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SHORT COMMUNICATION

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Expression pattern of a basic helix-loop-helix transcription factor *Xhairy2b* during *Xenopus laevis* development

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Abstract We report on the temporal and spatial expression pattern of the *Xenopus laevis hairy2b* (*Xhairy2b*) transcription factor. *Xhairy2b* transcripts are present maternally, and expressed throughout the prospective ectoderm prior to the gastrula stage. During gastrulation, *Xhairy2b* expression is restricted to the deep layer of the Spemann organizer and three distinct regions in the prospective neuroectoderm, neural plate border, notoplate and anterior neural plate. At later stages, *Xhairy2b* expression is localized to prechordal plate, presomitic mesoderm, neural tube, neural crest derivatives and several tissue territories of the central nervous system. The analyses of *Xhairy2b* and several other hairy-related genes suggest potential roles for *Xhairy2b* in the formation of boundaries in neural tissue territories.

Keywords *Xenopus* · *Xhairy2b* · bHLH · Neural crest · Central nervous system

Hairy-related genes encode basic helix-loop-helix (bHLH) transcriptional repressors and are known to play important roles in developmental processes, such as neurogenesis and myogenesis. In the course of screening for novel transcription factors in *Xenopus*, we isolated a new member of the hairy-related gene family, *Xhairy2b*. The amino acid sequence and the expression pattern of *Xhairy2b* are most similar to that of *Xhairy2a* (92.8% amino acid identity; Turner and Weintraub 1994; Davis et al. 2001). However the nucleotide sequence of *Xhairy2b* is only 70% identical with *Xhairy2a* within 5′ and 3′ UTR. From these results and due to the pseudotetraploid nature of the *Xenopus laevis* genome (Graf and Kobel 1991), they are likely to represent alternative copies of the same gene.

To better understand the roles of *Xhairy2* genes in early development, the temporal and spatial expression pattern were analyzed by both northern blotting and whole-mount in situ hybridization. Northern blot analysis reveals that relatively low levels of *Xhairy2b* transcripts are present maternally (Fig. 1 lane 1). A slight increase in *Xhairy2b* expression is observed in early to mid gastrula stages (stage 10–11; Fig. 1 lanes 5 and 6), and the expression becomes stronger from late gastrula stage onward (stage 12; Fig. 1 lanes 7–11).

Whole-mount in situ hybridization demonstrates that *Xhairy2b* is expressed both in mesoderm and in neuroectoderm. The mesodermal expression of *Xhairy2b* is restricted to the prechordal plate mesoderm, while the ectodermal expression of *Xhairy2b* is restricted to three

