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EXPRESSION NOTE

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Developmental expression of the *Xenopus Iroquois*-family homeobox genes, *Irx4* and *Irx5*

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Abstract We have isolated and characterized the developmental expression of the *Xenopus Iroquois 4* (*Irx4*) and *Iroquois 5* (*Irx5*) homeodomain transcription factors. *Irx4* is expressed in a subset of cells in the neural retina and the developing hindbrain and also, specifically, in the ventricle of the heart. *Xenopus Irx5* is expressed in the developing midbrain, hindbrain, neural tube, and also in the retina.

Keywords Retina · Midbrain · Hindbrain · Heart · Ventricle

Members of the *Drosophila Iroquois* gene family appear to act as pre-patterning genes, reinforcing regionality and imposing specific positional identity (Gomez-Skarmeta et al. 1998). Similarly, the vertebrate *Iroquois* homeobox genes (*Irx*) show localized expression patterns in the developing embryo and may also function as pre-patterning genes (Gomez-Skarmeta et al. 1998). More specifically, vertebrate *Irx* genes exhibit highly regulated patterns of expression in the developing nervous system (Bellefroid et al. 1998; Gomez-Skarmeta et al. 1998; Goriely et al. 1999), brain (Bosse et al. 1997, 2000; Cohen et al. 2000), eye (Bosse et al. 1997) and heart (Bao et al. 1999; Bosse et al. 2000; Christoffels et al. 2000).

In a screen for *Iroquois* genes expressed in the *Xenopus* embryo, we have isolated the orthologues of *Irx4* and *Irx5*. The deduced sequences of the *Xenopus* Irx4 and Irx5 proteins are presented in Fig. 1, aligned with the corresponding orthologues identified in mouse, human and chicken. The *Xenopus* Irx4 protein contains 496 amino acids and has a predicted molecular weight of 55.5 kDa. The *Xenopus* Irx4 protein shares 60% and 64% overall identity with the human and mouse orthologues, respectively. The *Xenopus* and chick Irx4 proteins are 69% identical overall. The *Xenopus* Irx5 protein is 474 amino acids long with a predicted molecular weight of 51.8 kDa, and is 61% identical to murine Irx5, the only currently identified orthologue (Bosse et al. 2000).

We have examined the early developmental expression of *Xenopus Irx4* and *Irx5* using whole-mount in situ hybridization (Fig. 2). *Irx4* transcripts are first detected at stage 20 in several discrete clusters of cells within the hindbrain (Fig. 2A). By stage 26 intense *Irx4* staining appears in a region of the hindbrain above the otic vesicle (Fig. 2B) and this persists in later stage embryos (Fig. 2C–E, N). Additionally, *Irx4* staining is present in isolated cells throughout the hindbrain (Fig. 2A–E, O). Starting at stage 28, expression of *Irx4* is also detected in a subset of retinal cells lining the optic cup (Fig. 2C, D, M) and this domain of expression persists during later development (data not shown). Expression of *Irx4* in cardiac tissue is first detected at very low levels in the late tailbud embryo at about stage 36 (data not shown) and increases during subsequent development (Fig. 2E). Cardiac expression is initially limited to a lateral sub-region of the ventricular myocardium (Fig. 2F), rather than to all ventricular muscle. This cardiac expression of *Irx4* resembles that previously described for the transcription factor *Xenopus dHand* (Angelo et al. 2000). The similarity between *dHand* and *Irx4* expression in the myocardium is not surprising because *dHand* is believed to be a modulator of *Irx4* expression in the myocardium (Bruneau et al. 2000). As development proceeds expression of *Irx4* expands to include all ventricular muscle (Fig. 2G).

