Biochemical and Biophysical Research Communications 415 (2011) 11–16

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# 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*

ARTICLE INFO

*Article history:*
Received 26 September 2011
Available online 6 October 2011

*Keywords:*
*Homez*
Primary neurons
*Xenopus laevis*
Transcription factor
Primary neurogenesis

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).

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.09.138

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

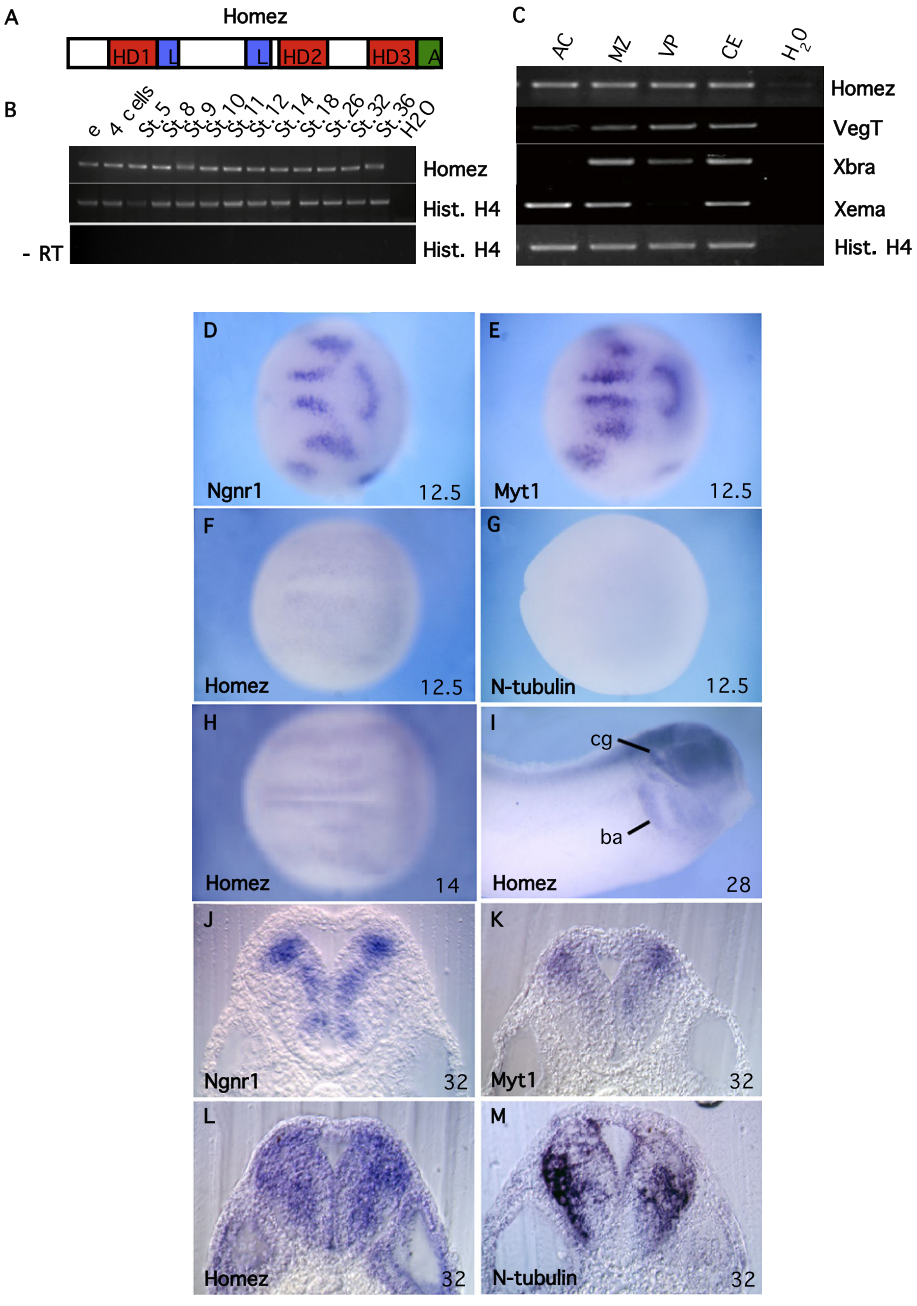


**Fig. 1.** Homez structure and gene expression during embryogenesis. (A) Shematic representation of the Homez protein. HD, homeodomain; L, leucine repeats, A, acidic domain. (B) Temporal expression of *Homez* by RT-PCR. RNA was extracted from embryos at the indicated stages. (C) RT-PCR analysis of *Homez* 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.

previously include: Ngnr1 [2], noggin, tBr, NotchICD and Su(H)DBM [10]. For *in situ* analysis, embryos were injected in one cell of two-cell stage embryos, and fixed at neurula or tadpole stage. For animal cap assays, synthesized mRNA was microinjected into the animal region of each blastomere of 4-cell-stage embryos. Animal caps were dissected at blastula stage (st.9) and cultured until neurula stage (st.15) for RT-PCR.

