Zebrafish BarH-like genes define discrete neural domains in the early embryo

Alicia Colombo a,1, Germán Reig a,1, Marina Mione b, Miguel L. Concha a,∗

a Anatomy and Developmental Biology Program, Faculty of Medicine, Institute of Biomedical Sciences, Universidad de Chile, Independencia 1027, Santiago, Chile

b Istituto FIRC di Oncologia Molecolare (IFOM), Via Adamello 16, I-20139 Milan, Italy

Abstract

BarH (Barhl) genes encode for highly conserved homeodomain-containing transcription factors involved in critical functions during development, including cell fate specification, migration and survival. Here, we report the dynamic and restricted expression of three zebrafish barhl within the developing central nervous system. barhl2 becomes expressed in the late gastrula as a transverse diencephalic domain located immediately caudal to the prospective eyes. At early somitogenesis, barhl1.1 and barhl1.2 are expressed in the diencephalon in domains that partially overlap with the ventral and dorsal aspects of barhl2 expression, respectively. At later stages, expression of all zebrafish barhl shows large extent of overlap in the pretectum, tectum and dorsal hindbrain. The presence of a unique territory of barhl2 expression in the dorsal telencephalon and the high levels of expression in the retina are both consistent with expression reports of other Barhl2 orthologues, and support the subdivision of vertebrate Barhl into two paralogue groups based on the phylogenetic analysis of nucleotide and amino acid sequences.

Keywords: BarH; Barhl; Homeodomain proteins; FIL domains; Zebrafish; Central nervous system; Eye

1. Results and discussion

BarH1 and BarH2 encode for homeodomain-containing proteins that play essential roles during embryogenesis in Drosophila including morphogenesis and fate determination of the eye and external sensory organs (Hayashi et al., 1998; Higashijima et al., 1992a,b; Kojima et al., 1991, 1993; Lim and Choi, 2003, 2004), regional pre-patterning of specific structures of the nervous system such as the notum (Sato et al., 1999), and formation and specification of the distal leg segments (Tsuji et al., 2000). Vertebrate homologues have been identified in medaka (Poggi et al., 2002), Xenopus (Patterson et al., 2000), rat (Saito et al., 1998), mouse (Bulfone et al., 2000; Saba et al., 2003; Mo et al., 2004) and human (Bulfone et al., 2000) (Fig. 1), and appear to share some functional properties with the fly counterparts, such as the ability to regulate bHLH proteins (Saito et al., 1998; Sato et al., 1999). Different reports have named these genes as either BarH (bb) (e.g. Saito et al., 1998; Patterson et al., 2000) or BarH-like (Barhl) (e.g. Bulfone et al., 2000). Confusingly, assignment of paralogue numbers in the two nomenclatures is inverted. For example, mouse BarH1 (mbh1) is the same as mouse Barhl2 (mBarhl2) and viceversa, and the same apply for Xenopus BarH genes. In this paper, we have adopted the Barhl nomenclature as it has a HGNC-approved symbol (HGNC:954). Alternative/original names for each of these genes are indicated in the Experimental Procedures section of this article.

Vertebrate Barhl are expressed primarily in the developing nervous system where they appear to control subtype cell identity, migration and survival. Members of the Barhl2 group confer commissural neuron identity on dorsal cells in the spinal cord (Saba et al., 2003, 2005), are involved in the specification of retina ganglion (Poggi et al., 2004) and glycineric amacrine (Mo et al., 2004) cells, and have pro-apoptotic activity in cells of the gastrula that pattern the neural plate (Offner et al., 2005). Vertebrate Barhl members, on the other hand, regulate the migration of cerebellar granule cells (Li et al., 2004), and are involved in the survival of specific cell types within the cerebellum (Li et al., 2004) and cochlea (Li et al., 2002).

Here, we report the expression of three zebrafish barhl, which we have named barhl1.1, barhl1.2 and barhl2, based on the comparative analysis of both nucleotide and amino acid sequences (Fig. 1). Protein sequence alignment of Barhl from zebrafish (Dr BARHL1.1, Dr BARHL1.2, Dr BARHL2), medaka (Ol BAR), Xenopus (Xl BARHL2, Xl BARHL1), rat

* Corresponding author. Tel.: +56 2 978 6875; fax: +56 2 978 6368.
E-mail address: mconcha@med.uchile.cl (M.L. Concha).
1 These authors contributed equally to this work.
(Rn BARHL2), mouse (Mm BARHL1) and human (Hs BARH1, Hs BARH2) revealed high degree of amino acid identity within three regions: one containing around 100 amino acids encompassing the homeodomain (Fig. 1A) and two regions located on the N-termini containing FIL domains (FIL1 and FIL2) (Saito et al., 1998) (Fig. 1B). FIL domains have been found in homeoproteins of the Engrailed, Goosecoid, Nk-1, Nk-2 and Msh classes, where they have been associated with transcriptional repression activity (Jaynes and O’Farrell, 1991; Smith and Jaynes, 1996). Phylogenetic analysis based on either Maximum-Likelihood (Fig. 1C) or Neighbour (data not shown) methods, and considering nucleotide sequences encoding for the extended homeodomain region depicted in A. The tree indicates, zebrafish has two barhl1 genes (named barhl1.1 and barhl1.2) and only one barhl2 gene.

