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The homeobox leucine zipper gene *Homez* plays a role in *Xenopus laevis* neurogenesis

Rym Ghimouz, Isabelle Bar, Julie Hanotel, Beatica Minela, Marc Keruzore, Aurore Thelie, Eric J. Bellefroid *

Laboratoire de Génétique du Développement, Université Libre de Bruxelles, Institut de Biologie et de Médecine Moléculaires (IBMM), Rue des Profs. Jeener et Brachet 12, B-6041 Gosselies, Belgium

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ABSTRACT

The *Homez* gene encodes a protein with three atypical homeodomains and two leucine zipper motifs of unknown function. Here we show that during neurula stages, *Xenopus Homez* is broadly expressed throughout the neural plate, the strongest expression being detected in the domains where primary neurons arise. At later stages, *Homez* is maintained throughout the central nervous system in differentiating progenitors. In accordance with this expression, *Homez* is positively regulated by neural inducers and by Ngnr1 and negatively by Notch signaling. Interference with *Homez* function in embryos by injection of an antisense morpholino oligonucleotide results in the specific disruption of the expression of late neuronal markers, without affecting the expression of earlier neuronal and early neurectodermal markers. Consistent with this finding, *Homez* inhibition also interferes with the expression of late neuronal markers in *Ngnr1* overexpressing animal cap explants and in Notch inhibited embryos. In gain of function experiments, *Homez* inhibits the expression of late neuronal markers but has no effect on earlier ones. These data suggest a role for *Homez* in neuronal development downstream of proneural/neurogenic genes.

1. Introduction

Following neural induction, neurectodermal cells gradually differentiate into neurons and glia. In amphibian, a number of neurons are born shortly after gastrulation in the developing neural plate that are responsible for the early movements and responses of the larvae. Those neurons, termed primary neurons, are organized in three longitudinal domains on each side of the midline. As the neural plate closes to form the neural tube, the medial, intermediate and lateral domains of primary neurons will give rise to motor-, inter-, and Rohon-Beard sensory neurons, respectively. Due to their accessibility and the amenability of the frog embryo to manipulations, these primary neurons constitute an excellent system to elucidate the cascade of molecular events that control vertebrate neurogenesis [1].

Studies in the recent years have shown that the commitment of progenitor cells to a neuronal fate is driven by basic-helix-loop-helix proneural factors. Neurogenin related 1 (Ngnr1) is one of the earliest expressed proneural factors that define the three prospective patches of primary neurons in the neural plate [2]. Ngnr1 is sufficient to activate in nonneural ectoderm a network of downstream differentiation factors such as *Myt1* [3]. Ngnr1 also activates lateral inhibition mediated by the Delta-Notch pathway, which restrict the number of cells within the domains of primary neurogenesis that are allowed to differentiate. The precise role of most of the Ngnr1 downstream effectors in neurogenesis remains poorly defined [4].

Homez encodes an unusual nuclear protein with three atypical homeodomains and two leucine zipper motifs conserved in vertebrate genome sequences. *Homez* is broadly expressed in adult tissues. During embryogenesis, *Homez* has a more restricted expression pattern with strong expression in the developing nervous system [5]. It is most closely related to members of the ZHX family of transcription factors containing several zinc fingers and five homeoboxes that have been implicated in several deseases, including nephrotic syndrome and hepatocyte carcinogenesis [6,7]. Despite their clinical importance, the developmental role of these factors remains unknown. Here we show that *Homez* is expressed during primary neurogenesis in *Xenopus*. Using loss and gain of function approaches, we have investigated its *in vivo* regulation and function.

2. Materials and methods

2.1. Plasmid construction and design of antisense morpholino oligonucleotide

A *Xenopus laevis Homez* cDNA clone in the pCMV-Sport6 vector was obtained from RZPD (Acc. BC071005, Unigene Xl. 47067). For *in situ* hybridization probe synthesis, the plasmid was cutted by

* Corresponding author. Fax: +32 2 650 97 33.
E-mail address: ebellefr@ulb.ac.be (E.J. Bellefroid).

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Sal1 and transcribed with T7.For RNA injection, the ORF region was amplified using the 5′ primer AGAGGCCTACCCCTACCACCAAC and 3′ primer ACGAGCTCACAAACGAGCATTTAGTCCTG. The resulting fragment was digested with StuI and XbaI and inserted at the StuI and XbaI site of the polylinker of the pCS2Flag vector. The *Homez* antisense morpholino oligonucleotide 5′ GGGTCATTCTCAGCTTATGAAGGTA (initiation codon is underlined) was designed against the translation initiation. A standard control morpholino oligo (SC MO) from GeneTools was used as a control.

