Available online at www.sciencedirect.com
SCIENCE DIRECT
Gene 347 (2005) 43–54
GENE
SECTION
FUNCTIONAL GENOMICS
www.elsevier.com/locate/gene

Two promoters control the mouse *Nmp4/CIZ* transcription factor gene

Marta Alvarez[a], Rita Shah[a], Simon J. Rhodes[b], Joseph P. Bidwell[a,*]

[a]*Department of Anatomy and Cell Biology, Indiana University School of Medicine, Medical Science Bldg 5035, 635 Barnhill Drive, Indianapolis, IN 46202, United States*
[b]*Department of Biology, Indiana University–Purdue University Indianapolis, IN 46202, United States*

Received 23 July 2004; received in revised form 27 September 2004; accepted 14 October 2004
Available online 21 January 2005
Received by A.J. van Wijnen

Abstract

Nmp4/CIZ proteins (*n*uclear *m*atrix *p*rotein *4*/*c*as *i*nteracting *z*inc finger protein) contribute to gene regulation in bone, blood, and testis. In osteoblasts, they govern the magnitude of gene response to osteotropic factors like parathyroid hormone (PTH). *Nmp4/CIZ* is recurrently involved in acute leukemia and it has been implicated in spermatogenesis. However, these conserved proteins, derived from a single gene, are expressed in numerous tissues indicative of a more generalized housekeeping function in addition to their tissue-specific roles. To address how *Nmp4/CIZ* expression is governed, we characterized the 5′ regulatory region of the mouse *Nmp4* gene, located on chromosome 6. Two adjacent promoters P_1 [−2521 nucleotide (nt)/−597 nt] and P_2 (−2521 nt/+1 nt) initiate transcription of alternative first exons (U_1 and U_2). Both promoters lack TATA and CCAAT boxes but contain initiator sites and CpG islands. Northern analysis revealed expression of both U_1 and U_2 in numerous adult tissues consistent with the constitutive and ubiquitous activity of a housekeeping gene. Sequence analysis identified numerous potential transcription factor-binding sites significant to osteogenesis, hematopoeisis, and gonadal development. The promoters are active in both osteoblast-like cells and in the M12 B-lymphocyte cell line. Low doses of PTH attenuated P_1/P_2 activity in osteoblast-like cells. The *Nmp4/CIZ* promoters are autoregulated and deletion analysis identified regions that drive P_1 and P_2 basal activities as well as regions that contain positive and negative regulatory elements affecting transcription. The *Nmp4/CIZ* promoters comprise a genomic regulatory architecture that supports constitutive expression as well as cell- and tissue-specific regulation.

Keywords: Autoregulation; Leukemia; Osteoblast; Parathyroid hormone

1. Introduction

Nmp4/CIZ proteins (*n*uclear *m*atrix *p*rotein *4*/*c*as *i*nteracting *z*inc finger protein) have significant roles in the development and homeostasis of connective, hematopoietic, and gonadal tissues (Nakamoto et al., 2004; Shen et al., 2002; Martini et al., 2002; Bidwell et al., 2001). They contribute to the transcriptional regulation of Runx2, a central lineage regulator of osteoblast differentiation (Shen et al., 2002), and mediate synergy control of osteoblast gene expression, i.e., they govern the amplitude of transcription induction (Shah et al., 2004). For example, *Nmp4/CIZ* proteins suppress the increase in activity of specific osteoblast genes responding to parathryoid hormone (PTH), prostaglandin E_2, and bone morphogenetic protein 2 (Shah et al., 2004; Shen et al., 2002). For PTH, the mechanism of synergy control appears to involve hormone-induced changes in *Nmp4/CIZ* DNA-binding activity along target genes (Shah et al., 2004). Additionally, they influence the expression of numerous matrix metalloproteinases

Abbreviations: 5′ RACE, rapid amplification of cDNA ends; βgal, β-galactosidase; EST, expressed sequence tag; EWSR1, Ewing sarcoma gene; Nmp4/CIZ, nuclear matrix protein 4/cas interacting zinc finger protein; PCR, polymerase chain reaction; PTH, parathyroid hormone; RLM-RACE, 5′ RNA ligase-mediated rapid amplification of cDNA; RPA, ribonuclease protection assay; TAF15, TBP-associated factor 15.

* Corresponding author. Tel.: +1 317 278 1142; fax: +1 317 278 2040.
E-mail address: jbidwell@iupui.edu (J.P. Bidwell).

0378-1119/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.gene.2004.10.025

(MMPs) in fibroblasts, including *MMP-3*, *-5*, and *-7* (Nakamoto et al., 2000), consistent with a role in connective tissue extracellular matrix turnover (Bidwell et al., 2001). *Nmp4/CIZ* is frequently involved in acute leukemia through fusion with the TET-proteins Ewing sarcoma gene (EWSR1) or TBP-associated factor 15 (TAF15), suggestive of a role in lymphoid and myeloid development (Martini et al., 2002). Finally, mice harboring a disrupted *Nmp4/CIZ* gene manifest deficits in spermatogenesis and male fertility defects (Nakamoto et al., 2004).

