Biochemical and Biophysical Research Communications 381 (2009) 537–543

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Identification and characterization of an alternative promoter of the human PGC-1α gene

Toyo Yoshioka [a,2], Kenjiro Inagaki [a,2], Tetsuya Noguchi [a,*], Mashito Sakai [a], Wataru Ogawa [a], Tetsuya Hosooka [a,1], Haruhisa Iguchi [b], Eijiro Watanabe [b], Yasushi Matsuki [b], Ryuji Hiramatsu [b], Masato Kasuga [a,c]

[a] Division of Diabetes, Metabolism, and Endocrinology, Department of Internal Medicine, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan
[b] Genomic Science Laboratories, DainipponSumitomo Pharma Co. Ltd., 4-2-1 Takatsukasa, Takarazuka 665-8555, Japan
[c] Research Institute, International Medical Center of Japan, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan

ARTICLE INFO

Article history:
Received 13 February 2009
Available online 20 February 2009

Keywords:
PGC-1α gene
Alternative promoter
Skeletal muscle
Exercise
cAMP response element
E-box
Muscle-specific transcription factor
CREB

ABSTRACT

The transcriptional regulator peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) controls mitochondrial biogenesis and energy homeostasis. Although physical exercise induces PGC-1α expression in muscle, the underlying mechanism of this effect has remained incompletely understood. We recently identified a novel muscle-enriched isoform of PGC-1α transcript (designated PGC-1α-b) that is derived from a previously unidentified first exon. We have now cloned and characterized the human PGC-1α-b promoter. The muscle-specific transcription factors MyoD and MRF4 transactivated this promoter through interaction with a proximal E-box motif. Furthermore, either forced expression of Ca^{2+}- and calmodulin-dependent protein kinase IV (CaMKIV), calcineurin A, or the p38 mitogen-activated protein kinase (p38 MAPK) kinase MKK6 or the intracellular accumulation of cAMP activated the PGC-1α-b promoter in cultured myoblasts through recruitment of cAMP response element (CRE)-binding protein (CREB) to a putative CRE located downstream of the E-box. Our results thus reveal a potential molecular basis for isoform-specific regulation of PGC-1α expression in contracting muscle.

Introduction

Peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) is a master regulator that directs mitochondrial biogenesis and the formation of insulin-sensitive type I (slow-twitch) myofibers in skeletal muscle [1]. Endurance exercise, which improves insulin sensitivity, has been shown to induce PGC-1α expression in muscle and to enhance oxidative metabolism in rodents and humans [2–4], implying that feasible measures to up-regulate PGC-1α expression or activity selectively in skeletal muscle may prove effective for the prevention or treatment of insulin resistance.

Several molecular pathways have been postulated to mediate the stimulatory effect of exercise on PGC-1α expression in skeletal muscle. Activation of Ca^{2+}- and calmodulin-dependent protein kinase IV (CaMKIV) and the protein phosphatase calcineurin A (CnA) in response to an increase in the cytosolic Ca^{2+} concentration may thus result in transactivation of the PGC-1α gene through cAMP response element (CRE)-binding protein (CREB) and the myocyte enhancer factor 2 family of transcription factors, respectively [5]. The p38 mitogen-activated protein kinase (p38 MAPK) and β_2-adrenergic receptor, both of which are activated by physical exercise, have also been implicated in the induction of muscle PGC-1α expression [6,7].

We have identified an alternative first exon (designated exon 1b) of the mouse PGC-1α gene that is spliced to the canonical exon 2 and contributes both the 5′ untranslated region and the first 12 codons to a novel isoform of PGC-1α transcript (PGC-1α-b mRNA) (H. Iguchi, E. Watanabe, and Y. Matsuki, unpublished observation). The same splicing variant was independently described by another laboratory [8]. The PGC-1α-b mRNA was found to produce functional protein with transcriptional activity; it was up-regulated in skeletal muscle in response to exercise to a much greater extent than was the previously described PGC-1α-a transcript [8]. These findings suggested that the molecular mechanism by which exercise induces PGC-1α expression in skeletal muscle may be more complex than that proposed on the basis of previous studies with

* Corresponding author. Fax: +81 78 382 2080.
E-mail address: noguchi@med.kobe-u.ac.jp (T. Noguchi).
[1] Present address: Division of Endocrinology, Diabetes, and Metabolism, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02215, USA.
[2] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.02.077

the promoter corresponding to the PGC-1α-a mRNA [5,6,9–11]. To address this issue, we have now cloned and characterized an alternative promoter for the human PGC-1α gene.

Materials and methods

Cloning and in silico sequence analysis of an alternative enhancer–promoter region of the human PGC-1α gene. An ~3-kb fragment (designated P1) upstream of the first translation initiation site within exon 1b of the human PGC-1α gene was amplified by the polymerase chain reaction (PCR) with human genomic DNA (Clontech) as a template and the isoform-specific forward primer 5′-ATTTACTTTGGATACTGCCCTATTGAGTGA-3′ and reverse primer 5′-TGGTATCATGAGATGAGGGAACACTCACAA-3′. The resulting PCR product was subcloned into the pT7-Blue vector (Takara Bio, Otsu, Japan) and sequenced with the use of a Thermo Sequenase II Dye Terminator Cycle Sequencing Kit (Amersham Pharmacia Biotech) and a DNA autosequencer (ABI 373, PE Applied Biosystems). Potential binding sites for transcription factors were predicted with the AliBaba2 program, which searches highly correlated sequence fragments versus the profiling data of TRANSFAC resources [12].