Irx5 transcripts are first detected at about the time of neural tube closure (stage 19) in two prominent bands in the developing nervous system corresponding to regions of the midbrain, hindbrain, neural tube and also in the

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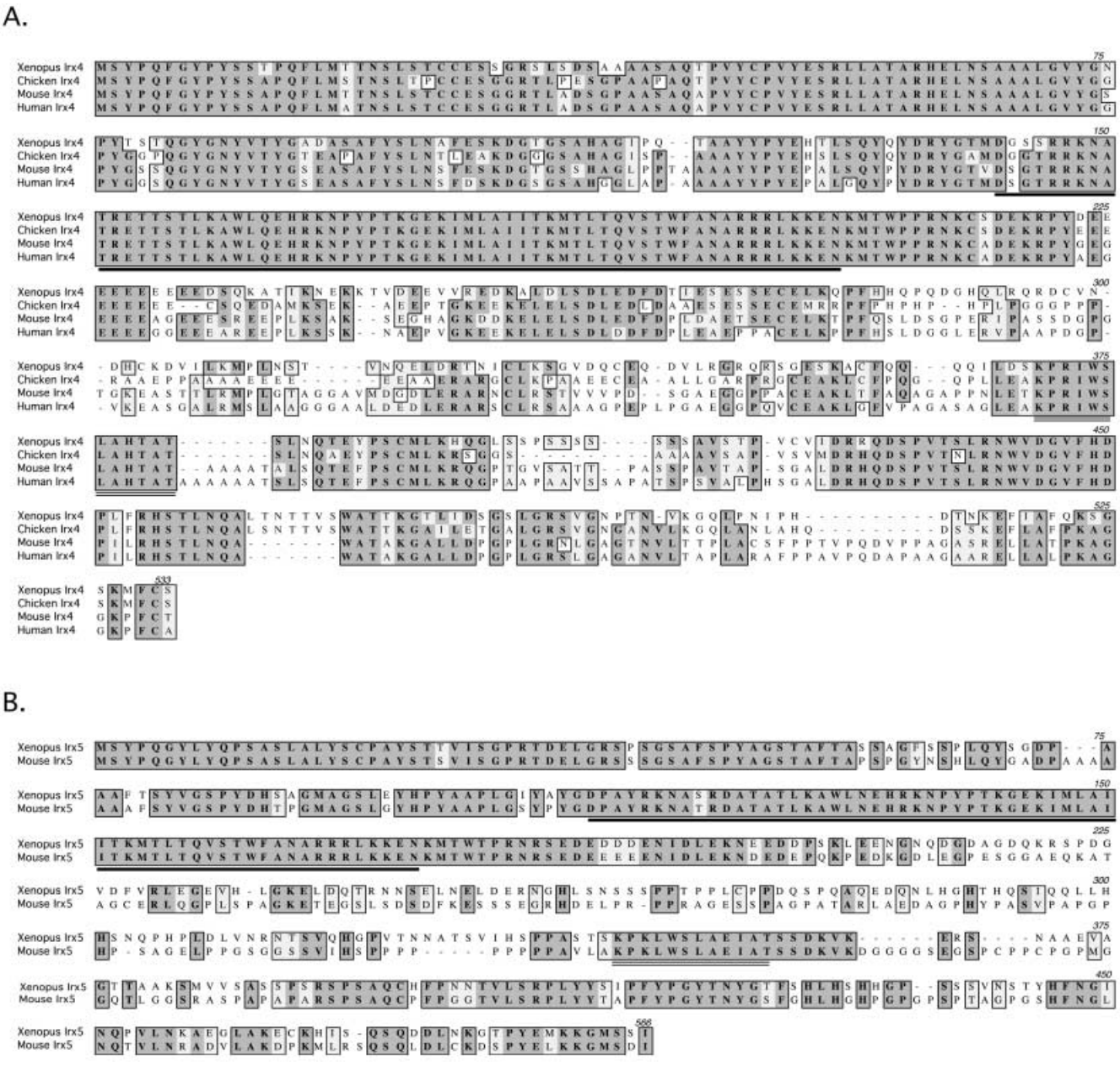

