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Hong Wang
University of Massachusetts Medical School

Jovenal T. San Agustin
University of Massachusetts Medical School

George B. Witman
University of Massachusetts Medical School

See next page for additional authors

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Novel Role for a Sterol Response Element Binding Protein in Directing Spermatogenic Cell-Specific Gene Expression

Hang Wang,[1] Jovenal T. San Agustin,[2] George B. Witman,[2] and Daniel L. Kilpatrick[1]*

Department of Physiology[1] and Department of Cell Biology,[2] University of Massachusetts Medical School, Worcester, Massachusetts

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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 CREMτ is an activator of several genes expressed in haploid spermatids and is required for completion of spermiogenesis (5, 28). CREMτ 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, CREMτ 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

* Corresponding author. Mailing address: Department of Physiology, University of Massachusetts Medical School, 55 Lake Ave. N, Worcester, MA 01655-0127. Phone: (508) 856-6274. Fax: (508) 856-5997. E-mail: Daniel.Kilpatrick@Umassmed.edu.

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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 X58550) 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 SmaI 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 squalene synthase (SQS) 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 oligodeoxynucleotides used for generating various DNA probes and competitors for electrophoretic mobility shift assays (EMSAs) 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-1spc cells, which were cultured in 13% FBS. One percent nonessential amino acids (AA) also was included for GC-4spc and GC-1spc cells. All cells were incubated with 5% CO_2 at 37°C. For sterol depletion studies, cells were freshly plated in DMEM-PS-AA medium containing 10% FBS. Twenty-fours later, they were rinsed with 1× phosphate-buffered saline and then were cultured for an additional 10 h in DMEM-PS-AA containing 5% lipoprotein-deficient FBS (Sigma, St. Louis, Mo.), 50 μM compactin, and 50 μM sodium mevalonate with (sterol loaded) or without (sterol depleted) cholesterol (10 μg/ml) and 25-hydroxycholesterol (1 μg/ml). ALLN protease inhibitor (Calbiochem, La Jolla, Calif.) at 25 μg/ml was added to the culture medium 1 h prior to extraction of nuclear proteins.

For promoter studies, DNAs for promoter constructs (0.5 μg), pCMV7 or pCMV-BP2gc (10 ng), and pRL-null normalization plasmid (0.1 μg) were cotransfected with Trans-Fast reagent (Promega). Cell extracts were then analyzed 40 to 48 h later with the Dual Luciferase reporter assay system (Promega). All promoter data (expressed as relative firefly luciferase light units [RLU]) were normalized with *Renilla* luciferase activity and are reported as the means ± standard errors of four to eight independent experiments. The expression vector pKAc (0.1 μg) for the protein kinase A c subunit also was included in *proacrosin* promoter studies. Student's *t* test was used to evaluate data significance.

Transgenic mice. Transgenes containing wild-type or mutant rat *proacrosin* promoter-luciferase sequences as well as a simian virus 40 poly(A) signal were released from their parent pGL3 vectors with SalI and ApaI and gel purified prior to injection. The genotype of offspring was determined by PCR for luciferase sequences (data available on request). Testes and somatic tissues from adult (2 to 3 months) male transgenic founders or F_1 mice were extracted and assayed for luciferase activity. Protein concentration was determined with Bradford reagent (Bio-Rad Laboratory, Hercules, Calif.).

Immunohistochemistry. Immunostaining was performed on paraffin-embedded sections of adult mouse testes as described in a previous study (2) with slight modifications. Briefly, deparaffinized testis sections (5 μm) were rehydrated and subjected to antigen retrieval and blocking with the biotin blocking system (DakoCytomation, Carpenteria, Calif.) and 20% normal swine serum–5% fatty acid-free bovine serum albumin. Sections were incubated with a rabbit antiluciferase antibody (0.5 μg/ml; Cortex Biochem Inc., San Leandro, Calif.), and bound antibody was detected with biotinylated swine anti-rabbit immunoglobulin G and alkaline phosphatase-conjugated streptavidin together with the Fuchsin substrate system (DakoCytomation). Hematoxylin was used as a counterstain.

