoter activity that they exhibit.

Finally, what is the significance of SREBP2gc expression for cholesterol synthesis during spermatogenesis? Recent studies have shown that loss or inhibition of the function of dhcr24, a terminal reductase in the cholesterol biosynthetic pathway, disrupts spermatogenesis (41, 51). Several cholesterol biosynthesis genes also are specifically up-regulated during late spermatogenesis (46, 48), which likely involves trans activation by SREBP2gc. However, a number of observations indicate that enhancement of cholesterol synthesis per se is not the role of this transcription factor in meiotic and haploid germ cells. For one thing, not all cholesterol biosynthetic genes are coordinately up-regulated during late spermatogenesis (46). Accordingly, cholesterol synthesis actually declines in pachytene spermatocytes and round spermatids (36), as does testicular cholesterol content during sexual maturation (46). These facts further argue that SREBP2gc has major functions distinct from cholesterol synthesis and are consistent with the switch to a sterol-independent mechanism of SREBP2 production in these spermatogenic stages. While this may involve an increased synthesis of certain cholesterol intermediates, such as T-MAS (46), it is likely that a major role of SREBP2gc is to regulate a totally new set of promoters uniquely expressed in spermatocytes and spermatids.

ACKNOWLEDGMENTS

This work was supported by Public Service Grant RO1 DK36468 and Center Grant DK32520. We thank George Gagnon and Rachel Stock for their excellent assistance with several aspects of this work. The mouse pgk-2 promoter-LacZ plasmid was provided by Y. Nakainani (Kanazawa University, Ishikawa, Japan), SQS gene promoter plasmids were obtained from I. Shechter (Uniformed Services University of the Health Sciences, Bethesda, Md.), and the human CYP51 gene promoter construct was provided by D. Rozman (University of Ljubljana, Ljubljana, Slovenia). GC-4se cells were kindly provided by Wolfgang Englel (University of Göttingen, Göttingen, Germany).

REFERENCES

developmentally regulated messenger ribonucleic acids in mouse spermato-
genic cells. Endocrinology 137:638–646.

36. Potter, J. E., C. F. Millette, M. J. James, and A. A. Kandutsch. 1981. Elevated cholesterol and dolichol synthesis in mouse pachytene spermo-

37. Rozman, D., M. Fink, G. M. Fimia, P. Sassone-Corsi, and M. R. Waterman. 1999. Cyclic adenosine 3,5'-monophosphate(cAMP):cAMP-responsive ele-
ment modulator (CREM)-dependent regulation of cholesterologenic lan-
testerol 14α-demethylase (CYP51) in spermatids. Mol. Endocrinol. 13:1951–
1962.

hybrid assay identifies YY1 as a binding factor for a proacrosin promoter ele-


452.


42. Sogawa, K., H. Imataka, Y. Yamasaki, H. Abe, and Y. Fujii-
Kuriyama. 1993. cDNA cloning and transcriptional properties of a novel GC

monophosphate-responsive element-binding protein-like gene exclusively

44. Stromstedt, M., M. R. Waterman, T. B. Haugen, K. Tasken, M. Parvinen,
and D. Rozman. 1998. Elevated expression of lanosterol 14α-demethylase (CYP51) and the synthesis of oocyte meiosis-activating sterols in postmeiotic


46. Tascou, S., K. Nayernia, A. Samanli, J. Schmidtle, T. Vogel, W. Engel, and
P. Burfeind. 2000. Immortalization of murine male germ cells at a discrete
stage of differentiation by a novel directed promoter-based selection strat-

specific transcripts of rat farnesyl pyrophosphate synthetase are development-

of a homebox gene related to Drosohila cut is expressed in mouse testis.

8478–8490.

Gozlan-Kelner, I. Spivak, O. Moshkin, E. Fridman, Y. Becker, R. Skaliter, P.


52. Yamagata, K., K. Murayama, M. Okabe, K. Toshimori, T. Nakashishi, S.
10474.


actions activate transcription of the mouse protamine 2 gene during sper-

Multiple elements influence transcriptional regulation from the human tes-

Selectionive transcription of rat proenkephalin fusion genes from the spermat-