Available online at www.sciencedirect.com
SciVerse ScienceDirect
Theriogenology 77 (2012) 881–887
Theriogenology
www.theriojournal.com

Differential expression of cyclic-AMP responsive element modulator (CREM) mRNA isoforms during testicular development of boars

B.L. Sartini*, M. Eerhart, S. Green

Department of Fisheries, Animal and Veterinary Science, University of Rhode Island, Kingston, Rhode Island 02881, USA.

Received 11 March 2011; received in revised form 2 September 2011; accepted 11 September 2011

Abstract

The cyclic-AMP responsive element modulator (CREM) transcription factor, a key regulator of spermatogenesis, can activate or repress gene expression by differential expression of alternatively spliced mRNA isoforms. The objective of this study was to report the sequences of the predominant CREM mRNA isoforms with leader exons B, $\theta1$, $\theta2$, in addition to the relative expression of these transcripts, in immature and adult boar testes. Activator CREM isoforms $\tau\gamma$ (containing the B exon), $\theta1\tau\gamma$ and $\theta2\tau\gamma$, were expressed in the adult boar testes, but not in immature testes. The CREM $\theta2\tau\gamma$ isoform had the highest expression levels in the adult boar testes compared to the $\tau\gamma$ and $\theta1\tau\gamma$ isoforms ($\theta2\tau\gamma = 4.94 \pm 1.49$ in contrast to $\tau\gamma = 1.66 \pm 0.79$ and $\theta1\tau\gamma = 2.32 \pm 0.72$; mean $\pm$ SD; $P < 0.01$). Interestingly, the predominant CREM mRNA isoforms in adult boar testes contained the C and γ exons; these exons are not present in human and rodent CREM mRNA, demonstrating species-specific expression. In contrast to the adult CREM isoforms, only CREM mRNA isoforms with the B exon ($\tau1$ and α) were amplified in the 21 d (immature) boar testes. Further understanding of CREM mRNA isoform expression and regulation during boar spermatogenesis could contribute to our knowledge of spermatogenic efficiency in this species.
© 2012 Elsevier Inc. All rights reserved.

Keywords: CREM; Testis; Spermatogenesis; Male fertility; Promoter; Pig

1. Introduction

Male fertility is a limiting factor to the reproductive efficiency of livestock production; current methods are insufficient to predict *in vivo* variation of sire sperm potential [1]. Based on recent transcriptome analysis of male germ cells, pre-mRNA processing, including alternative splicing, is prevalent in spermatogenesis and inaccurate splicing can impact fertility [2,3]. For example, inefficient alternative splicing of the cyclic-AMP responsive element modulator (CREM) transcription factor, a key regulator of post-meiotic male germ cell gene expression, results in impaired sperm development leading to subfertility in men and stallions [4–7]. The CREM gene sequence and mRNA isoform expression profile has not been reported in the boar and could contribute to our knowledge of the efficiency of spermatogenesis and fertility in this species.

In mice and humans, the CREM gene undergoes extensive mRNA splicing, generating up to 20 mRNA isoforms which encode for proteins functionally categorized as activators or repressors of downstream gene expression [reviewed in 8,9]. In general, the activator CREM mRNA isoforms include combinations of exons encoding the KID domains (Exons E and/or F), glutamine rich transactivation domains (Exons C and/or

* Corresponding author Tel.: +1-401 874 2667; fax: +1-401 874 7575
E-mail address: blsartini@uri.edu (B.L. Sartini).

0093-691X/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2011.09.013

G), and DNA-binding domains (Exons H and Ia and/or Ib). Activator CREM mRNA and protein levels are highest post-meiotically, and in some cases translationally delayed, to initiate downstream spermatid gene expression [10–12]. Repressor CREM mRNA and protein are expressed at higher levels in the immature testis and typically lack a transactivation domain, therefore suppressing downstream gene expression [11,13]. This developmental switch from repressor CREM isoform expression in the immature testis to activator CREM isoforms in the adult testis is in part regulated by FSH and is necessary for normal spermatogenesis [13,14].

