Available online at www.sciencedirect.com

SCIENCE DIRECT

Developmental Biology 277 (2005) 508–521

DEVELOPMENTAL BIOLOGY

www.elsevier.com/locate/ydbio

Genomes & Developmental Control

Functional role of a novel ternary complex comprising SRF and CREB in expression of *Krox-20* in early embryos of *Xenopus laevis*

Takashi Watanabe[a,b,1], Ikuko Hongo[b], Yoshiaki Kidokoro[a], Harumasa Okamoto[b,*]

[a]*Institute for Behavioral Sciences, Gunma University School of Medicine, Maebashi 371-8511, Japan*
[b]*Neuroscience Research Institute, National Institute of Advanced Industrial Science and Technology (AIST) Central 6, Tsukuba 305-8566, Japan*

Received for publication 25 October 2003, revised 21 August 2004, accepted 21 August 2004
Available online 5 October 2004

Abstract

Krox-20, originally identified as a member of "immediate-early" genes, plays a crucial role in the formation of two specific segments in the hindbrain during early development of the vertebrate nervous system. Here we cloned a genomic sequence of *Xenopus Krox-20* (*XKrox-20*) and studied functions of a promoter element in the flanking sequence and associated transcription factors, which function in early *Xenopus* embryos. Using the luciferase reporter assay system, we showed that the 5′ flanking sequence was sufficient to induce luciferase activities when the reporter construct was injected into embryos at the eight-cell stage. Deletion and mutagenesis analyses of the 5′ flanking sequence revealed a minimal promoter element that included two known subelements, a CArG-box and cAMP response element (CRE) within a stretch of 22 bp nucleotide sequence (−72 to −51 from the transcription initiation site), both of which were essential for the promoter activity. The gel mobility shift assay indicated that these two subelements bound to some components in whole cell extracts prepared from stage 20 *Xenopus* embryos. Antibody supershift and competition experiments revealed that these components in cell extracts were serum response factor (SRF) and a member of CRE binding protein (CREB) family proteins that bound the CArG-box and CRE, respectively. They appeared to assemble on the minimal promoter element to produce a novel ternary complex. When we injected mRNA of a dominant-negative version of *Xenopus* SRF (XSRFΔC) into animal pole blastomeres at the eight-cell stage, expression of *XKrox-20* in the nervous system as well as the minimal promoter activity was strongly suppressed. Suppression by XSRFΔC was counteracted by coexpressed wild-type XSRF. These results indicate that XSRF functions as an endogenous activator of *XKrox-20* by forming a ternary complex with CREB on the minimal promoter element.
© 2004 Elsevier Inc. All rights reserved.

Keywords: *Xenopus*; *XKrox-20*; *egr-2*; Immediate-early genes; CArG-box; CRE; SRF; CREB; Ternary complex; Hindbrain; Neuroectoderm; Promoter; Ets-binding site; Ets family transcription factor; CBP; Rhombomere

Introduction

The *Krox-20* gene encodes a transcription factor that contains three consecutive C_2H_2 zinc finger domains (Chavrier et al., 1988; Vesque and Charnay, 1992). During early development of the vertebrate nervous system, *Krox-20* is expressed in the neuroectoderm and plays a crucial role in development of rhombomeres 3 (r3) and 5 (r5) in the hindbrain, the neural crest (Bradley et al., 1993; Wilkinson et al., 1989), and Schwann cells (Topilko et al., 1994). In rhombomeres, the sites of *Krox-20* expression overlap with those of a variety of transcription factor genes such as *Hox* genes (Nonchev et al., 1996a,b), *EphA4 (Sek-1)* (Irving et al., 1996), and *Kreisler*/*mafB* (Frohman et al., 1993; Marin and Charnay, 2000a,b; McKay et al., 1994). Since *Krox-20* protein has been shown to directly bind to *cis*-elements in some *Hox* genes (Chavrier et al., 1990; Nonchev et al., 1996a,b; Vesque et al., 1996) and *EphA4 (Sek-1)* (Theil et al., 1998; Vesque et al., 1996), *Krox-20* appears to

* Corresponding author. Neuroscience Research Institute, National Institute of Advanced Industrial Science and Technology (AIST) Central 6, 1-1-1 Higashi, Tsukuba 305-8566, Japan. Fax: +81 298 61 6482.
E-mail address: h-okamoto@aist.go.jp (H. Okamoto).
[1] Present address: Department of Neuronal Signaling, Tokyo Institute of Psychiatry, 2-1-8 Kamikitazawa, Setagaya-ku, Tokyo 156-8585, Japan.

0012-1606/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2004.08.051

contribute to the formation of r3 and r5 by regulating expression of these transcription factors (Seitanidou et al., 1997). Indeed, analysis of phenotypes of knockout mice has revealed that deletion of *Krox-20* results in a total loss of r3 and r5, although overall segmentation in other parts of the hindbrain is maintained (Schneider-Maunoury et al., 1993, 1997).