Promoter sequence analysis. To identify possible SREBP2gc response elements within the rat, mouse, and human *proacrosin* promoters, sequences obtained from GenBank were searched for known sterol response element (SRE) half-sites with OMIGA, version 2.0, software (Oxford Molecular Ltd.). These were also compared to an NNCNNNCNAN motif often associated with SREs (45).

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 spermatogonium-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) also was 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 protein 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 protein 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 repressed proteolytic processing of endogenous SREBP precursors. SREBP2gc may have similar effects on the generation of ma-

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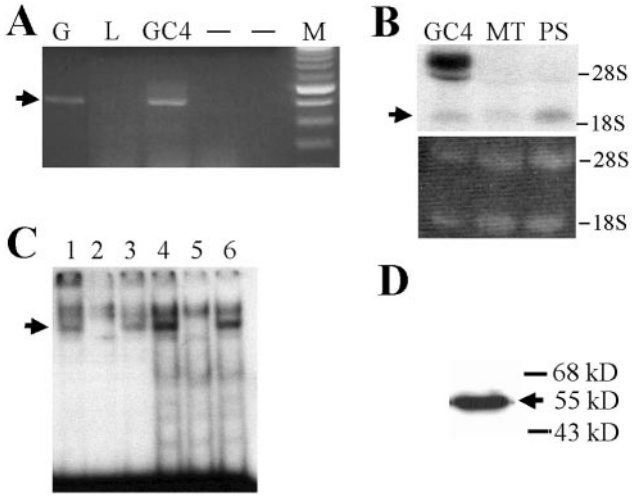

FIG. 1. GC-4spc cells express SREBP2gc. (A) RT-PCR analysis of SREBP2gc mRNA. One microgram of total RNA from adult mouse spermatogenic cells (G) and liver (L) and GC-4spc cells was analyzed. —, no RNA template and no reverse transcriptase negative controls; M, DNA size ladder. Primers that specifically detect the mouse SREBP2gc transcript were used (50) (available on request). (B) Northern analysis using total RNA from GC-4spc cells (15 μg), 21-day-old mouse testis (MT; 20 μg), and purified mouse pachytene spermatocytes (PS; 20 μg). Arrow, SREBP2gc mRNA. Ethidium bromide staining is shown below the Northern analysis results. (C) EMSA of SREBPs in GC-4spc cells. Lanes 1 to 3, 2 μg of GC-4spc nuclear extract; lanes 4 to 6, 2 μg of adult mouse germ cell nuclear extract. Lanes 1 and 4, no competitor; lanes 2 and 5, wild-type SRE-1 competitor; lanes 3 and 6, mutated SRE-1 competitor. Arrow, specific SREBP complex. (D) Western analysis of nuclear extracts (30 μg) from GC-4spc cells for SREBP2 proteins. A single, major band of ~55 kDa (arrow), identical in size to that for SREBP2gc, was detected.

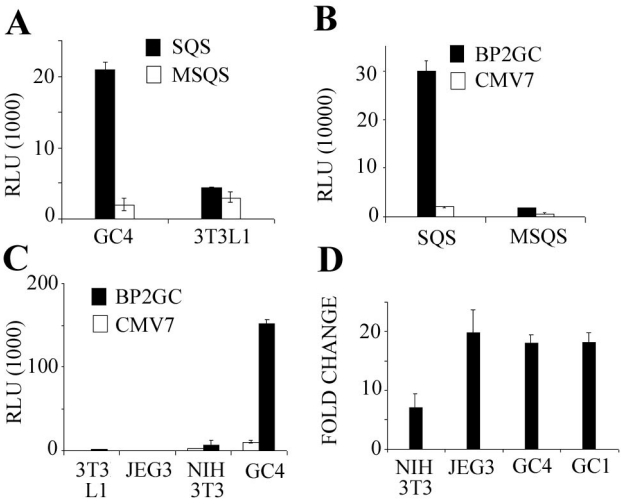

FIG. 3. Expression of SREBP transcriptional activity in GC-4spc cells. (A) GC-4spc and 3T3L1 cells were transfected with human SQS gene promoter constructs containing either wild-type or mutated (MSQS) SRE sites, and luciferase activity was determined. (B) Cotransfection of GC-4spc cells with wild-type 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 CYP51 (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.