Whole-mount *in situ* hybridization analysis was performed as described using digoxigenin-labeled antisense probes [8]. Plasmids used for probe synthesis were *Ep. Keratin* [11], *N-tubulin* [1], *Myt1* [3] *Ngnr1* [2], *Sox3* [12], *CRMP4* [13].

### 2.3. Immunolocalization

Embryos were fixed in MEMFA. Whole-mount immunolocalization was performed using a primary mouse monoclonal anti-Flag antibody (Sigma F-3165) at 1/1000 and an anti-mouse peroxidase-conjugated secondary antibody (Sigma A9044) at 1/500. Staining was performed with DAB/$H_2O_2$ (Sigma D4293).

### 2.4. Semi-quantitative RT-PCR

RNA extraction (Quiagen) and reverse transcriptase-PCR analysis were performed as described by the manufacturers. The following primers were used: *Homez* (forward 5′AGAGGGTGTGGCTT CAGAGA and reverse 5′CTCTTCCTCAGACGGAATGC); *Ep. Keratin* (forward 5′-CTCACTTTGCCAGCACTCTG-3′ and reverse 5′-GTGATAG CAATGGCCTTCGT-3′); *Sox2* (forward 5′-GCACAACTCGGAGATCAG-CAAG-3′ and reverse 5′-CGTTGATGTAAGTCTGCGAGCTGC-3′); *Sox3* (forward 5′-CAGGTATGACATGAGCGGCC-3′ and reverse 5′-TATCTC GCAGGTCTCCCAGG-3′);*N-tubulin* (forward 5′-GTGGCTGTCAA-CATGGTGCC-3′ and reverse 5′-GTGGAATGTCACAAACTGCG-3′); *CRMP4* (forward 5′-GAAAGCCATTGGAAAGGACA-3′ and reverse 5′-ATCCCACGGCTATTCTTCCT-3′); *Myt1* (forward 5′-GGAAG GAAG AAAACCCGAG-3′ and reverse 5′-TAGGAGGCTGCTGTCTTGGT-3′); *BTBD6* (forward 5′-CACTAGACCCAGGCTGCCTT-3′ and reverse 5′-CAGTGTGCTGGAGTTGGA-3′); *Muscle actin* (forward 5′-GCTGACA-GAATGCAGAAG-3′ and reverse 5′-TTGCTTGGAGGAGTGTGT-3′); *Histone H4* (forward 5′-CGGGATAACATTCAGGGTATCACT-3′ and reverse 5′-ATCCATGGCGGTAACTGTCTTCCT-3′).

