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The gonadotropin-regulated long-chain acyl CoA synthetase gene: A novel downstream Sp1/Sp3 binding element critical for transcriptional promoter activity

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Abstract

The 79 kD gonadotropin-regulated testicular long chain acyl-CoA synthetase gene (GR-LACS) is a hormone-regulated member of the acyl-CoA synthetase family that is expressed abundantly in Leydig cells and to a lesser extent in germinal cells of the adult testis. GR-LACS possesses an ATP/AMP binding domain and the fatty acyl-CoA synthetase (FACS) signature motif. To gain insights into the transcriptional regulation of GR-LACS in gonadal cells, we determined the genomic organization of the gene, including the upstream flanking sequences. The mouse GR-LACS gene spans over at least 45 kb and the coding region is encoded by exons 1–14. All exon–intron junction sites correspond to the consensus splice sequence GT-AG. Exon 7 and 11 comprise the conserved ATP/AMP binding domain and the FACS signature motif, respectively. Primer extension and S1 nuclease analyses demonstrated four transcriptional start sites located at −266/−216 bp 5′ to the ATG codon. The minimal promoter domain resides within −254/−217 bp 5′ to ATG codon, and upstream sequences to −404 bp (−1035/−405 bp) contribute to the inhibition of transcription in the expressing mouse Leydig tumor cells. Removal of −217/−1 bp, containing a 23 nt GC rich sequence (−112/−90) with an Sp1/Sp3 binding element, within the 1st exon of this TATA-less promoter, significantly reduced GR-LACS gene transcription. Transcriptional activity was abolished by a 2 nt mutation of this element. Thus, functional analyses of this promoter domain indicate that transcription of GR-LACS gene requires an Sp1/Sp3 binding element downstream of the transcriptional start sites which is essential for basal promoter activity.
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Keywords: GR-LACS; Genomic organization; Transcription; Testis

1. Introduction

Fatty acid utilization is initiated by activation of various fatty acids by Acyl CoA synthetases (ACS) to produce acyl-CoA thioesters that are intermediate participants in lipid metabolism. ACSs are classified biochemically by the carbon length of their fatty acids substrates, as short chain (C2–C4), medium chain (C6–C10), long chain (C12–20) and very long-chain (≥22 C) ACSs. These enzymes have been implicated in a variety of biological processes, ranging from membrane transport, signaling and mitochondrial metabolism to gene transcription (Black et al., 1985; Shrago, 2000).

Gonadotropin-regulated long chain fatty Acyl-CoA synthetase (GR-LACS) is a 79 kD protein that was cloned in our laboratory from a rat cDNA Leydig cell library (Tang et al., 2001). This protein, which is transcriptionally down-regulated by gonadotropin and is capable of activating long-chain fatty acids, is a new member of the long-chain fatty

Abbreviations: GR-LACS, gonadotropin-regulated long-chain fatty acyl CoA synthetase; MLTC, mouse Leydig tumor cell; BAC, bacterial artificial library; ES, embryonic stem cells; cDNA, DNA complementary to RNA; PCR, polymerase chain reaction; EMSA, electrophoresis mobility shift assay; Acc#, accession number.

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acyl-CoA synthetase (LACS) family. GR-LACS has sequence identity with two conserved regions of the LACS and luciferase families, namely the ATP/AMP binding domain and the 25 aa FACS signature motif, but shares low overall amino acid sequence similarity (23–28%) with other known members of these families (Tang et al., 2001). However, rat GR-LACS displayed high sequence similarity (>95% homology) with mouse and human species, including mouse lipidosin (Moriya-Sato et al., 2000) and human/mouse bubblegum (Steinberg et al., 2000). In contrast to rat GR-LACS and mouse lipidosin, which exhibit long-chain ACS activity, human bubblegum (BG) was reported to possess very long-chain ACS activity (Steinberg et al., 2000). However, in a mouse neuroblastoma cell line (Neuro2a), mouse lipidosin/BG appears to have a minor role in very long-chain fatty acid activation (Pei et al., 2003). A minimal very long chain ACS activity was observed in the microsomes of COS-7 cells overexpressing lipidosin/BG (Fraisl et al., 2004). BG was hypothesized to play a central role in X-linked adrenoleukodystrophy (Pei et al., 2003; Steinberg et al., 2000), a neurodegenerative disorder associated with elevated plasma levels of very long-chain fatty acids. From studies in rat (Tang et al., 2001), mouse (Fraisl et al., 2004; Moriya-Sato et al., 2000) and human (Steinberg et al., 2000), the expression pattern of GR-LACS gene appears to be tissue- and species-specific. Our earlier studies (Tang et al., 2001) indicated that GR-LACS mRNA is abundantly expressed in the adult rat Leydig cell and to a lesser degree in seminiferous tubules, ovary and brain. However, it is not detectable in the adrenal gland of adult rats. In contrast to the rat, lipidosin/ GR-LACS is expressed in mouse adrenal cortex (Moriya-Sato et al., 2000), and is present in the mouse ovary and brain in a significant higher amount than in the testis (Tang et al., 2001; Li et al., unpublished).