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 spermatocytes 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 (>10-fold difference between wild-type and SRE mutant constructs), which was not the case in transfected 3T3L1 cells (Fig. 3A). This indicated the presence of endogenous SREBP transcriptional activity selectively in GC-4spc cells. Further, cotransfected SREBP2gc dramatically increased SQS promoter activity in this cell line, which also required the SRE site (Fig. 3B). Thus, GC-4spc cells express active SREBP2gc protein and are suitable for studying its transcriptional activity in a spermatogenic cell-like environment, including its possible regulation of germ cell-specific gene expression.

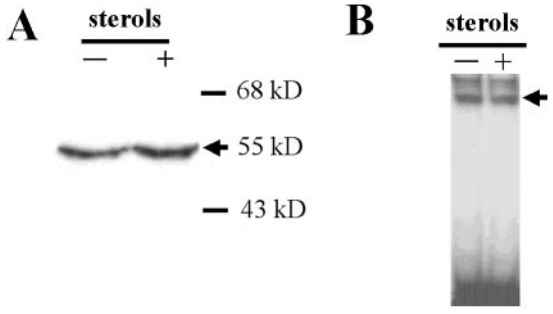

FIG. 2. Levels of SREBP2gc protein and SRE DNA binding activity are insensitive to sterols. GC-4spc cells were cultured in either sterol-loaded (+) or sterol-depleted (−) medium (see Materials and Methods). Nuclear protein was then assayed by Western analysis (A; 30 μg per lane) or EMSAs (B; 6 μg per lane) using the SRE-1 probe. Arrows, SREBP2gc protein or specific DNA binding complex in each case.

SREBP2gc activates a spermatogenic cell-specific promoter. Proacrosin is an acrosomal zymogen for a protease implicated in sperm competition and sperm-oocyte interactions (1, 29) and in the dispersal of acrosomal components upon onset of the acrosome reaction (53). It is encoded by a spermatogenic cell-specific gene first expressed in spermatocytes and then highly up-regulated in spermatids (21), at which time mRNA translation occurs (31). Since both the *proacrosin* and *pgk-2* promoters are transcribed in pachytene spermatocytes and in GC-4spc cells, we examined their potential regulation by SREBP2gc in cotransfection studies. *pgk-2* promoter activity was not stimulated by SREBP2gc in any of the cell lines tested (results not shown). However, strong activation of the *proacrosin* promoter was observed in GC-4spc cells (Fig. 3C). In con-

