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Biochimica et Biophysica Acta 1759 (2006) 240-246

Promoter paper

Partial characterization of the mouse α -sarcoglycan promoter and its responsiveness to MyoD

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Received 19 August 2005; received in revised form 20 April 2006; accepted 26 April 2006 Available online 9 May 2006

Abstract

The mouse α -sarcoglycan gene is expressed in muscle cells during differentiation, but its transcriptional regulation is not understood. We have characterized the promoter region of the mouse α -sarcoglycan gene. This region is composed of positive and negative regulatory elements that respond to the myogenic differentiation environment. Accordingly, MyoD transactivates the α -sarcoglycan full-length and the proximal promoter. Chromatin immunoprecipitation assays revealed that MyoD, TFIID, and TFIIB interact with the distal promoter in C2C12 myoblasts, a stage at which the α -SG promoter appears to drive basal activity. In myotubes, such factors are located concomitantly at the distal promoter and at a DNA region around the proximal promoter. In agreement with these results, TFIID and TFIIB co-immunoprecipitate with MyoD. We conclude that the α -SG promoter is activated by MyoD, which interacts with TFIID and TFIIB in a protein complex differentially located at the distal promoter and around the proximal promoter during myogenic cell differentiation. © 2006 Elsevier B.V. All rights reserved.

Keywords: α-Sarcoglycan promoter; MyoD transactivation; Core promoter

The sarcoglycan complex is composed of α -, β -, γ -, and δ -sarcoglycans (SGs) and sarcospan, which are transmembrane proteins [1,2]. Additionally, ϵ - and ζ -SG were further discovered [3,4]. This complex is present in tissues such as striated and smooth muscle, retina, and endothelium [5–8]. β -, δ - and ϵ -SGs are widely expressed [1]; γ -SG is present in striated and smooth muscle [9], whereas α -SG has been detected solely in striated muscle [10]. The SG complex forms part of the dystrophin associated glycoprotein complex (DGC), its physiologic relevance highlighted by the fact that mutations disrupting the corresponding coding genes cause diverse muscular dystrophies [1,11]. In particular, α -, β -, γ - and δ -SGs deficiencies lead to autosomal recessive type 2D, 2E, 2C, and 2F muscular dystrophies, respectively, which are characterized by

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sarcolemmal structure disruption [12]. Evidence suggests a relationship between the SG complex structure and the transcriptional regulation of SG gene expression. For example, ordered recruitment of sarcoglycans is critical for the appropriate assembly of the complex, which determines its correct localization at the sarcolemma. This suggests a differential sarcoglycans requirement for SG complex assembly [13,14]. Also, overexpression of the γ sarcoglycan gene leads to muscular dystrophy with a similar phenotype to that observed in γ -SG-deficient mice [15], suggesting that fine modulation of the SG genes expression is critical for proper sarcolemmal structure and thus muscle physiology. In addition, α-SG has been detected exclusively in striated muscle [10], and its promoter region is up-regulated in C2/4 [16] and C2C12 myotubes ([17] and this report), explaining the increase of its mRNA during myogenic cell differentiation [18]. These facts suggest that the α -SG regulatory region is controlled by myogenic transcription factors.

Muscle-specific gene expression is regulated by the MyoD family of bHLH transcription factors (Myf5, MyoD, MRF4, and

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myogenin), also known as myogenic regulatory factors (MRFs) that recognize consensus sequence CANNTG, denominated E-box [19], and that play specific roles during myogenic commitment and differentiation [12]. Transfection of MyoD family member cDNAs in cells committed to different fates leads to expression of muscle genes [21–23]; therefore, these proteins are considered myogenic master regulators. Despite evidence sug-

gesting muscle-specific regulation of the α -SG gene expression and its relevance in muscle physiology, there are very little data with regard to the structure of α -SG gene promoter and nothing is known concerning its regulation by myogenic factor MyoD, considered the prototype of a master regulator [21]. Knowledge of the mechanisms by which sarcoglycan genes are transcriptionally regulated in muscle will lead us to better understand the processes