Fig. 1A, B Primary sequence of the *Xenopus* Irx4 and Irx5 proteins. The homeobox region of *Xenopus Irx3* (Bellefroid et al. 1998) was used as probe to isolate Iroquois clones from a stage 42 *Xenopus* embryo library. Amongst the clones isolated were the *Xenopus Irx4* and *Irx5* sequences (GenBank accession numbers AF338157 and AF338158). **A** Derived sequence of the *Xenopus* Irx4 protein aligned with the chicken, mouse and human Irx4 sequences (GenBank accession numbers AAD16100, AAF23886 and XP003825 respectively). The *Xenopus* and mammalian Irx4 protein sequences are 95% identical in the homeodomain (*underlined*) and 100% identical in the Iroquois box (*double underline*). The *Xenopus* and chicken protein sequence are 97% identical within the homeodomain and 100% identical in the Iroquois box. The Iroquois box is a unique region used to classify Irx family members (Bruneau et al. 2000). **B** Derived sequence of *Xenopus* Irx5 aligned with the mouse Irx5 sequence (GenBank accession number AF230074). The *Xenopus* and mouse Irx5 sequences are 98% identical in the homeodomain (*underlined*) and 100% identical in the Iroquois box (*double underline*)

optic vesicle (Fig. 2H, I). *Irx5* expression continues in these tissues during tailbud stages (Fig. 2J, K). Sectioning of stage 34 embryos reveals that *Irx5* is expressed throughout the retina of the eye (Fig. 2P, Q) in contrast to *Irx4* expression, which is limited to a subset of retinal cells of the optic cup (Fig. 2M). In the region of the hindbrain above the otic vesicle, *Irx4* (Fig. 2N) and *Irx5* (Fig. 2R) are predominantly expressed in non-overlapping domains. This raises the possibility that different *Irx* genes function in distinct subsets of neural tissue. Although *Irx5* expression has previously been described in the atria of chick embryos (Bosse et al. 2000) and in the endocardium and ventricular myocardium of mice embryos (Christoffels et al. 2000), we are unable to detect *Irx5* transcripts in the hearts of developing *Xenopus* embryos, at least up until stage 50 (data not shown).

