Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1783 (2008) 131 – 143
Biochimica et Biophysica Acta
BBA
www.elsevier.com/locate/bbamcr

DNMT1 interacts with the developmental transcriptional repressor HESX1

Ezat Sajedi [a], Carles Gaston-Massuet [a], Cynthia L. Andoniadou [a], Massimo Signore [a], Paul J. Hurd [b], Mehul Dattani [c], Juan Pedro Martinez-Barbera [a,*]

[a] *Neural Development Unit, Institute of Child Health, University College London, 30 Guilford Street, WC1N 1EH, London, UK*
[b] *The Wellcome Trust and Cancer Research UK Gurdon Institute, University of Cambridge, Cambridge, UK*
[c] *Clinical and Molecular Genetics Unit, Institute of Child Health, University College London, 30 Guilford Street, WC1N 1EH, London, UK*

Received 23 May 2007; received in revised form 28 August 2007; accepted 28 August 2007
Available online 7 September 2007

Abstract

Hesx1 is a highly conserved homeobox gene present in vertebrates, but absent from invertebrates. Gene targeting experiments in mice have shown that this transcriptional repressor is required for normal forebrain and pituitary development. In humans, mutations in *HESX1* impairing either its repressing activity or DNA binding properties lead to a comparable phenotype to that observed in *Hesx1* deficient mice. In an attempt to gain insights into the molecular function of HESX1, we have performed a yeast two-hybrid screen and identified DNA methyltransferase 1 (DNMT1) as a HESX1 binding protein. We show that *Dnmt1* is co-expressed with *Hesx1* within the anterior forebrain and in the developing Rathke's pouch. Mapping of the interacting regions indicates that the entire HESX1 protein is required to establish binding to a portion of the N-terminus of DNMT1 and its catalytic domain in the C-terminus. The HESX1–DNMT1 complexes can be immunoprecipitated in cells and co-localise in the nucleus. These results establish a link between HESX1 and DNMT1 and suggest a novel mechanism for the repressing properties of HESX1.

Keywords: DNA methylation; Repression; Homeobox; Forebrain; Pituitary; Mouse

1. Introduction

Hesx1 is a transcription factor that belongs to the paired class of homeobox genes. *Hesx1* is conserved in vertebrates, but it is absent from other animal groups, including amphioxus and ascidians. *Hesx1* is expressed in the rostral region of the developing vertebrate embryo, but expression has not been detected in any adult tissues or established cell lines, with the exception of mouse ES cells [1,2]. In mouse, *Hesx1* expression is very dynamic and is regulated by specific enhancers located in the 5′ and 3′ regions of the locus [3]. *Hesx1* transcripts are first detected in the anterior endoderm of the early gastrula embryo, but the most prominent sites of expression are the anterior neural ectoderm, which later gives rise to the forebrain, and Rathke's pouch, the primordium of the anterior pituitary gland [1,2]. The expression pattern of *Hesx1* orthologues in other vertebrates is highly conserved [4,5].

Previous research has shown that *Hesx1* is essential for normal forebrain and pituitary gland formation in mammals [6,7]. *Hesx1*-deficient mouse embryos show variable degrees of forebrain defects, including abnormalities in dorsal midline structures, namely the septum pellucidum, corpus callosum, and anterior and hippocampal commissures. *Hesx1* homozygous mutants also show pituitary dysplasia, anophthalmia or microphthalmia and defects in the olfactory bulbs. A comparable phenotype is observed in the congenital human disorder septo-optic dysplasia (SOD), a syndrome characterised by variable combinations of pituitary abnormalities, midline forebrain defects and optic nerve hypoplasia [6]. Indeed, it has been shown that mutations in human *HESX1* are associated with familial cases of SOD and other forms of hypopituitarism