Nmp4/CIZ proteins have a broad tissue distribution (Thunyakitpisal et al., 2001). In situ hybridization of rat embryo tissue sections (18 days postcoitus) showed significant *Nmp4/CIZ* expression not only in endochondral bone tissue but in neuronal cells of both the central and peripheral nervous system (Thunyakitpisal et al., 2001). Northern analysis revealed *Nmp4/CIZ* mRNA transcripts in adult rat heart, brain, spleen, lung, liver, skeletal muscle, kidney, and testis (Thunyakitpisal et al., 2001). Seven *Nmp4/CIZ* isoforms have been characterized (Thunyakitpisal et al., 2001; Nakamoto et al., 2000), all derived from a single gene that in mice is located on chromosome 6 (Alvarez et al., 2001; Thunyakitpisal et al., 2001). *Nmp4/CIZ* proteins are highly conserved with 95% identity between the rat and human proteins (Bidwell et al., 2001).

Nmp4/CIZ are novel nucleocytoplasmic shuttling proteins that localize to the nuclear matrix and chromatin nuclear compartments and, in the cytoplasm, appear to associate with $p130^{cas}$, a protein implicated in focal adhesion signaling (Thunyakitpisal et al., 2001; Nakamoto et al., 2000). Consistent with their nucleocytoplasmic shuttling activity, *Nmp4/CIZ* proteins have from 5 to 8 Cys_2His_2 zinc fingers, which mediate nuclear localization and DNA binding, and have an SH3-binding domain that promotes their interaction with the cytoplasmic protein $p130^{cas}$ (Torrungruang et al., 2002; Nakamoto et al., 2000; Feister et al., 2000). The *Nmp4/CIZ* zinc fingers are unique in their capacity for interacting with the narrow minor groove of their unusual poly(dA:dT) consensus sequence which may contribute to their context-specific functionality (Torrungruang et al., 2002).

To elucidate the mechanisms that contribute to the ubiquitous tissue distribution of the *Nmp4/CIZ* isoforms together with their marked significance to connective and hematopoietic tissues, we have characterized the 5′ regulatory region of the mouse *Nmp4/CIZ* gene. Two adjacent promoters initiate transcription of alternative first exons, both of which are expressed in various adult mouse tissues. Although these promoters exhibit characteristics of housekeeping genes, in silico analysis indicate they contain potential consensus elements for transcription factors that play significant roles in hematopoiesis, oseteogenesis, and gonadal development. Consistent with the *Nmp4/CIZ* role in bone homeostasis, both promoters are remarkably sensitive to PTH. Thus, *Nmp4/CIZ* promoter architecture drives constitutive basal expression in multiple tissues while providing the molecular framework for cell- and tissue-specific regulation.

2. Materials and methods

2.1. Cell culture

UMR 106-01 rat osteosarcoma cells and MC3T3-E1 murine osteoblast-like cells (provided by Dr. Laurie McCauley, University of Michigan, Ann Arbor, MI) were grown in MEM and α-MEM (Gibco BRL, Grand Island, NY), respectively. Media were supplemented with 100 μg/ml streptomycin, 100 IU/ml penicillin, 25 μg/ml amphotericin, 2 mM L-glutamine (Gibco BRL), and 10% fetal bovine serum (FBS; Sigma, St. Louis, MO). NIH-3T3 murine fibroblasts were grown in DMEM (high glucose, Gibco BRL) supplemented with 10% bovine calf serum (Sigma) and penicillin, streptomycin, amphotericin, and L-glutamine as described above. The B-lymphocyte cell line, M12, a generous gift from Dr. Mark Kaplan (Indiana University School of Medicine), was grown in RPMI-1640 supplemented as described above. All cells were maintained in humidified 95% air/5% CO_2 at 37 °C.

2.2. Rapid amplification of cDNA end (5′ RACE) and ribonuclease protection analysis (RPA) of mouse Nmp4/CIZ cDNA

Total RNA was harvested from MC3T3-E1 cells using the RNeasy protocol (Qiagen, Valencia, CA). The 5′ RNA ligase-mediated rapid amplification of cDNA (RLM-RACE) reactions were performed using the FirstChoice RLM-RACE protocol (Ambion, Austin, TX). The gene-specific primer R1 (Table 1) was used to obtain first strand cDNA from 10 μg of total RNA. Nested amplification of the cDNA was completed by using the GeneRacer™ 5′ Primer and gene-specific primer R2 (Table 1). Polymerase chain reactions (PCR) were carried out with an initial denaturation step of 94 °C for 3 min and 35 cycles of denaturing at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 1 min. The 5′ RACE reaction was also performed with mouse brain Marathon-Ready cDNA (Clontech, Palo Alto, CA) using R1/AP1 (adapter primer) and R2/AP2 for nested PCR following the manufacturer's protocol and conditions for the nested PCR reactions as described above. All PCR products were ligated into pCR®2.1-TOPO (Invitrogen, Carlsbad, CA) and sequenced on both strands by automated DNA sequencing (Biochemistry Biotechnology Facility [BBF], Indiana University School of Medicine).

To prepare probes for RPA, we used PCR amplification to obtain specific sequences from the mouse C57BL/6J BAC clone RPCI 346 E 12 (Alvarez et al., 2001). For obtaining genomic fragments containing U_1, we used the oligos −716/P1 and P1A/P1B (Table 1) and for genomic