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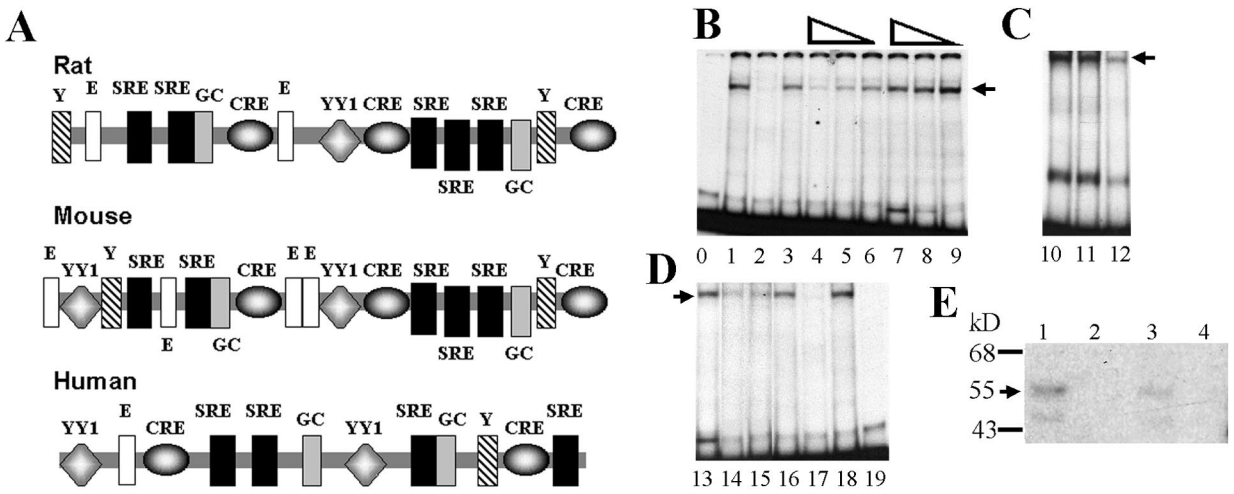

FIG. 4. Identification of SREBP2gc binding sites within the *proacrosin* promoter. (A) Organization of SREs within the rat, mouse, and human *proacrosin* promoters. GC, E, and Y boxes as well as CREs and YY1 sites also are shown. (B to D) Competitive EMSAs using adult mouse germ cell nuclear extracts (2 μg) and rat *proacrosin* SRE sites. Lanes: 0, no extract; 1, extract without competitor; 2, wild-type SRE-1 competitor; 3, SRE-1mut; 4 to 6, wild-type SREpa2; 7 to 9, mutated SREpa2; 10, no competitor; 11, mutated SREpa3; 12, wild-type SREpa3; 13, mutated SREpa4; 14, wild-type SREpa4; 15, wild-type SREpa5; 16, SRE-1mut; 17, wild-type SRE-1; 18, no competitor; 19, no extract. The mutated SREpa5 and SREpa4 competitors were identical (see Table 1). Arrows, specific SREBP complexes. (E) Southwestern analysis using SREpa2. Five micrograms of nuclear extract from adult mouse germ cells (lanes 1 and 3) and adult mouse liver (lanes 2 and 4) was probed with either wild-type SREpa2 (lanes 1 and 2) or SRE-1 (lanes 3 and 4) sequences. Arrow, germ cell-specific, 55-kDa SREBP2gc protein.

trast, 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[a] *proacrosin* promoters

SRE	Source[b]	Sequence[c]	Mutation[d]
SREpa1	M	GCACTTCAGCACAGATCAG (−123, −141)	
	R	TGGCACCTCAGCG (−133, −145)	
SREpa2	M	*ATGGGTTGG*TTGCACATGAGTACCTTCACCACCCTGAGGTCAG (−168, −209)	GATAAGATATAA-----GATACCGATA
	R	CTCATGAGT*ACCTCACCAC*CCTGAGGCGG (−170, −198)	
SREpa3	M	*GGCTGGCCAA* (−240, −249)	GGATAAGATA
	R	*GGCTCGCCAA* (−239, −248)	
SREpa4	M	*ACCTTTCCATACTAT* (−782, −796)	GGATAAGATAG------AAGATA
	R	*GCCTTTCCATGCTAT*AAGAGG (−763, −784)	
SREpa5	M	*CTGGATGGGTAGGA* (−844, −857)	GATAAGATAAG--A
	R	*CTCGATGGGTAGGA* (−822, −837)	

[a] Human SREs: *TTGCAGGCCAGGC* (−13, −24), *ACCTGGCCTGACT* (−97, −109), GGGTGATGTGGGG (−262, −274), and GTCTGCAGTGGAC (−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 NNCNNNCNAN 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.