## 3. Results and discussion

### 3.1. Identification and developmental expression of Homez

Through BLAST searches of the *X. laevis* EST databases, we identified an EST encoding a 514 amino acid protein with 35–40% overall identity to human and mouse *Homez* proteins (Genbank Accession No. BC071005, Unigene MGC80043) (Supplementary Fig. 1A). As the human and mouse proteins, it contains three homeodomains, two leucine repeats and a C-terminal acidic domain (Fig. 1A). Homology between the identified *Xenopus* and human and mouse Homez proteins is mainly limited to the first and second homeoboxes and the acidic domain (72–72% in homeodomain 1, 83–85% in homeodomain 2 and 31–25% in the acidic domain). In the closely related *Xenopus tropicalis* species, the corresponding gene (Genbak Accession No. NM001142664.1) is encoded by two exons, as in the human and mouse genomes. To study its subcellular localization, we constructed a plasmid encoding a Flag-tagged version of the protein. Flag tagged protein localization was studied in the ectoderm of injected gastrula embryos. As mouse *Homez*, the *Xenopus* protein was detected in the nucleus, further supporting its identification as *X. laevis Homez* (Supplementary Fig. 1B).

The expression pattern of *Homez* during embryogenesis was investigated by RT-PCR analysis with RNA isolated from various developmental stages of development and whole-mount *in situ* hybridization. Using RT-PCR, *Homez* was detected maternally and maintained throughout the developmental stages studied (Fig. 1B). In dissected early gastrula stage embryos, *Homez* transcripts were detected at similar levels in animal, equatorial and vegetal pole regions (Fig. 1C). By whole-mount *in situ* hybridization, *Homez* transcripts were first detected at late gastrula early neurula stage (st. 12.25–12.5). From that stage, *Homez* was found in the developing neural plate, the strongest signals being detected within the domains of primary neurogenesis. Expression of *Homez* in the primary neurons follows that of *Ngnr1* and *Myt1* already detectable at 10.5 and 11.5, respectively, and precedes that of *N-tubulin*, a terminal pan-neuronal marker [1,3] (Fig. 1D–H). At tailbud stages, *XHomez* was detectable in the brain, spinal cord, cranial nerves, eyes and branchial arches. (Fig. 1I). The zebrafish *Homez* ortholog has a similar embryonic expression profile (Zfin ZDB-IMAGE-0140107).

As cells differentiate, they move away peripherally from the ventricular zone [14]. During this process, *Ngnr1* that defines the proliferating cells of the ventricular zone is switched off while early differentiating postmitotic markers such as *MyT1* encoding a zinc finger transcription factor induced by Ngnr1, and *N-tubulin*, are sequentially turned on. *Myt1* is found in the region immediately adjacent to the ventricular zone (the subventricular zone) while *N-tubulin* is found exclusively in the outermost differentiated layer of cells of the marginal zone. [2,3] (Fig. 1J–L). In transverse sections through the hindbrain at the level of the otic vesicle, *XHo-*