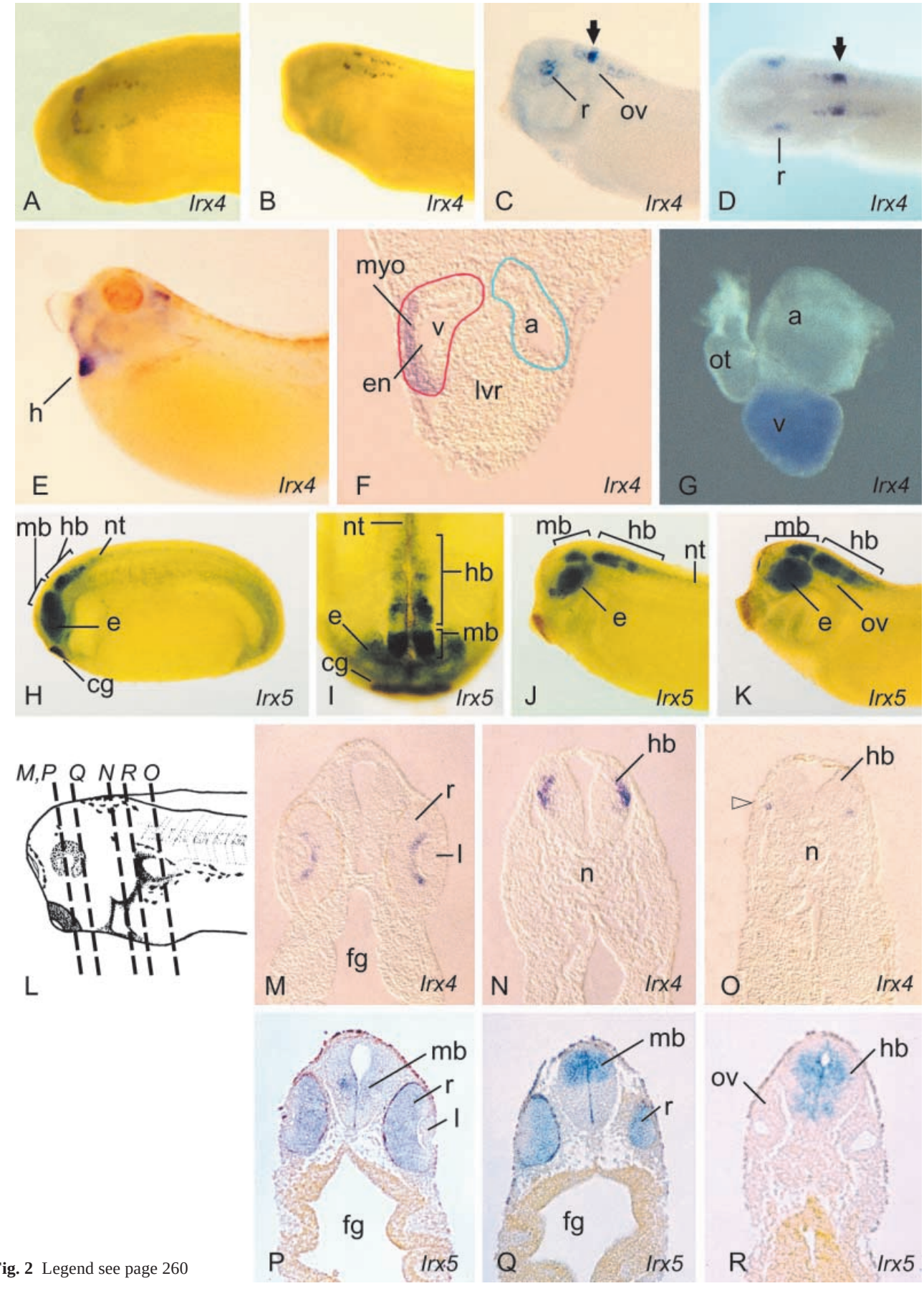

Fig. 2 Legend see page 260

◀ **Fig. 2** Developmental expression of *Irx4* and *Irx5* in the *Xenopus* embryo. All embryos were assayed by whole-mount in situ hybridization as described by Gerber et al. (1999). **A** Dorsal view of stage 21 embryo showing *Irx4* expression in discrete cells of the hindbrain. **B**, **C** Lateral views of *Irx4* stained embryos at stages 26 and 32, respectively. *Arrow* in **C** indicates an intense region of expression in the hindbrain dorsal to the otic vesicle. **D** Dorsal view of embryo shown in **C**. *Arrow* indicates intense region of expression in the hindbrain. **E** Lateral view of *Irx4* expression at stage 41, showing transcripts in the developing heart. **F** Transverse section through the heart of stage 41 embryo, showing *Irx4* expression restricted to a subdomain in the ventricular myocardium. The ventricular and atrial myocardia are *outlined in red and blue* respectively. **G** *Irx4* staining of an isolated stage 50 heart showing expression throughout the ventricle. **H** Lateral view of a stage 21 embryo showing *Irx5* expression in eye, midbrain, hindbrain and neural tube. **I** Dorsal view of same embryo shown in **H**. **J** *Irx5* expression in a stage 26, and **K** a stage 32 embryo, lateral views. **L** Diagram of a stage 34 embryo (lateral view) showing position of transverse sections (*M*–*R*). **M**–**O** Transverse sections through an *Irx4*-stained stage 34 embryo showing expression in a subset of the neural retina and in the anterior and posterior hindbrain. *Open arrowhead* in **O** indicates expression in isolated cells of hindbrain. **P**, **Q** Transverse sections through an *Irx5*-stained stage 34 embryo showing expression throughout the neural retina and in the midbrain. **R** *Irx5* expression at stage 34, showing hindbrain expression immediately dorsal to otic vesicle. *a* Heart atria, *cg* cement gland, *e* optic vesicle or eye, *en* endocardium, *fg* foregut, *h* heart, *hb* hindbrain, *l* lens, *lvr* liver, *mb* midbrain, *myo* myocardium, *n* notochord, *nt* neural tube, *ot* heart outflow tract, *ov* otic vesicle, *r* retina, *v* heart ventricle

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