Krox-20 was originally identified as a member of "immediate-early" genes that include proto-oncogenes, such as c-*fos* and c-*Jun* (reviewed in Herschman, 1991). Indeed, in NIH3T3 cells, the expression of mouse *Krox-20* was induced within 15 min following serum stimulation in the absence of de novo protein synthesis (Chavrier et al., 1988). By deletion analysis and mutagenesis, serum responsiveness of the promoter region of human *Krox-20* homologue *egr-2* was traced to a CArG-box (sequence of the form CC $(A/T)_6$ GG) in the transient transfection assay performed in NIH3T3 cells (Rangnekar et al., 1990). A similar CArG-box was identified as a regulatory element in the promoter region of a variety of genes including c-*fos* and *egr-1* (Herschman, 1991). These promoters have been shown to be activated by binding of a serum response factor (SRF) to the CArG-box (reviewed in Panitz et al., 1998; Shore and Sharrocks, 1995). It was suggested that the CArG-box in the *egr-2* promoter region also bound SRF (Rangnekar et al., 1990). However, it is still not known whether the CArG-box and SRF are also involved in the expression of *Krox-20* in the neuroectoderm during early development of the vertebrate nervous system.

In *Xenopus* embryos, *Krox-20* is also expressed in the neuroectoderm at specific sites along the anteroposterior (A/P) axis: its expression starts at the late gastrula stage, and the sites of expression are confined to r3, r5, and adjacent neural crest regions at later stages (Bradley et al., 1993; Nieto et al., 1992). Here we cloned a genomic sequence of *Xenopus Krox-20* (*XKrox-20*) and identified a minimal promoter element that was active in early *Xenopus* embryos. The minimal promoter element included two known transcription-factor-binding sites, a CArG-box and a core sequence of cAMP response element (CRE), within a stretch of 22 bp nucleotide sequence. CRE has been shown to mediate the transcriptional response to an elevated level of cAMP by binding to a member of CRE binding protein (CREB) family (Gonzalez et al., 1989; Ziff, 1990). Both SRF and CREB proteins are expressed in *Xenopus* embryos at gastrula and neurula stages (Lutz et al., 1999; Mohun et al., 1991). In this study, we showed that SRF and a member of CREB family proteins assembled on the minimal promoter element to produce a novel ternary complex. In addition, a dominant-negative version of *Xenopus* SRF (XSRFΔC) suppressed expression of endogenous *XKrox-20* in the nervous system. We conclude that the identified minimal promoter element plays a role in transcriptional activation of *Krox-20* during early *Xenopus* development by forming a novel ternary complex with SRF and CREB proteins.

Materials and methods

Animal care

Methods for keeping frogs and obtaining embryos have been described previously (Mitani and Okamoto, 1989).

Cloning of XKrox-20 genomic DNA from Xenopus library

Probe for *XKrox-20* in screening a genomic library (STRATAGENE) was prepared using PCR that was performed on cDNA synthesized from *Xenopus* neurula stage mRNA. A pair of primers was designed within 0.8 kbp 5′ region of previously reported *XKrox-20* cDNA (Bradley et al., 1993), which was devoid of the zinc finger domains: U, 5′-CACAGCTGCCAAGGACATCTAAGG-3′ and D, 5′-AAGAGCGCCGAGTAGTCGGG-3′. The cloned cDNA probe was used for screening of about 10^7 recombinant phage.

5′ RACE

Using 5′-Full RACE Core Set (TaKaRa), 5′ RACE was performed according to the manufacture′s recommendations. RT primer was designed about 200 bp downstream of translation initiation site and its 5′ end was phosphorylated: RT, 5′-CAGAGATCGCTTGTC-3′. Two pairs of primers were designed for nested PCR: U1, 5′-GCAGTTTATCTATAGCTTTGGCGGCCATGG-3′ and D1, 5′-CAGTGACTTTCAGTAGTTTTCTGCAGCAGATCC-3′ and U2, 5′-GCAAGTGCCCCCCAATATCAACTCAACC-3′ and D2, 5′-GCGTCTGTCACCATCTTCCCTAATGTGG-3′.

Construction of expression plasmids

A genomic sequence cloned in a recombinant phage, which contained the longest 5′ flanking sequence, was cut out by *Not*I and subcloned into the pBluescript II SK (−) for further analyses. To prepare reporter constructs containing 5′ flanking sequence of various length, the *Nco*I fragment (−1010 to +218) was cloned into a luciferase reporter vector pGL3-Basic (Promega) using *Nco*I site in the vector (−1010/Luc). Constructs, −3987/Luc and −3210/Luc, were then generated by serial addition of appropriate restriction fragments to −1010/Luc. −232/Luc was generated by removing *Sma*I fragment (one end at −232 and the other upstream end in the vector) from −1010/Luc. Sequences of the intron and 3′ flanking regions were amplified by PCR. These PCR products were subcloned into the pGEM-T (Promega) and then checked by sequencing (ABI). Correct clones were recloned into *Bam*HI site in the reporter vector pGL3. A pair of primers for the intron was designed: U, 5′-GGATCCGTAAGGGCTGTGTTTGTTTATGG-3′ and D, 5′-GGATCCCTGCGGGGGAAAGGACATTGG-3′. A pair of primers for the 3′ flanking region was designed: U, 5′-GGATCCCACTTGCACAGGGACAGGGTACC-3′ and D,

Download English Version:

https://daneshyari.com/en/article/10934408

Download Persian Version:

https://daneshyari.com/article/10934408

Daneshyari.com