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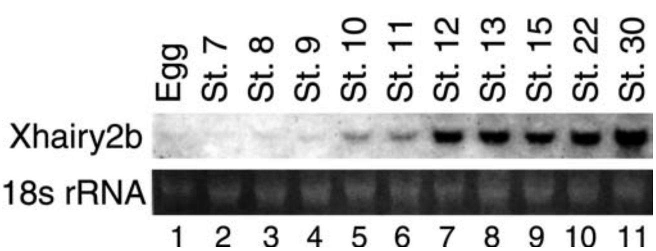
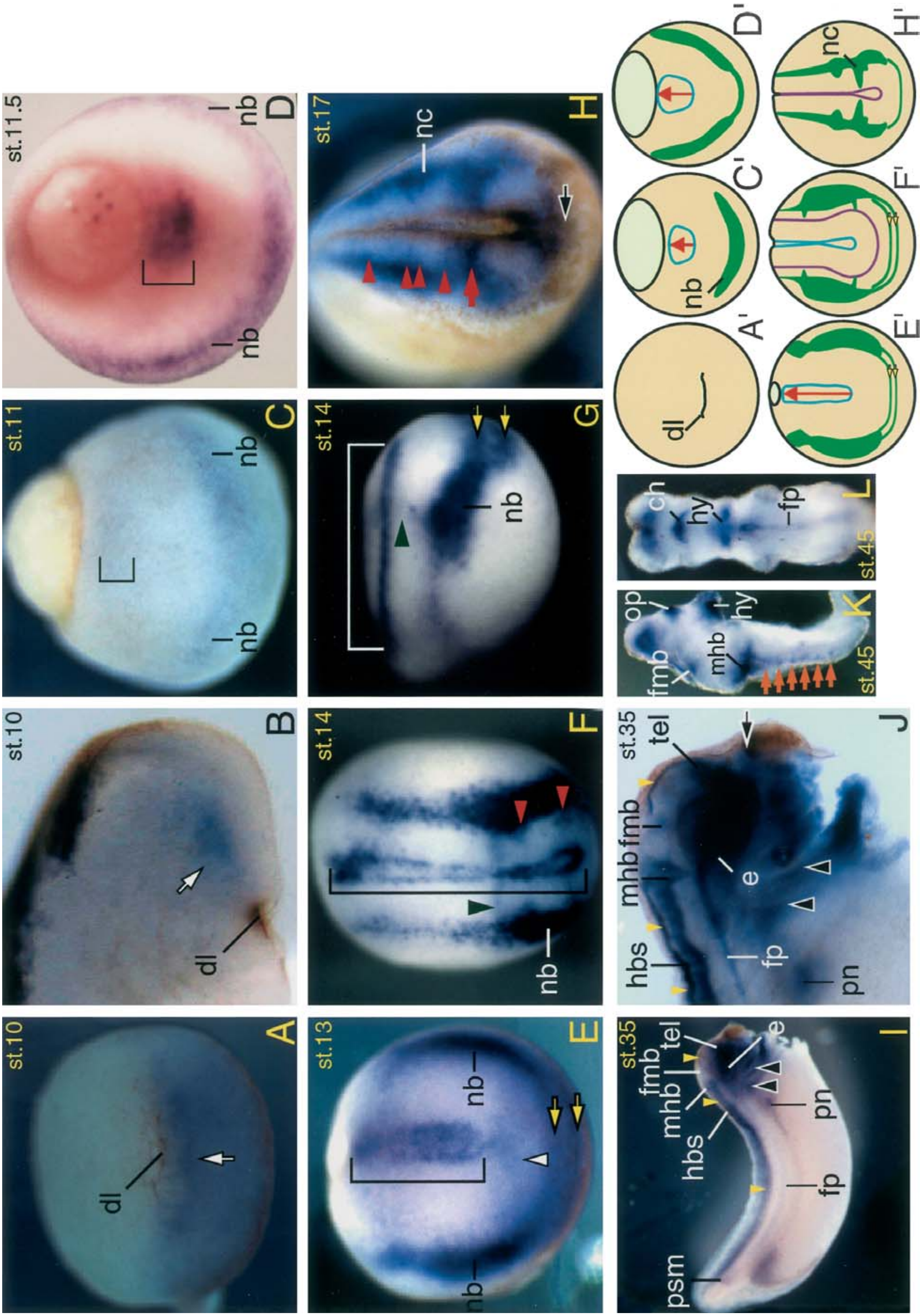


Fig. 1 Temporal expression pattern of *Xhairy2b* during *Xenopus* early development. Northern blot hybridization was performed using total RNA isolated from embryos of the indicated stages. Ethidium bromide-stained 18S rRNA is shown as a loading control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distinct regions, the neural folds, the dorsal midline and the anterior neural plate.

Probably because of its maternal expression, the *Xhairy2b* signal is observed in the entire prospective ectodermal region prior to the gastrula stage (data not shown). This expression disappears during early gastrulation. *Xhairy2b* transcripts are first detected in the deep layer of the dorsal blastopore lip at stage 10 (Fig. 2A, B, white arrow). In mid to late gastrulae (stage 11–13), the signal is observed in the prechordal plate mesoderm (Fig. 2E, white arrowhead; Fig. 3A, B, white bracket), but not in the more posterior chordamesoderm (Fig. 3B, D, cm). This mesodermal expression of *Xhairy2b* is maintained at least until stage 23 (data not shown), suggesting that prechordal plate may remain until early tailbud stages, though it is reported that the prechordal plate cells disperse into mesenchyme at stage 21 (Nieuwkoop and Faber 1994). In addition to these expression domains, there is a stripe of *Xhairy2b* expression in the segmenting presomitic mesoderm (Fig. 2F, G, green arrowhead; I, psm) as reported for *Xhairy2a* (Turner and Weintraub 1994; Davis et al. 2001).

