Available online at www.sciencedirect.com

SCIENCE DIRECT

Gene 344 (2005) 287–297

GENE
SECTION
FUNCTIONAL GENOMICS

www.elsevier.com/locate/gene

Functional characterization of the human *SOX3* promoter: identification of transcription factors implicated in basal promoter activity

Natasa Kovacevic Grujicic, Marija Mojsin, Aleksandar Krstic, Milena Stevanovic*

Institute of Molecular Genetics and Genetic Engineering, Vojvode Stepe 444a, P.O. BOX 23, 11010 Belgrade, Serbia and Montenegro

Received 23 August 2004; received in revised form 6 October 2004; accepted 5 November 2004
Available online 10 December 2004
Received by R. Di Lauro

Abstract

SRY-related HMG-b*ox* genes (*Sox* genes) constitute a large family of developmentally regulated genes involved in the decision of cell fates during development and implicated in the control of diverse developmental processes. *Sox3*, an X-linked member of the family, is expressed in the central nervous system (CNS) from the earliest stages of development. It is considered to be one of the earliest neural markers in vertebrates playing the role in specifying neuronal fate. The aim of this study has been to determine and characterize the promoter of the human *SOX3* gene and to elucidate molecular mechanisms underlying the regulation of its expression. In this study, we have isolated and performed the first characterization of the human *SOX3* promoter. We have identified the transcription start point (*tsp*) and carried out the structural and functional analysis of the regulatory region responsible for *SOX3* expression in NT2/D1 cell line. Using promoter–reporter constructs, we have determined the minimal *SOX3* promoter region that confers the basal promoter activity, as well as two regulatory elements which have positive effects on the promoter activity. We have investigated in detail the functional properties of three conserved motifs within the core promoter sequence that bind transcription factors specificity protein 1 (Sp1), upstream stimulatory factor (USF) and nuclear factor Y (NF-Y). By mutational analysis, we have shown that all three sites are of functional relevance for constitutive *SOX3* expression in NT2/D1 cells. We have also shown that, besides the TATA motif, at least one other essential regulatory element is required for the basal transcription of the human *SOX3*. Taken together, data presented in this paper suggest that transcription factors such as Sp1, USF and NF-Y could function as key regulators for the basal activation of the human *SOX3* gene.

Keywords: Neural development; NT2/D1 cell line; Transcription start point; Reporter gene assay; Transcription factor binding site

1. Introduction

SRY-related HMG-b*ox* genes (*Sox* genes) constitute a large family of developmentally regulated genes involved in the decision of cell fates during development and implicated in the control of diverse developmental processes (Pevny and Lovell-Badge, 1997; Wegner, 1999). They encode a group of proteins that carry a DNA-binding HMG domain and additional domains involved in transcriptional regulation (Pevny and Lovell-Badge, 1997). SOX proteins display properties of both classical transcription factors and architectural components of chromatin (Pevny and Lovell-Badge, 1997). They perform their functions in a complex interplay with other transcription factors in a manner highly dependent on cell type and promoter context (Kamachi et al., 2000).

SOX genes show diverse and dynamic patterns of expression and have been recognized as key players in the regulation of embryogenesis and nervous system development (Pevny and Lovell-Badge, 1997). It has been shown

Abbreviations: CAT, chloramphenicol acetyltransferase; *tsp*, transcription start point; nt, nucleotide(s); bp, base pair(s); *SOX* genes, *SRY*-related HMG-b*ox* genes; EC, embryonal carcinoma cells; RA, retinoic acid; Sp1, specificity protein 1; NF-Y, nuclear factor Y; USF1, upstream stimulatory factor 1; EMSA, electrophoretic mobility shift assay; CNS, central nervous system.

* Corresponding author. Tel.: +381 11 3976212; fax: +381 11 3975808.
E-mail address: stevanov@eunet.yu (M. Stevanovic).

0378-1119/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.gene.2004.11.006

that developing nervous system expresses high levels of *Sox* genes, including *Sox1*, *Sox2*, *Sox3*, *Sox14* and *Sox21* (Collignon et al., 1996; Uchikawa et al., 1999).

