Available online at www.sciencedirect.com

SCIENCE DIRECT

Biochimica et Biophysica Acta 1729 (2005) 94 – 104

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

http://www.elsevier.com/locate/bba

Multiple proteins are involved in the protein–DNA complex in the proximal promoter of the human α1(III) collagen gene (*COL3A1*)

Tomoaki Yoshino[a,b], Hideaki Sumiyoshi[c], Toshitaka Shin[c], Noritaka Matsuo[c], Yutaka Inagaki[d], Yoshifumi Ninomiya[a], Hidekatsu Yoshioka[c,*]

[a]*Department of Molecular Biology and Biochemistry, Okayama University Graduate School of Medicine and Dentistry, Okayama 700-8558, Japan*
[b]*Department of Medicine and Medical Science, Okayama University Graduate School of Medicine and Dentistry, Okayama 700-8558, Japan*
[c]*Department of Anatomy, Biology and Medicine, Faculty of Medicine, Oita University, 879-5593 Oita, Japan*
[d]*Department of Community Health, Tokai University School of Medicine, Kanagawa 259-1193, Japan*

Received 27 September 2004; received in revised form 25 March 2005; accepted 8 April 2005
Available online 27 April 2005

Abstract

We have characterized the proximal promoter of the human α1(III) collagen gene (*COL3A1*). Transient transfection assays using a series of chimeric constructs linked to the luciferase gene indicated that the segment from −96 to −34 is necessary to activate transcription. Electrophoretic mobility shift assays (EMSAs) showed that the multiple proteins form the DNA–protein complex in different combinations depending on the cell types. A competition assay using mutant oligonucleotides showed that the sequence 5′-GCTCTCATATTTCAGAA-3′ (−79 to −63 bp) is critical for DNA–protein complex formation. This sequence is contained in the B element of mouse α1(III) collagen gene (*Col3a1*) reported by Ruteshouse and de Crombrugghe (J. Biol. Chem., 1993). In the rhabdomyosarcoma cell line, A204, at least two proteins of 92–118 kDa and 40–52 kDa are involved in the DNA–protein complex bound to this motif.

Keywords: Type III collagen; Promoter; Transcription; DNA binding protein

1. Introduction

Type III collagen is a member of the fibrillar collagen family [1–5]. The molecule is a homotrimer composed of a single type of α chain. It is widely distributed in soft connective tissues such as fetal skin and blood vessels. This molecule is also detected during the repair process of wound healing, reflecting the immaturity of tissues and increased vascularity. It is thought to play an important role in defining tissue architecture and mechanical properties, although its function is not entirely understood. Mutations in the *COL3A1* gene cause type IV Ehlers-Danlos syndrome, a disease leading to aortic rupture in early adult life [6–8]. The absence of type III collagen in a mutant mouse model causes rupture of the blood vessels [9]. Electron microscopic analysis of the vessels revealed that collagen fibrils were missing in the media of the aorta and were irregular in size in the adventitia of the aorta and skin.

Type III collagen is a component of the small argyrophilic collagen fibers that characterize reticular connective tissues [10]. Type III collagen is coexpressed with type I collagen in most tissues. Studies with monoclonal antibodies suggest that type III is a component of the striated fibrils, along with collagen type I [11]. Type III collagen can be present on banded collagen fibrils regardless of fibril diameter [12]. It can also form heterotypic fibrils with type I collagen. The expression of the ratio of type III to type I collagen varies spatially and temporally. It seems likely that transcriptional events are involved in the specific expression of the type III

Abbreviations: BBF, B element binding factor; AGPC, acid guanidium phenol chloroform; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; vSMC, vascular smooth muscle cells; ssDNA, salmon sperm DNA

* Corresponding author. Tel.: +81 97 586 5670; fax: +81 97 549 6302.
E-mail address: hidey@med.oita-u.ac.jp (H. Yoshioka).