Xhairy2b expression appears in the outer limit of the floor plate (notoplate; Fig. 2C–G, bracket; Fig. 3A, B, black bracket) overlying the presumptive chordamesoderm in mid-gastrula stage embryos (Fig. 3B, D, cm). This expression domain gradually elongates posteriorly during gastrulation (Fig. 2C′–E′, red arrow) and can be seen until the tadpole stages (stage 45; Fig. 2I, J, L, fp).

Shortly before stage 11, ectodermal expression of *Xhairy2b* is detected as a narrow stripe (Fig. 2C, nb; C′, green region). This stripe curves and forms the neural plate border during gastrulation (Fig. 2D–G, nb; D′, E′, green region). The neural plate border contributes to the neural crest, and *Xhairy2b* expression appears to be localized to these neural crest cells (Fig. 2H, nc). These expression patterns of *Xhairy2b* are similar to the patterns of *Xsna*, the earliest prospective neural crest marker. While *Xsna* is first detected at mid-late gastrula stage 11, and is required for the early step of specification and migration of the neural crest (Essex et al. 1993; Mayor et al. 1993), the onset of *Xhairy2b* expression in the neural plate border precedes this expression. Recent molecular analyses reveal that neural induction occurs shortly after the onset of gastrulation, and the earliest neural markers, such as *Xotx2* and *XHR1*, are observed at stages between 10.5 and 11 (Blitz and Cho 1995; Pannese et al. 1995; Shinga et al. 2001). In addition, it is also proposed that an initial step in the induction of the neural crest occurs soon after the onset of gastrulation (reviewed in Aybor and Mayor 2002). Taken together, it is reasonable to regard *Xhairy2b* which is first expressed at the neural border prior to stage 11 as the earliest marker for prospective neural crest.

During neurula stages (stage 13–18), while *Xsna* expression is down-regulated in the anterior neural fold which gives rise to telencephalon (Eagleson and Harris 1990; Mayor et al. 1993), *Xhairy2b* expression is still observed in the entire neural fold (Fig. 2E–H; E′, F′, H′, green region). During this period, an additional stripe of *Xhairy2b* expression appears in the anterior neural fold (Fig. 2E, G, E′, F′, yellow arrow). These expression patterns of *Xhairy2b* appear to represent the telencephalon (Fig. 2I, J), the dorsal border of the cement gland (Fig. 2H, J, 3E, black arrow) and the branchial arches (Fig. 2I, J, black arrowhead) seen in tadpole stage embryos (stage 35–45).

In neurulae (stage 14–18), *Xhairy2b* expression is found as a keyhole-shaped band (Figs. 3C, black arrowhead; 2F′, H′, purple line) and several spike-shaped bands in the superficial layer of neural plate (Fig. 2F, H, red arrowhead). These expression patterns of *Xhairy2b* are similar to the pattern described for *Xsna* and *Xslug*. The *Xsna*- or *Xslug*-expressing cells in the superficial layer are known to contribute to the roof plate of the neural tube (Essex et al. 1993; Mayor et al. 1995). In fact, the expression of *Xhairy2b* is observed in the roof plate at later stages (Fig. 2I, J, yellow arrowhead), indicating that *Xhairy2b*-expressing cells in the superficial layer may take the fate of roof plate. In contrast to the expression of other spike-shaped bands, the most remarkable one, which is localized in the prospective forebrain-midbrain boundary (Figs. 2H, 3F, G red arrow), is detected not only in the superficial layer but also in the deep layer (Fig. 3E, red arrow). In tailbud (stage 35), *Xhairy2b* expression is observed in the forebrain-midbrain, midbrain-hindbrain and hindbrain-spinal cord boundaries (Fig. 2I, J), but is down-regulated in the forebrain-midbrain and hindbrain-spinal cord boundaries at later tadpole stages (stage 45; Fig. 2K and data not shown). In addition, *Xhairy2b* is also expressed in the pronephros, eye (Fig. 2I, J), optic chiasm