GR-LACS is not related to X-linked adrenoleukodystrophy, since its activity is specific for long-chain fatty acids and is completely devoid of very long chain ACS activity. In addition, there is no difference in mouse BG mRNA expression between the wild type and adrenoleukodystrophy protein (ALD) null mice, which accumulate very long chain fatty acids (Fraisl et al., 2004). Furthermore, double knockout mice for ALD protein and very long-chain acyl CoA synthetase (XALD/Vlcs) did not display a more severe X-ALD phenotype (Heinzer et al., 2003).

Since GR-LACS is constitutively expressed in the steroid-producing rat testicular Leydig cells and is down-regulated during desensitization by gonadotropin, we hypothesize that GR-LACS contributes to the provision of energy requirements and biosynthesis of steroid precursors, and participates through acyl-CoA's multiple functions in the regulation of the male gonad. To further understand the functional role of GR-LACS and gain insights into its basal and hormonally mediated transcriptional regulation, we isolated and characterized the mouse GR-LACS gene. We have elucidated the genomic organization of the mouse GR-LACS gene, as well as characterized its promoter and basal transcriptional regulation.

2. Materials and methods

2.1. Isolation and characterization of mouse GR-LACS gene

Genomic clones were isolated from a mouse genomic BAC library derived from the ES 129/SVJ strain (Genome Systems, Inc, St. Louis, Mo), using two mouse GR-LACS cDNA probes corresponding to nucleotide positions +1/+320 bp and +1870/+2140 bp (Tang et al., 2001). Six clones were selected to deduce the organization of the complete mouse GR-LACS gene. Each clone was digested with either EcoR1 or PstI, electrophoresed, blotted and hybridized to the selected probes. Positive DNA fragments were subcloned into pZERO-2 cloning vector (Invitrogen, Carlsbad, CA) and sequenced by the dideoxy chain termination procedure using Thermo sequenase kit (USB, Cleveland, OH).

2.2. Primer extension, S1 nuclease analysis

For primer extension analysis, ^{32}P-end-labeled oligonucleotides complementary to nucleotides −120/−87 ($m-87$) and +1/+21 ($m+21$) of the mouse GR-LACS cDNA (Moriya-Sato et al., 2000; Tang et al., 2001) were annealed to total RNA of adult mouse testis. For S1 nuclease mapping analysis, ^{32}P end-labeled PCR genomic fragments (−622/−87 and −622/+21) were annealed to mouse testis total RNA as previously described (Tsai-Morris et al., 2004). All the extended products and protected fragments were separated on a 6% polyacrylamide, 8 M urea gel. The transcriptional start sites were determined by comparison with known sequence products as markers.

2.3. Transient expression analyses of mouse GR-LACS gene

GR-LACS/luciferase fusion plasmids were constructed using serial deleted GR-LACS 5′ flanking sequences (Fig. 3) into pGL3 basic vector (pGLB) at either the site of SacI/XhoI (p-3326/-1GL), EcoRI/XhoI (p-1661/-1GL), NcoI/XhoI (p-1035/-1GL) or SacI/XhoI (p-404/-217GL, p-343/-1GL, p-305/-1GL, p-254/-1GL and p-217/-1GL). Fusion constructs with a 217 bp deletion (−217/−1 bp) of the 5′ untranslated region (UTR) (p-1035/-217GL and p-404/-217GL), and the GC-box deletion (−112GGCACCGCC-112GGCACCGCCCACCTC-90) of the p-217/-1 construct were generated by PCR. Also, a GC-box mutant construct was generated by PCR with specific primer sets containing the mutated sequences (GC-boxX: GGCACCGaaACCTC). Each clone was verified by sequence analysis using the dideoxy chain termination method.

Transient expression studies using the Lipofectamine Plus reagent (BRL, Gaithersburg, MD) were performed