Expression of zebrafish barhl is highly dynamic and restricted to discrete domains within the developing central nervous system. Transcripts of barhl2 are first detected at 80–90% epiboly as bilateral stripes extending across the medio-lateral axis of the neural plate (Fig. 2A). At this stage, expression of barhl1.1 and barhl1.2 is still undetectable. Double labelling of barhl2 in combination with early markers of the prospective midbrain (pax2, Fig. 2A), telencephalon (emx1, Fig. 2B), eye field (rxb, Fig. 2C), and dorsal epithalamus (flh, Fig. 2D) indicate that barhl2 demarcates a transverse diencephalic domain within the anterior neural plate, located immediately caudal to the prospective eyes (Fig. 2E). After closure of the neural tube, the bilateral stripes of expression fuse at the midline and barhl2 defines a diencephalic domain perpendicular to the axis that comprises...
all the dorso-ventral extent of the neural tube. At this stage (about 1–2 somites), expression of barhl1.2 becomes detectable as a transverse diencephalic domain similar to barhl2 but excluded from the most dorsal and ventral aspects of the neural tube. A few hours later barhl1.1 expression appears restricted to the ventral diencephalon. By 10–14 somites, the three zebrafish barhl define complementary and partially overlapping domains within the diencephalon. barhl2 is detected in the prospective epithalamic and thalamic regions, and in a ventral diencephalic domain that move towards anterior to finally cover the posterior hypothalamus/posterior tuberculum (Fig. 2F,G). barhl1.1 and barhl1.2, on the other hand, are expressed in the prospective posterior hypothalamus/posterior tuberculum (Fig. 2K,L) and dorsal thalamus (Fig. 2P,Q), respectively. From around 18 h post-fertilisation (hpf), several novel domains of barhl expression appear in the brain. barhl2 transcripts become detectable in the dorsal telencephalon (where expression is maintained until around 40 hpf) (Fig. 2G,H). Interestingly, telencephalic barhl2, and the most ventral part of the hypothalamic barhl1.1 expression domain,
overlap with neurogenin1 (Fig. 2U–Y), a finding that is consistent with the proposed ability of vertebrate Barhl to regulate bHLH proteins (Saito et al., 1998; Sato et al., 1999). At 18 hpf, barhl1.1 is seen in a small subset of cells in the prospective cerebellum (Fig. 2L). At this stage, expression of barhl1.1 is also detectable along the dorsal hindbrain (prospective clusters of interneurons), in a domain that is later shared with barhl1.2 and barhl2 (from around 36 hpf; see Fig. 2I,N,S). At 24 hpf, barhl1.1 transcripts are also seen in a small subset of cells within the olfactory bulb (data not shown). At 36 hpf, novel domains of barhl expression become detectable in the pretectal area (barhl1.1, barhl1.2 and barhl2), latero-ventral epithalamus (barhl1.2), tectum and retina (barhl1.2) (Fig. 2H,M,R). barhl1.1 and barhl2 also show expression in the tectum and retina from around 48 hpf (Fig. 2J,O). By 60 hpf, all zebrafish barhl share overlapping expression domains in the tectum, pretectum and hindbrain (Fig. 3), whereas barhl2 maintains a unique expression domain in the dorsal diencephalon and is detected in the retina at considerably higher levels than barhl1.1 and barhl1.2 (compare Fig. 3C′–C′′ with 3F,F′,I,I′).

In summary, zebrafish barhl show a combination of unique and overlapping expression domains that support a subdivision into two paralogue groups based on both nucleotide and amino acid sequence analysis (Fig. 1). Comparison of Barhl expression domains between mouse and zebrafish is consistent with this subdivision and reveals that Barhl2 members have unique territories of expression in the telencephalon, epithalamus, and (at least at high levels) in the retina (Table 1).