2.2. Microinjection, animal cap assay, and whole-mount in situ hybridization

Xenopus embryos were obtained from adult frogs by hormone induced egg-laying and *in vitro* fertilization using standard methods [8] and staged according to Nieuwkoop and Faber [9]. Synthetic mRNAs were made using Sp6 mMESSAGE mMACHINE. (Ambion). The template for generating *Homez* mRNA was obtained by linearizing the pCS2Flag-*Homez* with NotI. Templates described

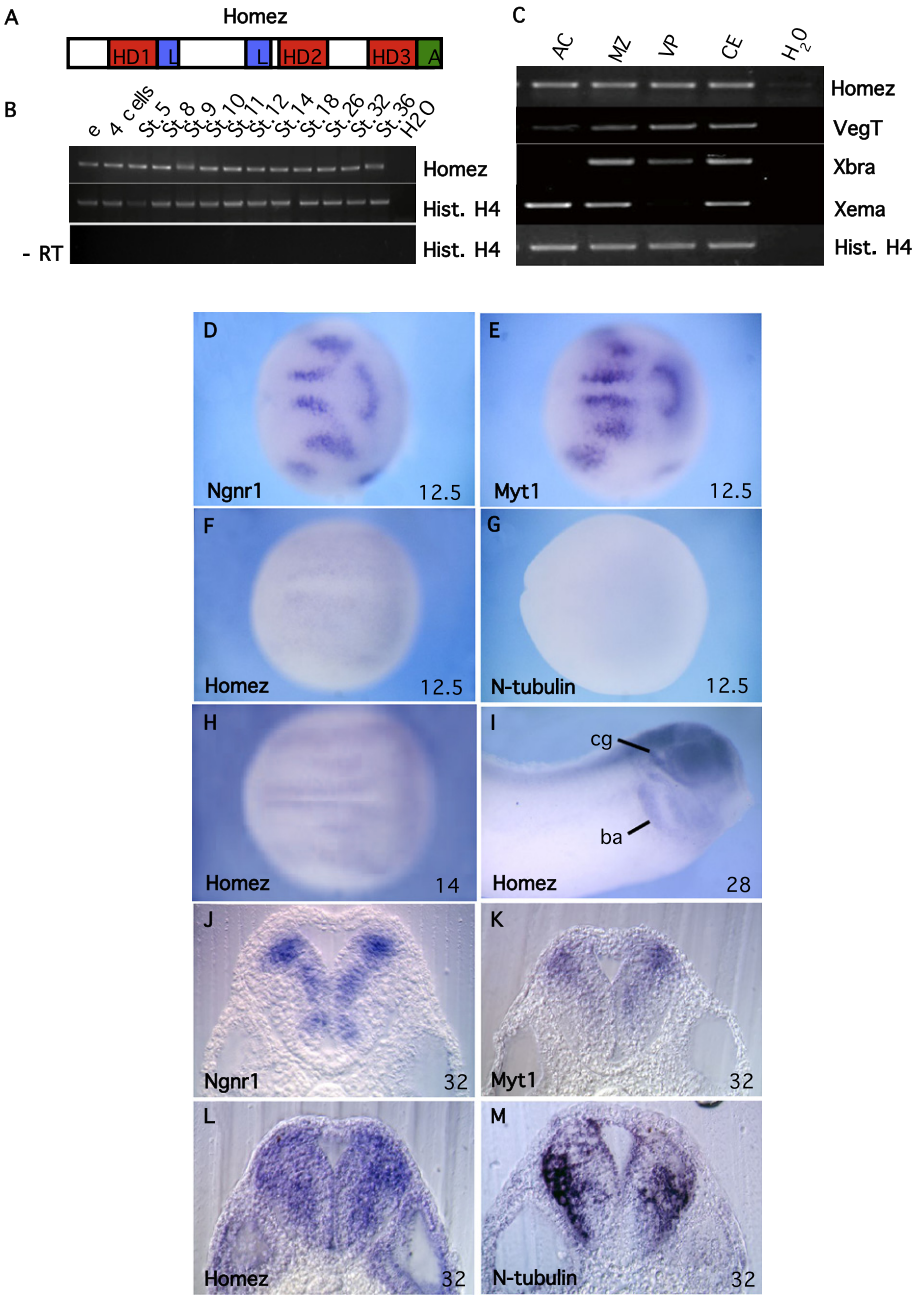

Fig. 1. Homez structure and gene expression during embryogenesis. (A) Shematic representation of the Homez protein. HD, homeodomain; L, leucine repeats, A, acidic domain. (B) Temporal expression of *Homez* by RT-PCR. RNA was extracted from embryos at the indicated stages. (C) RT-PCR analysis of *Homez* expression in dissected explants of stage 10.5 embryos. The endodermal *VegT*, mesodermal *Xbra* and ectodermal *Xema* markers were used as dissection controls. In B and C, *Histone H4* was used as a loading control. -RT, control RT-PCR without reverse transcriptase. (D-I) Spatial expression of *Homez* compared to that of *Ngnr1*, *Myt1* and *N-tubulin* analyzed by whole-mount *in situ* hybridization. Nieuwkoop-Faber stages are indicated. (D–H) Dorsal views. (I) Lateral view. Stripes of *Homez* expression in the domains of primary neurogenesis in the posterior neural plate are indicated (l, lateral; i intermediate; m, medial). Note that Homez is activated in the domains of primary neurogenesis at E12.5 when N-tubulin is not yet detectable. (J–M) *Homez* expression compared to that of *Ngnr1*, *Myt1* and *N-tubulin* in the neural tube of stage 32 embryos. Sections at the level of the otic vesicle are shown. Note that *XHomez*$^{+}$ cells are detected more medially than *N-tubulin* in the marginal zone. Abbreviations: AC, animal caps; ba, branchial arches; cg, cranial ganglia; e, egg; CE, control embryo; MZ, marginal zone; VP, vegetal pole.