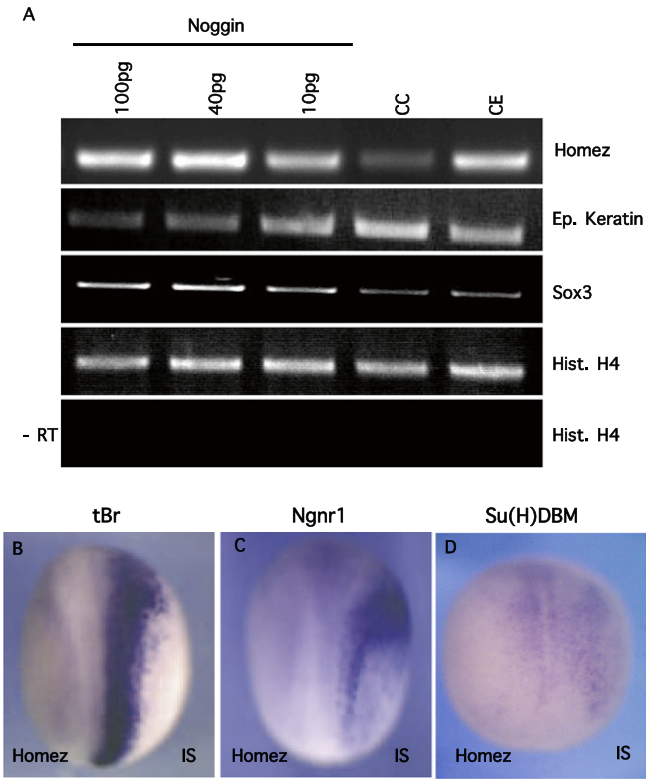


**Fig. 2.** *Homez* is regulated positively by attenuation of Bmps and Ngnr1 and negatively by Notch. (A) RT-PCR analysis of *Homez* expression in animal cap explants isolated from embryos injected with increasing doses of *noggin* mRNA or from uninjected control animal caps (CC). CE, control embryos. *Ep. Keratin* and the pan-neural *Sox3* gene were used as controls for neural induction. *Histone H4* was used as a loading control. -RT, control RT-PCR without reverse transcriptase. (B–D) *In situ* analysis of *Homez* expression in neurula embryos (dorsal views) injected unilaterally with the indicated mRNA and *LacZ* mRNA. Injected sides (IS) are indicated.