Fig. 2 Spatial expression pattern of *Xhairy2b*. Whole-mount in situ hybridization using antisense *Xhairy2b* RNA. Nieuwkoop-Faber stages of embryogenesis are indicated. **A**, **C–F** Dorsal view. Anterior is *down*. **B** Sagittal view of the embryo shown in **A**. Anterior to the *right*. **G** Lateral view of the embryo shown in **F**. Anterior to the *right*. **H** Anterior view. **I** Lateral view. Anterior to the *right*. **J** Higher magnification view of the embryo in **I. K**, **L** Lateral and ventral views of dissected brains. Anterior is *up*. *White arrow* expression in dorsal blastopore lip; *white arrowhead* expression in the prechordal plate mesoderm; *bracket* expression in the midline region of the neuroectoderm; *yellow arrow* expression as two stripe bands which is first detected at stage 12 on anterior neural fold; *yellow arrowhead* expression in the roof plate of the neural tube; *green arrowhead* presomitic mesodermal expression as a transverse line; *red arrowhead* expression as a spike-shaped band in the superficial layer of neuroectoderm; *red arrow* expression as the remarkable spike-shaped band among others in the deep layer of neuroectoderm; *black arrowhead* expression in the branchial arches; *black arrow* expression in dorsal limit of the cement gland; *orange arrow* expression in hindbrain rhombomeres. **A′**, **C′–F′**, **H′** Schematic diagram of the *Xhairy2b* expression pattern which is illustrated in **A**, **C–F**, **H**. *Green region* prospective neural plate border; *light blue line* notoplate expression; *red arrow* elongation movement of notoplate territory; *purple line* expression in the superficial layer cell of neural plate [*ch* optic chiasm, *dl* dorsal blastopore lip, *e* eye, *hbs* hindbrain-spinal cord boundary, *fp* floor plate (notoplate), *fmb* forebrain-midbrain boundary, *hy* hypophysis, *mhb* midbrain-hindbrain boundary, *nb* neural plate border, *nc* neural crest, *pn* pronephros, *psm* presomitic mesoderm, *tel* telencephalon]