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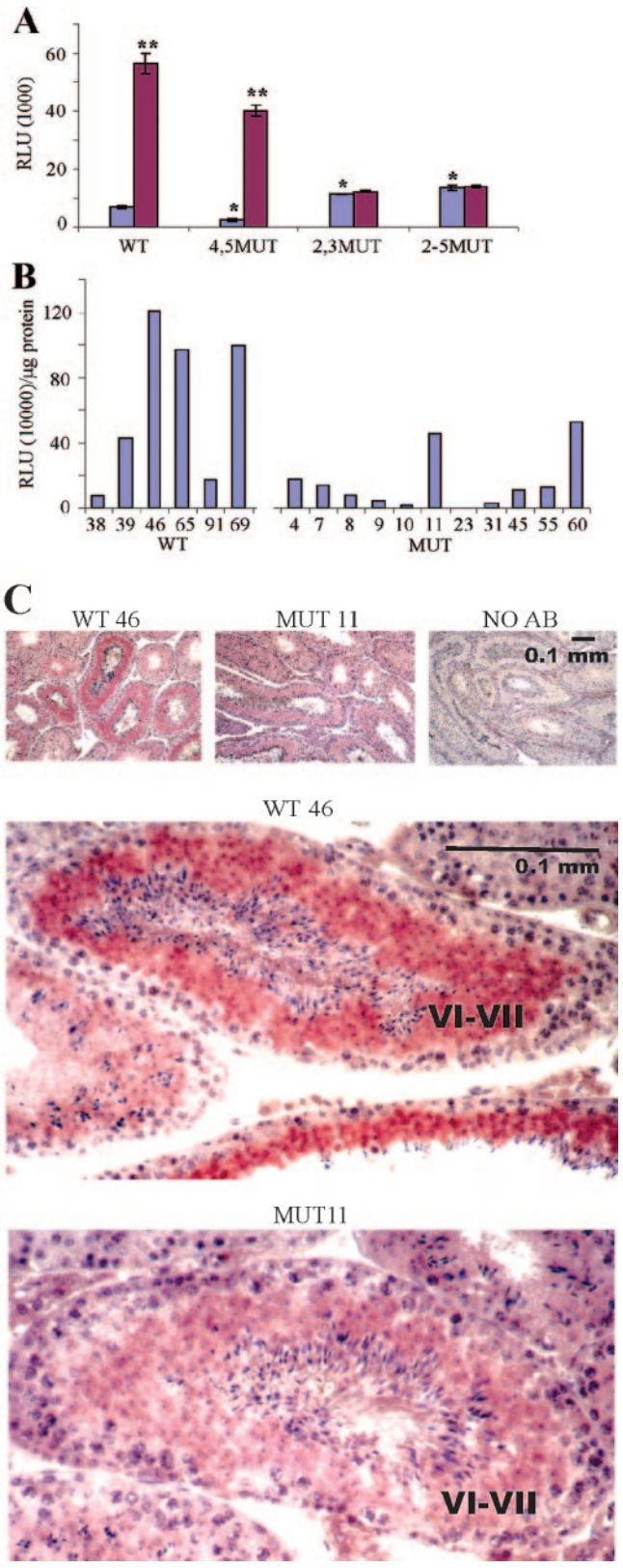

FIG. 5. SREBP2gc binding sites are required for *proacrosin* promoter activation in vitro and in vivo. (A) Activities of different *proacrosin* promoter plasmids in GC-4spc cells cotransfected with either empty pCMV7 (blue bars) or SREBP2gc (red bars) expression vectors. *, significantly different from basal activity for the wild-type (WT) promoter ($P < 0.01$); **, significantly different from basal activity for the respective promoter construct ($P < 3.00 \times 10^{-5}$). (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.

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 NNCNNNCNAN 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

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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 (CREMτ 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 CREMτ: 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 CREMτ, 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, CREMτ interacts with the phosphorylation-independent coactivator ACT, which is expressed only in haploid spermatogenic cells along with CREMτ (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 CREMτ 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 Y/CAAT- 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

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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.

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