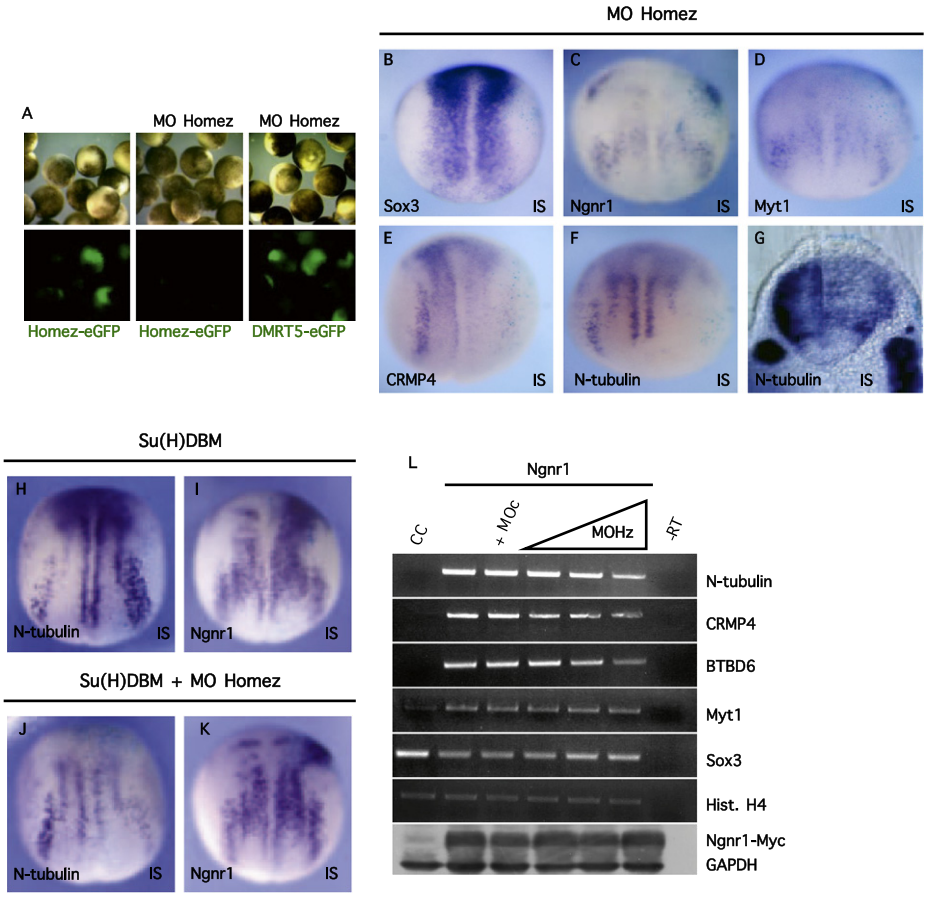


**Fig. 3.** Knockdown of Homez reduces the expression of late but not early neuronal markers. (A) *In vivo* translation of *Homez-eGFP* is specifically inhibited by the Homez MO. Embryos were injected with 500 pg of *Homez-eGFP* or, as a control, a *Dmrt5-eGFP* construct, alone or in combination with 10 ng of the Homez MO, as indicated. (B–F) Expression of the indicated neuronal genes in neurula embryos (dorsal views) injected unilaterally with 10 ng of the Homez MO and *LacZ* mRNA as a tracer. (G) Section of the neural tube at the level of the hindbrain of a MO Homez injected tadpole embryo stained with *N-tubulin*. (H–K) Dorsal views of embryos coinjected with 500 pg *Su(H)DBM* mRNA, with or without 10 ng of the Homez MO and probed as indicated. Injected sides (IS) are indicated. (L) RT-PCR analysis of the expression of the terminal neuronal markers *CRMP4*, *N-tubulin* and *BTBD6*, the early postmitotic marker *Myt1* and of the pan-neural markers *Sox3* in animal caps derived from embryos injected with 50 pg *Ngnr1* mRNA/blastomere alone or together with 2, 5, 5 or 10 ng of Homez MO or 10 ng of a standard control MO. Histone H4, internal positive control. -RT, control RT-PCR without reverse transcriptase. A control western blot shows that similar levels of Myc-Ngnr1 have been produced under each condition. GAPH, Glyceraldehyde 3-phosphate dehydrogenase.

*mez* was found widely transcribed in the marginal zone, the strongest signals being detected less laterally than *N-tubulin* (Fig. 1L and M). Thus, as during primary neurogenesis, *Homez* follows the expression of *Ngnr1* and *Myt1* and anticipates that of *N-tubulin* during secondary neurogenesis, suggesting a role in late steps of neuronal differentiation.

### 3.2. Homez is regulated by Bmps, proneural and neurogenic factors

*Homez* expression within the neural plate suggests that it could be regulated by neural inducer signals. Animal cap assays showed that the neural inducer noggin induces in a dose dependent manner *Homez* expression (Fig. 2A). *Homez* was also induced in embryos overexpressing tBR, a truncated form of the Bmp2/4 receptor (100%, $n = 8$) (Fig. 2B). Thus, as expected based on its expression within the entire neural plate, *Homez* is positively regulated by attenuation of Bmp signaling.

As *Homez* strongest signals are detected in the neural plate embryo where neurogenesis occurs, we next tested whether it expression is regulated by proneural and neurogenic genes. Misexpression of *Ngnr1* induced ectopic expression of *Homez* (100%, $n = 18$) (Fig. 2C). Inhibition of the Notch pathway by misexpression of a dominant-negative form of the DNA-binding protein SuH, Su(H)DBM [1,10] increased the density of staining in the domains of primary neurogenesis (100%, $n = 8$) (Fig. 2D). Thus, consistent with its widespread expression within the neural plate, *Homez* is positively regulated by attenuation of Bmps. Like that of other neuronal markers, it is also positively regulated by *Ngnr1* and negatively by lateral inhibition, further suggesting a role in neuronal differentiation.