-2605	GGTGAAGCACTGTTTCTTCCTCAGGGGTTCAAGGGCAGAGTGTTCTGCTGCCTGTTCTCAGAGTTAGACCCTGCAAGAGAA	80
-2525	GGGTAGATGCAGGAATGAAGGGCCAGAGGTGTCCACCATCAAAGGCATCATGGAAGAGCCGGGACCCGGGGCTGTGAACTT E-box GATA E-box	160
-2445	TCTGAACATTCAGCTGCATCAGCATGGCTTTGTAGAAACTTAGAAACTGCCAGGCCTCAGCGATATCAGCTGTAAAACA	240
-2365	TGGGCTATGGGAATATCTGTCTCATGGTACTGCTGTGGGTATGACATGGGTCAGAGCACATAAATGCTTGTGAGCCTTTT NFI	320
-2285	GGCACAGAGACGGACATCAGTCCTTCTCACCATCACACAAAGCCCCAGTCAGGCAGG	400
-2205	GGTATAGGTGGCTGAACATGTGCTGCTTTCAGTATTTTTGTGAGTCCTCTGCAGAGGCCAGCACAAATCGTGCAGCTTAT	480
-2125	AATTGTGGAAGCAGAGGCCTGGAGAGGGCTAGTTACTTAAGGCCACGTCTGCAGGAACTGTCAAGCTGGAACTCCAACCC	560
-2045	TTATGGAATTAGCCCCAGCTATGTGAGCTGGAGAGAGCCTCTCTGCTTACCTGTCCCCATTTGGCCGATACCGTCTGG	640
-1965	TGTCTCCAGCCGGACCTTCACATGGGAGATGTTGGTCTGCGGCTCAGGCTGGGGAACAGATCTTCTGCTTGTTAAG E-box E-box	720
-1885	GCAGTTGTCAGCCAGCCTCTACACTTACTAGTTGCTAGACCCCAGGTCAGGTGAGTGGCCCAGAGCACCTGTTCCCACAC NFI Sp1	800
-1805	$\tt CATGATGTTCTAAGGCTGGG{\ref{TTGGCACAGGGA}} \\ TGGGGGAGGGG{\ref{TGGGCACAGGGA}} \\ TGGGGAGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG$	880
-1725	${\tt TACAGGAGAAAAGAC} \underline{{\tt TTCAGAGAGGACGGAACACAT}} {\tt ATGACCTGCTCAAGGTCTGCCCAAGGGACAAGGCAGGAGGGGGACAAGGCAGGAGGGACAAGGCAGGAGG$	960
-1645	ACCAGGCAGAGAGCAGCTTGGATGGAGGCCAGGAAACTAAGAGGACACATTAAGCTAGGCCAGAGTCTAGGGATGTTG Sp1 Sp1 NFI	1040
-1565	GTGTGTGTGGGGTGGCGTGCGTTCAAA <mark>TTGGCCACAAACATAGAGGAAGGGCCAGAAAG</mark> CCATCGACTGGGGATGA E-box MEF2	1120
-1485	GCTGGGAGCTCAAGAACAATTCCAGTTCTTGAGATTTTAAACAACTGAGCTGGGGATGGGTTGGGGTAGGGGTGTTAGAG B-box	1200
-1405	AGATGCTCAGTGGCTAAGAGCACTCGCTCTTGCAAAGGATCTGGGTTTAGTTCCCAGCACCCACAGGGTGGCTCAC B-box GATA	1280
-1325	AACCACCTGATACTCCAATTCCAGAGGATTCCCCTCTTCTGATCTTTGTGGGCTCCAGATAAGCAAGGCATACATA	1360
-1245	E-box MEF2 CACAGGTAGGCCACCACTTGTACACAGAAAATAAAAATAAAACCTTAAAGCCTTAAGAGAGGGCCCAAGTAGCCAGCTTGG	1440
-1245 -1165	E-box MEF2 CACAGGTAGGCCACCACTTGTACACAGAAAATAAAAATAAACCTTAAAGCCTTAAGAGAGGGCCCAAGTAGCCAGCTTGG TGGCTTTGAAAACATACGCTGAGGGACTGGAGAGATGGCTCAGTGGTTAGGAGTACGGGTTGCTTTTCCTGAAGTCCTGG	1440 1520
	E-box MEF2 CACAGGTAGGCCACCACTTGTACACAGAAAATAAAAATAAAACCTTAAAGCCTTAAGAGAGGGCCCAAGTAGCCAGCTTGG	
-1165	E-box MEF2 CACAGGTAGGCCACCACTTGTACACAGAAAATAAAATAA	1520
-1165 -1085	E-box MEF2 CACAGGTAGGCCACCACTTGTACACAGAAAATAAAATAA	1520 1600
-1165 -1085 -1005	E-box MEF2 CACAGGTAGGCCACCACTTGTACACAGAAAATAAAATAA	1520 1600 1680
-1165 -1085 -1005 -925	E-box MEF2 CACAGGTAGGCCACCACTTGTACACAGAAAATAAAATAA	1520 1600 1680 1760
-1165 -1085 -1005 -925 -845	E-box MEF2 CACAGGTAGGCCACCACTTGTACACAGAAAATAAAATA	1520 1600 1680 1760 1840
-1165 -1085 -1005 -925 -845 -765	E-box MEF2 CACAGGTAGGCCACCACTTGTACACAGAAAATAAAATAA	1520 1600 1680 1760 1840
-1165 -1085 -1005 -925 -845 -765	E-box MEF2 CACAGGTAGGCCACCACTTGTACACAGAAAATAAAATAA	1520 1600 1680 1760 1840 1920 2000
-1165 -1085 -1005 -925 -845 -765 -685	E-box MEF2 CACAGGTAGGCCACCACTTGTACACAGAAAATAAAATA	1520 1600 1680 1760 1840 1920 2000
-1165 -1085 -1005 -925 -845 -765 -685 -605	E-box MEF2 CACAGGTAGGCCACCACTTGTACACAGAAAATAAAATAA	1520 1600 1680 1760 1840 1920 2000 2080 2160
-1165 -1085 -1005 -925 -845 -765 -685 -605 -525	E-box CACAGGTAGGCCACCACTTGTACACAGAAAATAAAATA	1520 1600 1680 1760 1840 1920 2000 2080 2160
-1165 -1085 -1005 -925 -845 -765 -685 -605 -525 -445	E-box MEF2 CACAGGTAGGCCACCACTGTACACAGAAAATAAAATAA	1520 1600 1680 1760 1840 1920 2000 2080 2160 2240 2320
-1165 -1085 -1005 -925 -845 -765 -685 -605 -525 -445 -365 -285	E-box MEF2 CACAGGTAGGCCACCACTTGTACACAGAAAATAAAATAA	1520 1600 1680 1760 1840 1920 2000 2080 2160 2240 2320 2400

Fig. 1. The α -sarcoglycan gene 5' regulatory region is composed of multiple putative transcription factor binding sites. Computer-assisted analysis of the 2.6 kb corresponding to the α -SG gene 5' sequence identified putative binding sites for transcription factors (boxes). MyoD recognition consensus sequences are indicated as E-boxes. Arrows under the sequence indicate the position of the primers employed to generate the deleted constructs. The sequence, not previously reported, is underlined. The sequence including the putative α -SG core promoter is shaded. Transcription initiation site is indicated as ± 1 .

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