Abbreviations: aa, amino acid; DNMT1, DNA methyltransferase 1; SAFB1, scaffold attachment factor beta 1; RNF2, ring finger protein 2; Lonp2, lon peptidase 2, peroxisomal; ZFP592, zinc finger protein 592; BTBD2, BTB (POZ) domain containing 2; SRFBP1, serum response factor binding protein 1; ZMIZ1, zinc finger MIZ-type containing 1; SOD, septo-optic dysplasia; TLE1, transducin-like enhancer of split 1; N-CoR, nuclear co-repressor; eh1, engrailed homology domain 1; GST, glutathione-*S*-transferase; Gal4DBD, Gal4 DNA binding domain; IVT, in vitro translated; HRP, horse radish peroxidase; PFA, paraformaldehyde; PcP, polycomb group; EZH2, enhancer of zeste homologue; ES cells, embryonic stem cells

* Corresponding author. Fax: +44 207 831 4366.
E-mail address: j.martinez-barbera@ich.ucl.ac.uk (J.P. Martinez-Barbera).

0167-4889/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2007.08.010

[combined pituitary hormone deficiency (CPHD) and isolated growth hormone deficiency (IGHD)] [8–16].

At the molecular level, there is evidence indicating that HESX1 can function as a transcriptional repressor in vitro and in vivo [10,11,17]. HESX1 contains two repressor domains, one located in the N-terminus (eh1 and HRPW motifs) and the other in the homeodomain (Fig. 1A). The N-terminal repressing domain binds TLE1, a mammalian orthologue of Groucho, whereas the homeodomain interacts with N-CoR [17]. TLE1 and N-CoR are proteins that mediate transcriptional repression through interactions with DNA binding transcription factors and histone deacetylases [17]. In *Xenopus*, the repressing activity of HESX1 is required for the regulation of both neural differentiation and patterning of the anterior neuroectoderm [18]. Recently, we have shown that the mechanism underlying the forebrain defects in the *Hesx1*-deficient embryos involves the ectopic expression of genes with caudalising activities within the anterior forebrain of the very early mouse embryo [19]. Normal pituitary organogenesis also requires the repressor functions of HESX1 [17]. Therefore, HESX1 is a critical transcriptional repressor that plays a broad role in the development of the forebrain and associated structures, such as the olfactory bulbs, eyes and pituitary gland.

It seems likely that HESX1 exerts its functions not only through the interaction with TLE1 and N-CoR, but further interacting proteins, which remain to be characterised. To search for novel HESX1-interacting proteins, we have performed a yeast two-hybrid screen on a 9.5–10.5 dpc (days post coitum) cDNA mouse library. We have identified DNMT1, a protein responsible for CpG methylation and repression of gene expression, as a HESX1 partner [20–22]. We have mapped the regions of the proteins involved in the interaction and show that HESX1–DNMT1 complexes are present in cells and that both proteins co-localise in the nucleus. We demonstrate, by in situ hybridisation and RT-PCR, that *Dnmt1* is actively transcribed in *Hesx1*-expressing cells in the forebrain and in Rathke's pouch of the developing mouse embryo. In transfected cells, the repressor activity of HESX1, which is mediated by the co-repressors TLE1 and N-CoR, cannot be enhanced by the addition of DNMT1 in a mammalian one-hybrid system. The link between HESX1 and DNMT1 suggests that HESX1 might repress transcription by an alternative mechanism, namely through CpG methylation of HESX1 target genes.

2. Experimental procedures

2.1. Plasmid constructs

Two bait vectors were constructed by fusing either the full-length HESX1 (aa 1–185) protein or the N-terminal half (aa 1–107, excluding the homeodomain), in frame to the Gal4 DNA binding domain in the pGBDU-C vector [23]. GST–HESX1 and GST–DNMT1 constructs were generated by cloning specific coding sequences into the pGEX-4T vector (Roche). Plasmids containing full-length cDNAs for the interactors were obtained from the IMAGE consortium (MRC Geneservice, Cambridge). These clones were 5706204 (*Dnmt1*), 4021046 (*Rnf2*); 6401542 (*Lonp2*); 5009612 (*Srfbp1*); 6413080 (*Btb2*); 6856060 (*Zmiz1*). An IMAGE clone (6826575) lacking 120 aa of the N-terminal part of *Zfp592* was also obtained. A full-length *Safb1* clone was kindly provided by Dr. Oesterreich