### 3.3. Homez knock-down inhibits late neuronal markers

To determine whether Homez is required for *Xenopus* primary neurogenesis, a morpholino antisense oligonucleotide was designated to interfere with *Homez* translation. Injection of the Homez MO specifically blocked the translation *in vivo* of its target mRNA (Fig. 3A). To assess the effects of Homez knockdown, embryos injected unilaterally at the two cell-stage with 10 ng of Homez MO together with b-galactosidase mRNA were analyzed by *in situ* hybridization in neurula stage embryos for the expression of the early neurectodermal marker *Sox3*, *Ngnr1*, *Myt1* and the terminal neuronal differentiation markers *N-tubulin* and *CRMP-4*. Expression of *Sox3* and *Ngnr1* was mainly not affected by the depletion of Homez in neurula embryos (*Sox3*, 91% unaffected, $n = 23$; *Ngnr1*, 82% unaffected, $n = 27$) (Fig. 3B and C). Expression of *Myt1* was mainly unchanged or only weakly affected in Homez MO injected embryos (79%, $n = 24$) (Fig. 3D). In contrast, *N-tubulin* and *CRMP-*

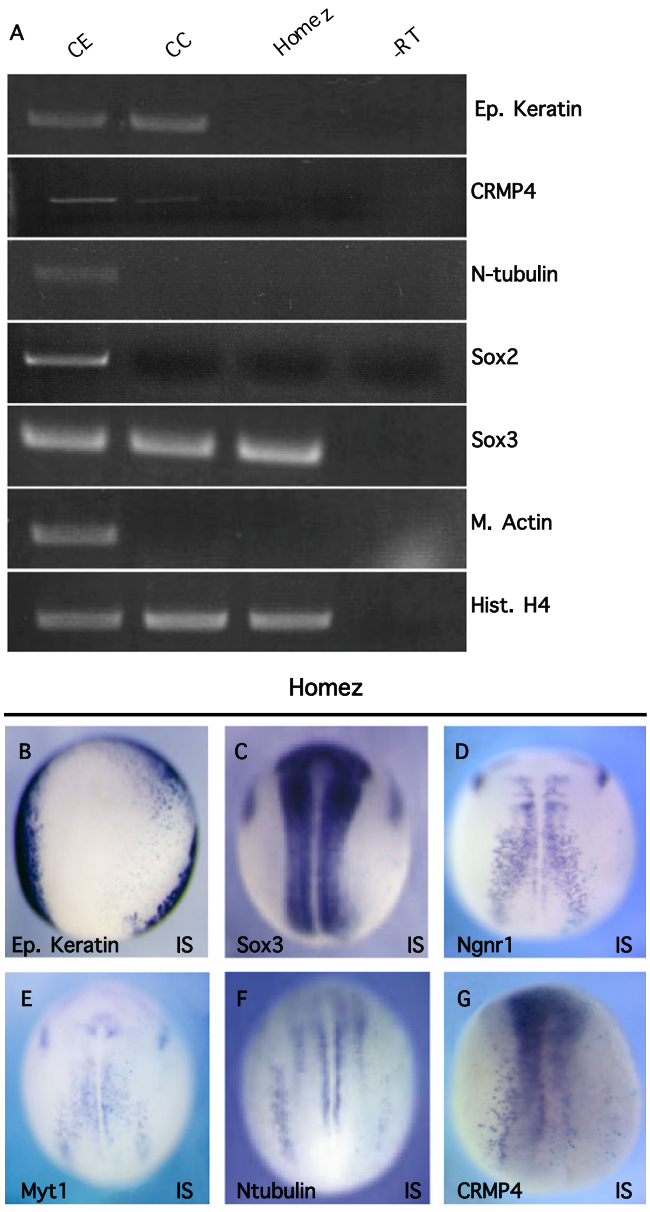


**Fig. 4.** Effect of *Homez* overexpression on the neuronal differentiation. (A) RT-PCR analysis of the expression of the indicated genes in animal caps from embryos overexpressing Homez (500 pg of *Homez* mRNA/blastomere) or from uninjected control embryos. Note that Homez blocks *Ep. Keratin* expression but is not sufficient to induce neural or neuronal markers in animal cap explants. CC, control caps; CE, control embryo. -RT, reverse transcriptase minus as a negative control. (B–G) Dorsal views of neurula embryos injected with 500 pg of *Homez* mRNA analyzed by *in situ* hybridization for the indicated markers. Injected sides (IS) are indicated. Note that overxexpression of *Homez* interferes with *N-tubulin* and *CRMP4* expression but does not affect *Ngnr1* and *Myt1*.

