### EXPRESSION NOTE

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

Received: 4 December 2000 / Accepted: 28 January 2001 / Published online: 14 March 2001 © Springer-Verlag 2001

**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  $\cdot$  Midbrain  $\cdot$  Hindbrain  $\cdot$  Heart  $\cdot$  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 prepatterning 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, hu-

Edited by R.P. Elinson

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man 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

#### A.

	76
Xenopus Irx4	MS Y P Q F G Y P Y S S T P Q F L M T T N S L S T C C E S S G R S L S D S A A A A S A Q T P V Y C P V Y E S R L L A T A R H E L N S A A A L G V Y G N
Chicken Irx4	M S Y P Q F G Y P Y S S A P Q F L M S T N S L T FIC C E S G G R T L FIE S G P A A P A Q T P V Y C P V Y E S R L L A T A R H E L N S A A A L G V Y G G
Mouse Irx4	M S Y P Q F G Y P Y S S A P Q F L M T T N S L S T C C E S G G R T L A D S G P A A S A Q A P Y Y C P Y Y E S R L L A T A R H E L N S A A A L G Y Y G S
Human Irx4	M S Y P O F G Y P Y S S A P O F L M A T N S L S T C C E S G G R T L A D S G P A A S A O A P Y Y C P Y Y E S R L L A T A R H E L N S A A A L G Y Y G G
indinari irx+	MS IF QFUIL IS SAF QFEMALOSES I CEES UUR LEADS UF AAS AQAFTICETTES REEALARBEAS AAAEUT 100
2000 CO. 100 CO. 100 CO.	150
Xenopus Irx4	PYT STQGYGNYVTYGADAS AFYSLNAFESKDGTGSAHAGTPQ - TAAYYPYEHTLSQYQYDRYGTMDGSSRRKNA
Chicken Irx4	PYGG PQGYGNYVTYGTEAPAFYSLNTLEAKDGGGSAHAGISP - AAAYYPYEHSLSQYQYDRYGAMDGGTRRKNA
Mouse Irx4	PYGSSQGYGNYVTYGSEASAFYSLNSFESKDGTGSSHAGLPPTAAAAYYPYEPALSQYPYDRYGTVDSGTRRKNA
Human Inx4	PYGGSQGYGNYVTYGSEASAFYSLNSFDSKDGSGSAHGGLAP- A A A A YYPYEP A LGQYP YDRYGTMDSGTRRKNA
	295
Xenopus Irx4	TRETTS TL KAWL QEHRKNPYPTKGEKIMLAIITKMTLTQVS TWFANARRLKKENKMTWPPRNKCS DEKRPYDE
Chicken Irx4	TRETTS TLKAWLQEHRKNPYPTKGEKIMLAIITKMTLTQVS TWFANARRLKKENKMTWPPRNKCS DEKRPYE EE
Mouse Irx4	TRETTS TEKAWE OE HRKNPYPTKGEKIMEAIITKMTETOVSTWFANARREKKENKMTWPPRNKCADE KRPYGE G
Human Irx4	TRETTS T L K A W L Q E H R K N P Y P T K G E K I M L A I I T K M T L T Q V S T W F A N A R R L K K E N K M T W P P R N K C A D E K R P Y A E G
Xenopus Irx4	E E E E E E E E E D S OK A T I KN E KK T VD E E V VR E D KA L D L S D L E D F D T I E S E S S E C E L KO P F H H O P O D G H O L R O R D C VN -
Chicken Irx4	EFEFE E CS O EDIAM KSEK AFEPTGKEEKELELS DLEDLDA AFESES SECEMER PEPHPHPHP HPILPGGGPPP.
Mouse Irx4	EEEEA GEEES REEPLKSAK SECHAGKDDKELELSDLEDFDPLDAETSECELKTPFOSLDSGPERIPASSDGPG
Human Irx4	EEEE G GEEEE AR EEPILKSISKNAEPVGKEEKELELS DL DJ DF DPLEA EPPALEELKPPFHS LD 3 G LER YPA APD GPL
Inditian # Are	EEEEOOEEEAKKEPEKSSK. SAEPOKEEKEEESDEDDEDEEAEPEAEEKPERSEDOOLEKVEAAEDOP
11 APR 11 - 11 11 APR 11 APR	375
Xenopus Irx4	- DHCKDVILKMPLNST VNQELDKTNICLKSGVDQCEQ - DVLRGRORSGESKACTQQ QQILDSKPRIWS
Chicken Irx4	RĂAEPPĂĂĂĂEĒĒ ĒĒĀĂERĀRĢCLKPĂĂEECĒĀ - ALLGĀRPRĢCĒĀKĒCFPQG - QPLLEĀKPRIWS
Mouse Irx4	T GKEAST T LRMPEGTAGGAVMDGD LERARNCLRSTVVVPD - SGAEGGPPACEAKLTFAQAGAPPNLETKPRIWS
Human Inx4	V K E A S G A L & M S L A A G G G A A L D E D L E R A R S C L R S A A A G P E P L P G A E G G P Q V C E A K L G F V F A G A S A G L E A K P R I W S
	450
Xenopus Irx4	LAHTAT
Chicken Irx4	LAHTAT
Mouse Irx4	LAHTAT AAAA TALS OTEFPS CMLKROGPTGVS ATT - PASSPAVTAP-SGALDRHODS PVTS LRNWVDGVFHD
Human Inx4	LAHTAT A A A A A A TS LS OTEFPS CML KR OGP A A PA A VS S A PA TS PS VALPHS GALDRHODS PVTS LR NWVD GYFHD
Xenopus Irx4	PLIFICHT TUNOALUTNTTVS WATTKGTEIDS GS LGRSVGNPTNI- VKGOEPNIPHDTNKEFTAFOKSG
Chicken Irx4	
Mouse Irx4	PILRHSTLNQA WATAKGALLDPGPLGRNLGAGTNVLTTPLACSFPPTVPQDVPPAGASRELLATPKAG
Human Irx4	PILRHSTLNQAWATAKGALLDPGPLGRSLGAGANVLTAPLARAFPPAVPQDAPAAGAARELLALPKAG
	533
Xenopus Irx4	S KM FCS
Chicken Irx4	S K M F C S
Mouse Irx4	GKPFCT
Human Irx4	GKPFCA
	C Key Minimum