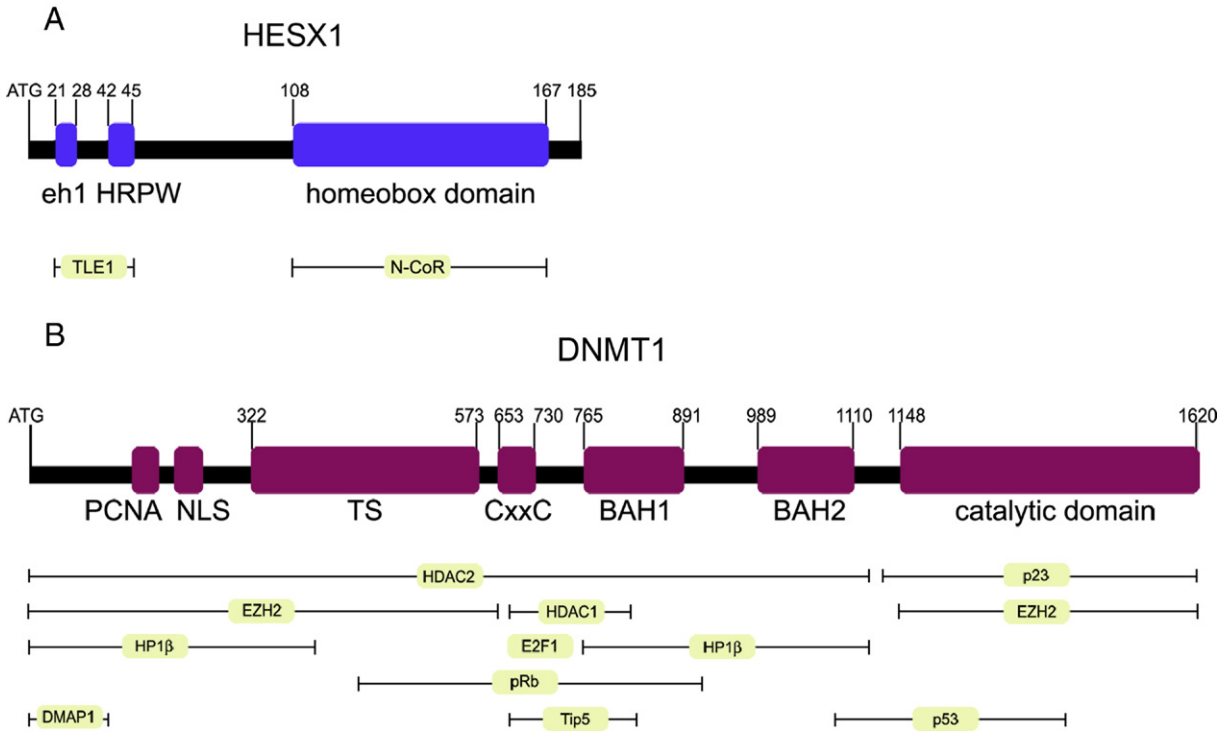

Fig. 1. Diagram of HESX1 and DNMT1 proteins showing their functional domains and interacting partners. (A) Two domains in the N-terminal half of HESX1 (eh1 and HRPW) interact with the repressor TLE1, whilst the homeodomain mediates interaction with the N-CoR. (B) DNMT1 contains multiple domains including (1) the PCNA binding motif; (2) the nuclear localisation signal (NLS); (3) the targeting sequence (TS); (4) the CxxC domain; (5) the polybromo domains 1 and 2 (BAH1 and 2); and (6) the catalytic domain. The regions involved in protein–protein interactions with previously reported DNMT1 partners are indicated. This image has been adapted from Spada et al. [21].