*4* expression was in most injected embryos strongly reduced (*N-tubulin*, 72% strongly inhibited, $n = 22$; *CRMP4*, 52%, $n = 25$) (Fig. 3E and F). A strong inhibition of *N-tubulin* expression was also observed in the neural tube of MO Homez-injected embryos at tadpole stages (Fig. 3G). Injection at the same dose of a standard MO; used as a negative control, did not alter CRMP4 or N-tubulin expression (data not shown). Injection of the *Homez* MO also efficiently inhibited N-tubulin and CRMP-4 expression induced by Su(H)DBM (N-tubulin, 63% inhibited, $n = 32$) (Fig. 3H and J); (CRMP4, 50% inhibited, $n = 18$) (data not shown). In contrast, Ngnr1 induction was not affected (73% unaffected, $n = 11$) (Fig. 3I and K).

To further assess the role of Homez in neuronal differentiation, we analyzed the effects of the depletion of Homez on neurogenesis induced by Ngnr1 in animal cap explants. We used increasing amounts of the Homez MO in the presence of a constant low level of Ngnr1 (50 pg mRNA) for induction of neuronal differentiation. As shown in Fig. 3L, coinjection of the MO Homez decreased the up-regulation of *N-tubulin*, *CRMP-4* and *BTBD6*, another late neuronal marker [15]. In contrast, the induction of *Myt1*, appeared not affected and *Sox3* appeared slightly upregulated. Together, these data indicate that Homez is necessary for the late phases of neuronal differentiation.

### 3.4. Overexpression of Homez inhibits late neuronal markers

To further approach Homez function, we carried out gain of function experiments using the Flag-tagged *Homez* construct. First, we overexpressed *Homez* in animal cap explants and carried out RT-PCR analysis for the expression of epidermal (*Ep. Keratin*), neural (*Sox2* and *Sox3*), and neuronal markers (*N-tubulin* and *CRMP4*). While *Ep. Keratin* expression was reduced in the explants derived from *Homez* injected embryos, none of the neural and neuronal marker tested was upregulated (Fig. 4A). We also analyzed the effects of *Homez* overexpression in embryos. Embryos were injected unilaterally at the two cell-stage with *Homez* mRNA and analyzed at neurula stage by *in situ* hybridization. As in caps, *Ep. Keratin* was inhibited by *Homez* (66%, $n = 24$) and *Sox3* was not affected (85% unaffected, $n = 35$) (Fig. 4A and B). *Ngnr1* and Myt1 were also mainly not affected by *Homez* overexpression (*Ngnr1*, 80% unaffected; *MyT1*, 75% unaffected, $n = 30$) (Fig. 4C and D). In contrast, *N-tubulin* and *CRMP-4* were strongly downregulated (*N-tubulin*, 57% inhibited, $n = 30$; *CRMP-4*, 66% inhibited, $n = 12$) (Fig. 4E and F). Thus, *Homez* is not sufficient to induce neuronal development. The maintenance of a high level of its expression appears incompatible with terminal neuronal differentiation.

Taken together, the temporal and spatial expression pattern of *Homez*, it regulation by *Ngnr1* and Notch signaling and the results of our gain and loss of function experiments support a late role for *Homez* during neurogenesis. The absence of any sequence similarity to *Homez* genes in invertebrates and its presence in all studied vertebrate species strongly supports the hypothesis that *Homez* originated early in vertebrate evolution. Whether *Homez* in other vertebrates also plays a role during neuronal maturation and its mode of action remain to be investigated.

## Acknowledgments

This research was funded by Grants from the FNRS, the FMRE, the ARC, IUAP and CIBLES Belgian Programs. A.T. is a postdoctoral FNRS fellow. R.G., J.H. and M.K. are research fellows from the Belgian FRIA.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2011.09.138.