Table 1
Comparison of expression territories of mouse (Mm) and zebrafish (Dr) barhl

<table>
<thead>
<tr>
<th></th>
<th>Mm Barhl1</th>
<th>Dr barhl1.1</th>
<th>Dr barhl1.2</th>
<th>Mm Barhl2</th>
<th>Dr barhl2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telencephalon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retina</td>
<td>Low levels</td>
<td>Low levels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithalamus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thalamus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypothalamus/posterior</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tuberculum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tectum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hindbrain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spinal cord</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>Bulfone et al. (2000)</td>
<td>This report</td>
<td>This report</td>
<td>Mo et al. (2004)</td>
<td>This report</td>
</tr>
</tbody>
</table>
2. Experimental procedures

2.1. Sequences analyses

mRNA and protein sequences were obtained from: *H. sapiens* Barhl1 (acc. Nos NM020064 and NP_064448), *H. sapiens* Barhl2 (acc. Nos NM_020063 and NP_064447), *M. musculus* Barhl1 (acc. Nos NM_019446 and NP_062319, also found as mBH2), *R. norvegicus* Barhl2 (acc. Nos NM_022956 and NP_075245), *X. laevis* Barhl1 (acc. Nos AF283692 and AAAG1451, found as XBH2), *X. laevis* Barhl2 (acc. Nos AF283691 and AAAG1450, found as XBH1), *X. tropicalis* Barhl1 (acc. No. ENSTETG00000002657), *X. tropicalis* Barhl2 (acc. No. ENSTETG000000023986), *Danio rerio* Barhl1.1 (acc. Nos AA592236 and AY596176, found as BarH1), *Danio rerio* Barhl1.2 (acc. Nos AAU10059 and AY596187, found as BarH2), *Danio rerio* Barhl2 (acc. Nos NP_991303 and NM_205740, found as B-H2), *O. latipes* Bar (acc. Nos AJ426046 and CA19778). Alignments were made using MegAlign software (DNASTAR, Inc.). Distance trees were drawn using the Maximum-likelihood and the Neighbour-joining program from the Phylip package, using nucleotide sequences encoding for the extended homeodomain region of Barhl depicted in Fig. 1A.

2.2. Cloning of zebrafish barhl

ESTs containing part of the coding sequence of zebrafish barhl1.2 (acc. Nos BI325578, BM023761, AI957963, BI840866) were identified through BLAST search using the nucleotide sequence encoding for the extended homeodomain region of Barhl depicted in Fig. 1A. Using the consensus sequence of these ESTs as a query, we obtained the full-length coding sequence of barhl2 in the Sanger Database (acc. No. ENSDARG0000004760). An EST containing part of the coding sequence of zebrafish barhl1.1 (acc. No. CA496570) was identified through BLAST search using nt. 700–1200 of *Hs* BARHL1 (acc. No. NP_991303 and NM_205740, found as B-H2), *O. latipes* Bar (acc. Nos AAU10059 and AY596187). Alignments were made using MegAlign software (DNASTAR, Inc.). Distance trees were drawn using the Maximum-likelihood and the Neighbour-joining program from the Phylip package, using nucleotide sequences encoding for the extended homeodomain region of Barhl depicted in Fig. 1A.

2.3. Zebrafish strains

*Danio rerio* of wild type and transgenic lines were from the ICBM Zebrafish Facility. The *tg(ngn-1-GFP)* line was donated by P. Blader (Blader et al., 2004).

2.4. Whole mount in situ hybridisation and immunohistochemistry

Gene and protein expression was detected using standard procedures (Jowett, 1999). Anti-sense mRNA probes used for whole mount in situ hybridisation were generated from plasmids containing either part (5'UTR and first exon of barhl1.2; 5'UTR and first two exons of barhl2) or the entire (barhl1.1) cDNA coding sequence. For antibody labelling, rabbit anti-GFP (Chemokine) was used at 1:1000 dilution.

2.5. Vibrotome sections

Embryos treated by whole mount in situ hybridisation were embedded in 4% agarose in PBS, oriented in frontal views with both eyes placed symmetrically, and mounted on a motorised vibrotome (Vibroslice MA752, Lafayette Instrument, Co.). 50 µm sections were placed on glass slides for digital photography.

Acknowledgements

We would like to thank Bianca Habermann (Max Planck Institute of Molecular Cell Biology and Genetics, Dresden) for assistance in bioinformatics, Maria Eugenia Cabreros for critical reading of the manuscript, Sara Mercurio for helpful suggestions in cloning of barhl1.2 Aldo Villalón and Maria Eugenia Cabreros for helping in vibrotome sections, Dina Silva for technical assistance with fish care, and to all members of our group for discussions. This work was supported by FONDECYT 1020902 and 7020902, Fundación Andes C-13760, ICM P01-007-F, and Wellcome Trust. A.C. was funded by a CONICYT PhD Scholarship. M.L.C. is a Wellcome Trust International Research Development Award Fellow.

References