Sox3, an X-linked member of the family (Stevanovic et al., 1993), is expressed in the central nervous system (CNS) from the earliest stages of development (Wood and Episkopou, 1999). It is considered to be one of the earliest neural marker in vertebrates playing the role in specifying neuronal fate (Brunelli et al., 2003). In *Xenopus*, *xSox3* is maternally expressed with zygotic transcripts appearing in the late blastula (Penzel et al., 1997). It has been shown that expression of the murine *Sox3* begins in a prestreak embryo, both in extra embryonic ectoderm and throughout the epiblast (Wood and Episkopou, 1999). From gastrulation onwards, the expression of *xSox3* is restricted to the presumptive neuroectoderm and subsequently to the CNS in a manner similar to that seen in mouse (Penzel et al., 1997; Wood and Episkopou, 1999). Transgenic studies in mouse have demonstrated that *Sox3* expression pattern in the developing neural tube can be dissected into different components, dependent on a series of modular elements that presumably transduce the action of transcription factors in specific regions of neural tube (Brunelli et al., 2003). By generating *Xenopus* embryos expressing mouse transgenes, it has been shown that some aspects of the regulation of *Sox3* during CNS development are evolutionary conserved. This indicates that mechanisms regulating early CNS patterning are conserved despite several substantial differences in neurogenesis between mammals and amphibians (Brunelli et al., 2003). Chick in ovo electroporation experiments demonstrated that expression of *Sox1*, *Sox2* and *Sox3* is a critical determinant of neurogenesis, keeping neural cells undifferentiated by counteracting the activity of proneural proteins (Bylund et al., 2003).

Finally, evidence for the developmental importance of the *SOX3* gene comes from the mutational analysis in humans. It has been demonstrated that *SOX3* is involved in X-linked mental retardation with growth hormone deficiency (Laumonnier et al., 2002). Additionally, a duplication of Xq26.1–q27.3 region, including the *SOX3* gene, is associated with X-linked hypopituitarism and variable degrees of mental retardation (Solomon et al., 2002). This is supported by the expression analysis in mouse that revealed that *Sox3* was required during formation of the hypothalamo–pituitary axis and specific central nervous system midline structures (Rizzoti et al., 2004). It is suggested that dysfunction of the SOX3 protein in patients with mental retardation and growth hormone deficiency disturbs cellular processes and function required for cognitive and pituitary development (Laumonnier et al., 2002).

Despite the mounting evidence that SOX3 is the key player in early developmental gene regulation, little is known about the transcriptional regulation of the *Sox3* gene itself. To date, the only data related to the control of *Sox3* gene expression came from the studies in which *cis* regulatory regions in the mouse *Sox3* promoter that direct tissue-specific heterologous marker gene expression in transgenic mice have been identified (Brunelli et al., 2003). Furthermore, it has been shown that human *SOX3* gene is transiently up-regulated during early stages of retinoic acid (RA)-induced neural differentiation of the embryonal carcinoma (EC) cell line NT2/D1 (Stevanovic, 2003).

Embryonal carcinoma (EC) cells, the stem cells of teratocarcinomas, resemble pluripotent stem cells from the early embryo (Andrews, 1988) providing the model of human embryonic development as well as tumor cell differentiation. Cell lines derived from such tumors, in particular, EC, have provided an invaluable in vitro resource in which molecular events regulating cell fate/lineage decision can be studied (Andrews, 1998). To date, the most widely characterized pluripotential EC cell line is NT2/D1 (Andrews, 1984), which displays the ability to differentiate along different somatic lineages dependent on the agent of morphogen. In the presence of RA, NT2/D1 irreversibly differentiates along the neuronal lineage (Andrews, 1984; Lee and Andrews, 1986) that, in recent years, has been exploited as a source of human postmitotic neurons for experimental studies (Hong et al., 1999).

In view of the fact that *SOX* genes are implicated in the control of nervous system development, RA-induced NT2/D1 cells provide an in vitro system for studying molecular basis of *SOX* gene regulation during neuronal differentiation. It has been shown that *SOX3* gene was expressed in NT2/D1 stem cells and that early phases of differentiation and neural induction, which take place within 48 h exposure to RA, involve up-regulation of the *SOX3* expression (Stevanovic, 2003).

Since the isolation and characterization of the human *SOX3* promoter has not been reported, the aim of this study has been to determine and characterize the promoter of the human *SOX3* gene and to elucidate molecular mechanism(s) underlying the regulation of its expression in the NT2/D1 cell line.

2. Materials and methods

2.1. Primer extension analysis

Primer extension analyses were performed with the following antisense primers:

PE5 5′ GCCCTGGGACTCCGTCGGAGCGGAGCTTGGGGG 3′ (−79)
PE17 5′ CTGTGGGCCAGCGAGTCCGGCGGGAA 3′ (−113)
R17ORF 5′ AGCTTGGGGGCCTGTGGGCCAG 3′ (−102)

The numbers indicated in parenthesis correspond to the distance in nucleotide (nt) from 5′ end of the sequence to