## References

[1] Chitnis D. Henrique, J. Lewis, D. Ish-Horowicz, C. Kintner, Primary neurogenesis in *Xenopus* embryos regulated by a homologue of the *Drosophila* neurogenic gene Delta, Nature 375 (1995) 761–766.
[2] Q. Ma, C. Kintner, D.J. Anderson, Identification of neurogenin a vertebrate neuronal differentiation gene, Cell 87 (1996) 43–52.
[3] E.J. Bellefroid, C. Bourguignon, T. Hollemann, O. Ma, D.J. Anderson, C. Kintner, T. Pieler, X-Myt1a *Xenopus* C2HC-type zinc finger protein with a regulatory function in neuronal differentiation, Cell 87 (1996) 1191–1202.
[4] K.A. Henningfeld, M. Locker, M. Perron, *Xenopus* primary neurogenesis and retinogenesis, Funct. Dev. Emb. 1 (2007) 26–36.

[5] D. Bayarsaihan, B. Enkmandakh, A. Makeyev, J. Greally, J.F. Leckman, F. Ruddle, *Homez* a homeobox leucine zipper gene specific to the vertebrate lineage, Proc. Natl. Acad. Sci. 100 (2003) 10358–10363.
[6] G. Liu, L.C. Clement, Y.S. Kanwar, C. Avila-Casado, S. Chugh, ZHX proteins regulate podocyte gene expression during the development of nephrotic syndrome, J. Biol. Chem. 281 (2006) 39681–39682.
[7] K. Yamada, H. Ogata-Kawata, K. Matsuura, N. Kagawa, K. Takagi, K. Asano, A. Haneishi, K. Miyamoto, ZHX2 and ZHX3 repress cancer markers in normal hepatocytes, Front. Biosci. 14 (2009) 3724–3732.
[8] H.L. Sive, R.M. Grainger, R.M. Harland, Early Development of *Xenopus laevis*. A Laboratory Manual, Cold Spring Harbor, New-York, 2000.
[9] P.D. Nieuwkoop, J. Faber, Normal table of *Xenopus laevis* (Daudin), North Holland, Amsterdam, 1967.
[10] D.A. Wettstein, D.L. Turner, C. Kintner, The *Xenopus* homolog of *Drosophila* suppressor of hairless mediates notch signaling during primary neurogenesis, Development 124 (1997) 693–702.
[11] E. Jonas, T.D. Sargent, I.B. Dawid, Epidermal keratin gene expressed in embryos of *Xenopus laevis*, Proc. Natl. Acad. Sci. USA 82 (1985) 5413–5417.
[12] R. Penzel, R. Oshwald, Y. Chen, L. Tackle, H. Grunz, Characterization and early embryonic expression of a neural specific transcription factor xSox3 in *Xenopus laevis*, Int. J. Dev. Biol. 5 (1996) 667–677.
[13] J. Souopgui, T.J. Klish, T. Pieler, K.A. Henningfeld, Expression and regulation of *Xenopus* CRMP-4 in the developing nervous system, Int. J. Dev. Biol. 51 (2007) 339–343.
[14] D. Parlier, A. Ariza, F. Christulia, F. Genco, J. Vanhomwegen, S. Kricha, J. Souopgui, E.J. Bellefroid, *Xenopus* zinc finger transcription factor IA1 (Insm1) expression marks anteroventral noradrenergic neuron progenitors in *Xenopus* embryos, Dev. Dyn. 237 (2008) 2147–2157.
[15] F.J. Bury, V. Moers, J. Yan, J. Souopgui, X.J. Quan, N. De Geest, S. Kricha, B.A. Hassan, E.J. Bellefroid, *Xenopus* BTBD6 and its *Drosophila* homologue lute are required for neuronal development, Dev. Dyn. 237 (2008) 3352–3360.