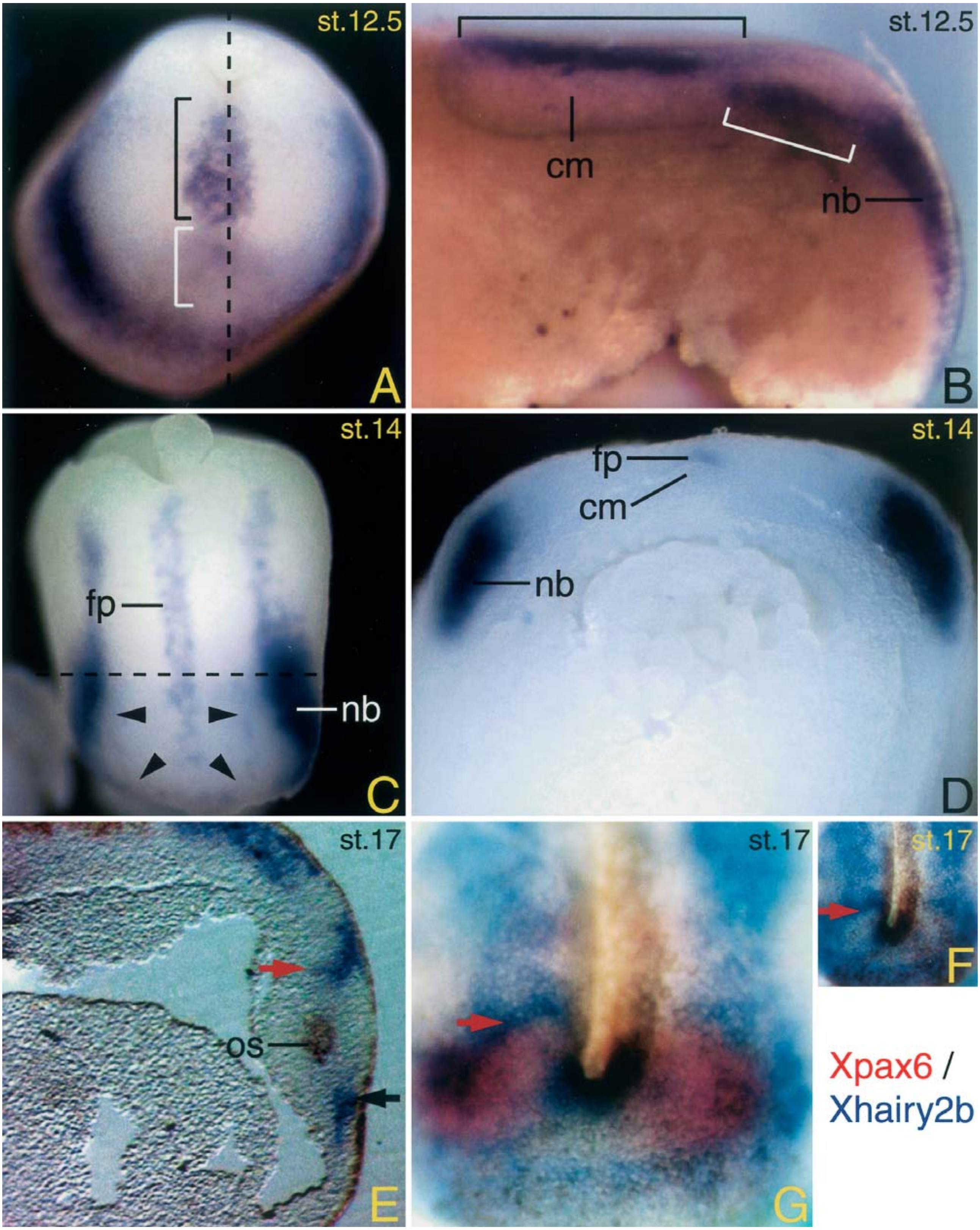

Fig. 3A–G *Xhairy2b* expression in the neuroectoderm and prechordal plate mesoderm. **A–E** Whole-mount in situ hybridization is performed with *Xhairy2b*. **A**, **C** Dorsal view. Anterior is *down*. **B** Sagittal view. Anterior to the *right*. **D** Transverse view. The *broken line* in **A** and **C** indicates the plane of section. **E** Sagittal view of the embryo shown in Fig. 2H. Anterior to the *right*. **F**, **G** Anterior view. **G** Double staining with *Xhairy2b* (*blue*) and dorsal forebrain marker *Xpax6* (*red*). Note: the anterior portion of the spike-shaped band (*red arrow*) is localized in the posterior position where *Xpax6* is expressed. *White bracket* expression in prechordal plate mesoderm; *black bracket* neuroectodermal expression; *black arrowhead* expression as the keyhole-shaped band in the superficial layer of neural plate; *black arrow* expression in the posterior limit of the prospective cement gland; *red arrow* spike-shaped expression in a deep layer of *Xhairy2b* [*cm* chordamesoderm (notochord), *os* optic stalk] and hypophysis (Fig. 2K, L) and hindbrain rhombomeres (Fig. 2K, orange arrow).

In this study, we show that *Xhairy2b* is dynamically expressed in the borders of several tissue territories, including the neural plate border, outer limit of floor plate and borders between major brain territories. Recent studies suggest that some hairy-related genes are expressed in the borders of several tissue territories. For instance, in *Drosophila*, *hairy* is expressed in several rows of eye disk cells anterior to the morphogenetic furrow and functions as a regulator of furrow progression and proneural tissue determination (Brown et al. 1995). *Drosophila hairy* is also expressed during the segmentation stage of early development to establish the segmented body plan (reviewed by St Johnston and Nüsslein-Volhard 1992). *Xenopus Hes-related1* (*XHR1*) is expressed in the midbrain-hindbrain isthmic region, and over-expression of a dominant-negative form of *XHR1* exhibits patterning defects in this region (Shinga et al. 2001). These results imply that the expression of hairy-related genes may play essential roles in the patterning of the tissue boundaries.

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