Four CREM promoter regions (P1–P4) have been identified that control, in part, stage-dependent CREM expression in male germ cells [4,10,12,13]. The P1 promoter controls constitutive expression of activator and repressor CREM isoforms with leader exon B in all stages of spermatogenesis. The P2, P3, and P4 promoters are regulated by cAMP in a stage-dependent manner. The P2 promoter controls expression of a short ICER repressor isoform that functions as a negative autoregulator of CREM expression [15]. The P3 and P4 promoters control transcription of activator CREM isoforms with the θ1 and θ2 leader exons, respectively. The P3 and P4 promoters appear to regulate the elevated activator CREM expression levels in spermatocytes and spermatids, although the functional difference between protein encoded by transcripts with θ1 and θ2 leader exons has not been determined [10,12]. The θ1 and θ2 mRNA levels decline in testis from infertile men, suggesting a role of these CREM isoforms in infertility [6].

The CREM mRNA isoform expression patterns differed among species examined [5], but have not been reported during boar spermatogenesis. Complete sequencing of mRNA isoforms is necessary to accurately interpret and validate transcriptome analysis (including microarray and RNA-Seq data). Additionally, molecular profiling of sire fertility requires identification of specific mRNA isoforms expressed in a tissue- or cell-specific manner. The objective of this study was to sequence the predominant CREM mRNA isoforms encoded by the B, θ1, and θ2 leader exons in testes of immature and adult boars, and to compare the relative expression levels of these isoforms in adult boar testes.

2. Materials and methods

2.1. Tissue collection

Immature boar testes (Landrace; n = 4 boars; 3 wk old) were collected at castration at the University of Rhode Island, Peckham Farm. Testes from adult boars (Landrace; n = 4 boars; 1–3 y old) with normal spermatogenesis, were collected at a local abattoir. All animal procedures were approved by the University of Rhode Island's Institutional Animal Care and Use Committee. At the time of collection, all tissue samples were immediately frozen with liquid nitrogen and stored at −80 °C until analysis. Additional tissue samples were fixed with Bouin's fluid, then embedded in paraffin. Testis sections were stained with hematoxylin and eosin to confirm that spermatogenesis was normal.

2.2. Sequencing strategy

To sequence the boar testis CREM isoforms encoded by the B, θ1, and θ2 leader exons, an initial attempt was made to amplify the 5′ untranslated region (UTR) of these CREM transcripts using 5′RACE. A reverse human exon H primer designed from previously reported human CREM sequence (accession BC041810; Table 1) was used in 5′ RACE reactions with adult boar testis RNA, according to the manufacturer's protocol (GeneRacer, Invitrogen, Carlsbad, CA, USA). Only the θ2 5′UTR was amplified in the 5′ RACE experiments; therefore, primers based on human CREM B and θ1 exons (accession AY292864 and AF417234, respectively) were designed and used in the RT-PCR reactions described below (forward and reverse primers in individual B and θ1 exons; Table 1) to obtain these additional boar CREM sequences. Finally, to distinguish individual mRNA isoforms, forward primers in B, θ1, and θ2 exons were used with a reverse primer in exon H.

2.3. RT-PCR

Total RNA was isolated from immature and adult boar testis tissue using TRI Reagent (Sigma-Aldrich, St. Louis, MO, USA). cDNA was synthesized from 1 μg isolated adult or immature testis RNA in a random primed reverse transcriptase (RT) reaction, following DNase treatment with gDNA Wipeout (QuantiTect Reverse Transcription; Qiagen, Valencia, CA, USA). For all primer pairs, cDNA was added to a PCR reaction mixture of 1× polymerase-specific reaction buffer, 1.5 mM $MgCl_2$, 10 mM dNTPs, 2.5 μmol forward and reverse primers (Section 2.2., Table 1), including either 2.5 U Taq polymerase (NEBioLabs, Ipswich, MA, USA) or 1 U Phusion polymerase (Thermo, Fisher Scientific, Pittsburgh, PA, USA). The PCR conditions were 94 °C for 5 min, 35 cycles of 94 °C (30 s), primer

Download English Version:

https://daneshyari.com/en/article/10892420

Download Persian Version:

https://daneshyari.com/article/10892420

Daneshyari.com