0167-4781/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbaexp.2005.04.003

Table 1
Primers used for PCR procedures

1. For generation of luciferase constructs of 5′ stepwise deletion 1 (see Fig. 1)
−587/+68: (sense; −587) 5′-ggagctcGGAAGCCATTCAAACATTGC-3′
−287/+68: (sense; −287) 5′-ggagctcCATTGTATGTATTAAAACAA-3′
−96/+68: (sense; −96) 5′-ggagctcTGAGGGGATGGGTGCGGCTC-3′
−34/+68: (sense; −34) 5′-ggagctcCGGTGCTGAAGGGCAGGGAA-3′
Common: (antisense; +68) 5′-gctcgagGTTCAAAGTAGCACCATCAA-3′
(Lowercase letters underlined indicate tagged the *Sac*I and *Xho*I sites in the sense and antisense primer for cloning.)
2. For generation of luciferase constructs of internal deletion 1 (see Fig. 3A)
⊿−130/−100: (antisense, −130) 5′-ccgaattcTATAGAAGACACAAC-3′
(sense, −100) 5′-ccgaattcACTGCTGAGGGGATG-3′
⊿−100/−80: (antisense, −100) 5′-ccgaattcAAAAGAAATGATAAATATTT-3′
(sense, −80) 5′-ccgaattcGGCTCTCATATTTCA-3′
⊿−80/−50: (antisense, −80) 5′-ccgaattcGCACCCATCCCCTCAGCAG-3′
(sense, −50) 5′-ccgaattcGTGAGGGAAGCCAAACTTT-3′
⊿−50/−20: (antisense, −50) 5′-ccgaattcTTTCCAGCCCCTTTCTG-3′
(sense, −20) 5′-ccgaattcGGCCAAAGCAAAGGAAT-3′
⊿−20/+10: (antisense, −20) 5′-ccgaattcTTAAATAGGAAAAAGTTTGG-3′
(sense, +10) 5′-ccgaattcTTTTATGACGGGCCC-3′
(Lowercase letters underlined indicate tagged *Eco*RI site in the primer for cloning.)
3. For generation of luciferase constructs of internal deletion 2 (see Fig. 3E)
⊿−100/−90: (antisense, −100) 5′-ccgaattcAAAAGAAATGATAAATATTT-3′
(sense, −90) 5′-ccgaattcGGATGGGTGCGGCTC-3′
⊿−90/−80: (antisense, −90) 5′-ccgaattcCCTCAGCAGTAAAAG-3′
(sense, −80) 5′-ccgaattcGGCTCTCATATTTCA-3′
⊿−80/−70: (antisense, −80) 5′-ccgaattcGCACCCATCCCCTCAGCAG-3′
(sense, −70) 5′-ccgaattcTTTCAGAAAGGGGCT-3′
⊿−70/−60: (antisense, −70) 5′-ccgaattcTATGAGAGCCGCACC-3′
(sense, −60) 5′-ccgaattcGGGCTGGAAAGTGAG-3′
⊿−60/−50: (antisense, −60) 5′-ccgaattcCTTTCTGAAATATGAGAG-3′
(sense, −50) 5′-ccgaattcGTGAGGGAAGCCAAACTTT-3′
(Lowercase letters underlined indicate tagged *Eco*RI site in the primer for cloning.)
4. For generation of oligonucleotides used for EMSA (see Fig. 6A)
Wild type: (wt sense; −96) 5′-aagcttGCTGAGGGGATGGGT-3′
(wt antisense; −31) 5′-aagcttAAAAGTTTGGCTTCC-3′
(Lowercase letters underlined indicate tagged *Hin*dIII site in the primer for cloning.)
m1: (m1 sense, −79) 5′-cctgcagGCTCTCATATTTCAGAAA-3′
(m1 antisense, −86) 5′-cctgcagCATCCCCTCAGCAGTAAA-3′
m2: (m2 sense, −73) 5′-cctgcagATATTTCAGAAAGGGGCT-3′
(m2 antisense, −80) 5′-cctgcagCGCACCCATCCCCTCAGC-3′
m3: (m3 sense, −67) 5′-cctgcagCAGAAAGGGGCTGGAAAG-3′
(m3 antisense, −74) 5′-cctgcagGAGAGCCGCACCCATCCC-3′
m4: (m4 sense, −62) 5′-cctgcagAGGGGCTGGAAAGTGAGG-3′
(m4 antisense, −69) 5′-cctgcagAATATGAGAGCCGCACCC-3′
m5: (m5 sense, −56) 5′-cctgcagTGGAAAGTGAGGGAAGCC-3′
(m5 antisense, −63) 5′-cctgcagTTCTGAAATATGAGAGCC-3′
(Lowercase letters underlined indicate tagged *Pst*I site in the primer for cloning and generating mutation.)

collagen gene [13]. Ruteshouse and de Crombrugghe identified two positive *cis*-regulatory elements, designated A and B, in the mouse α1(III) collagen promoter [14,15]. A factor related to Jun/AP-1 appeared to bind at the A element located between −122 and −106, whereas the factor binding to the B element located between −83 and −61 was a heat-resistant polypeptide, named the B element binding factor (BBF), with a molecular weight of approximately 95 kDa.

In the present study, we characterized the proximal promoter of the *COL3A1* gene. The segment from −96 to −34 was necessary for the activation of transcription. Multiple proteins in different combinations, depending on cell types, were found to form the DNA–protein complex at −79 to −63.

2. Materials and methods

2.1. DNA clone

The *COL3A1* clone containing the promoter fragment was provided by Dr. F Ramirez of the Medical College of Cornell University, New York, NY [16].

2.2. Cells and cell culture conditions

Human rhabdomyosarcoma A204 and mouse NIH3T3 cells were purchased from American Type Culture Collection (ATCC, Rockville, MD). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supple-