Expression vectors. Expression plasmids encoding MyoD or MRF4 (pCS2-MyoD and pCS2-MRF4) were kindly provided by P. Muñoz-Cánoves (Pompeu Fabra University, Barcelona, Spain); those encoding constitutively activated forms of CaMKIV (pSG5-CaMKIV-Δ317) or CnA (pCI-neo-CnA*) by E. Olson (University of Texas Southwestern Medical Center, Dallas); that for an activated form of MKK6b [pcDNA3-MKK6b(E)] by J. Han (Scripps Research Institute, La Jolla, CA); that encoding a dominant negative mutant of CREB (pCMV500-A-CREB) by C. Vinson (NCI, NIH, Bethesda, MD); and those encoding p300, CREB-binding protein (CBP), and a transcriptional repressor of p300 and CBP (pE1A) by N. Takahashi and T. Kawada (Kyoto University, Japan).

Transfection and luciferase assay. C2C12 myoblasts grown in 24-well plates (~1×10^4 cells per well) were transiently transfected with 100 ng of PGC-1α-b luciferase reporter construct, 100 ng of pME18S/lacZ (which encodes β-galactosidase), and 100 ng of various expression vectors. The corresponding empty vectors were used to maintain the total amount of transfected DNA constant. Forty-eight hours after transfection, luciferase activity in cell lysates was measured with the use of a PicaGene Luminescence Kit (Toyo Ink, Tokyo, Japan). Expression of reporter genes was normalized by the expression level of β-galactosidase determined with a Luminescent β-gal Detection Kit (Takara Bio) and was then expressed relative to the normalized value for cells transfected with the empty pGL3-basic plasmid.

Electrophoretic mobility-shift assay (EMSA). Double-stranded oligonucleotide probes (~10 pmol) for potential binding sites of transcription factors of the MyoD family (wild-type, GGAAC**CACC**

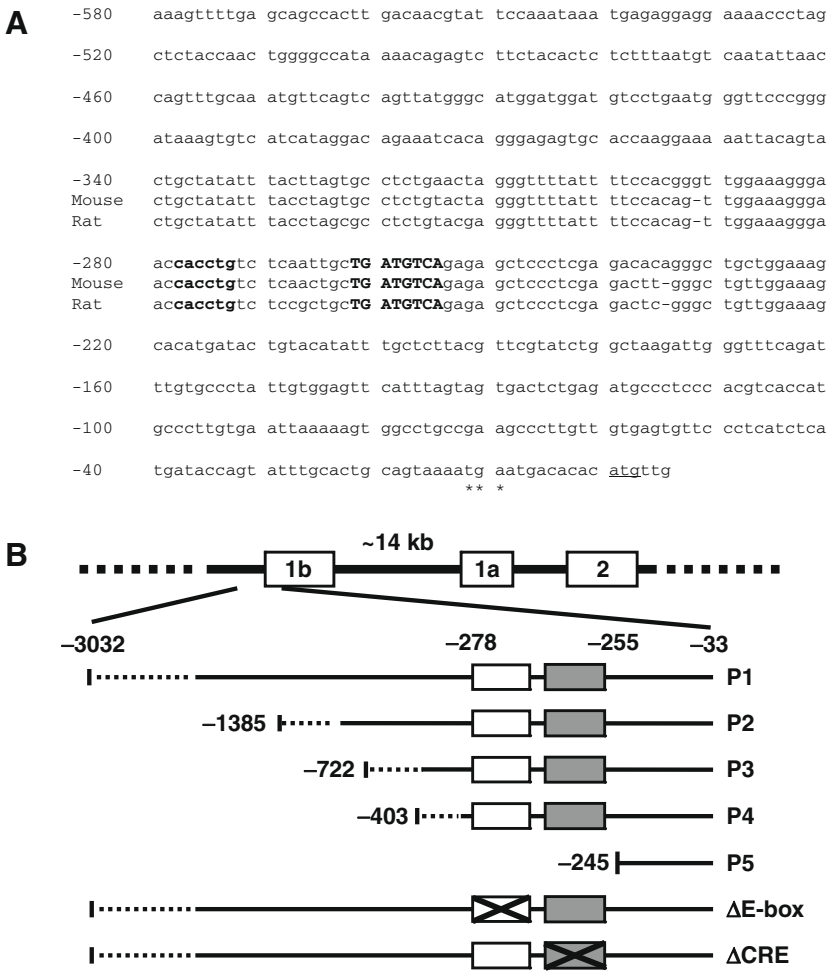

Fig. 1. Generation of luciferase reporter plasmids containing the PGC-1α-b promoter. (A) Nucleotide sequence of an alternative 5′ flanking region of the human PGC-1α gene and its alignment with the corresponding mouse and rat genes. A consensus E-box (bold) and a putative CRE (bold and uppercase) as well as initiation (underline) and stop (asterisks) codons are indicated. (B) Schematic representation of luciferase reporter plasmids for human PGC-1α-b. The ~3-kb wild-type fragment P1 contains the E-box (white box) and CRE (gray box); fragments P2 to P5 constitute a series of 5′ deletion mutants of P1; ΔE-box and ΔCRE consist of the ~3-kb fragment with the E-box or CRE, respectively, disrupted by site-directed mutagenesis. Numbers indicate nucleotide positions relative to the translation start site.