## Β.

Xenopus Irx5 Mouse Irx5	75 M S Y P Q G Y L Y Q P S A S L A L Y S C P A Y S T T V I S G P R T D E L G R S P M S Y P Q G Y L Y Q P S A S L A L Y S C P A Y S T S V I S G P R T D E L G R S S S G S A F S P Y A G S T A F T A P S P G Y N S H L Q Y G A D P A A A
Xenopus Irx5 Mouse Irx5	150 A A  F T  S Y V G S P Y D H S  A  G M A G S L  E  Y H P Y A A P L G   Y A  Y G D P A Y R K N A S R D A T A T L K A W L N E H R K N P Y P T K G E K I M L A T A A  A F  S Y V G S P Y D H T  P  G M A G S L  G  Y H P Y A A P L G  S Y  P  Y G D P A Y R K N A T R D A T A T L K A W L N E H R K N P Y P T K G E K I M L A T
Xenopus Irx5 Mouse Irx5	225 I T KMT L T Q V S T WF A N A R R R L K K E N KMT W T P R N R S E D E D D D E N I D L E K N E E D D P S KL E E N G N Q D G D A G D Q K R S P D G I T KMT L T Q V S T WF A N A R R R L K K E N KMT W T P R N R S E D E E E E E N I D L E K N D E D E P Q KP E D K G D L E G P E S G G A E Q K A T
Xenopus Irx5 Mouse Irx5	300 Y D F Y R L E GE VH - L G K E L D Q T R N N S E L N EL D E R NG HL S N S S S P P T P P L C P P D Q S P Q A Q E D Q N L H G H T H Q S I Q Q L L H A G C E R L Q G P L S P A G K E T E G S L S D S D F K E S S S E G R HD E L P R - P P R A G E S S P A G P A T A R L A E D A G P H Y P A S V P A P G P
Xenopus Irx5 Mouse Irx5	375 HIS N Q P H P ED L V N R N T S V Q MG P V T N N A T S V I H S P P A V I A K P K L W S L A E I A T S S D K V K E R S N A A E V A HIP - S A G E L P P G S G G S S V I H S P P P P P P P P A V L A K P K L W S L A E I A T S S D K V K D G G G G S E G S P C P P C P G P M G
Xenopus Irx5 Mouse Irx5	450 G Q T L G G S R A S P A P A R S P S A Q C P F P G G T V L S R P L Y Y S I P F Y P G Y T N Y G S F G H L H S H H G P S S S V N S T Y H F N G L G Q T L G G S R A S P A P A R S P S A Q C P F P G G T V L S R P L Y Y T A P F Y P G Y T N Y G S F G H L H G H P G P G P S P T A G P G S H F N G L
Xenopus Irx5 Mouse Irx5	N Q P V L N K A E G L A K E C K H T S - Q S Q D D L N K G T P Y E M K K G M S S N Q T V L N R A D V L A K D P K M L R S Q S Q L D L C K D S P Y E L K K G M S D I

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 (under*lined*) 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).

258

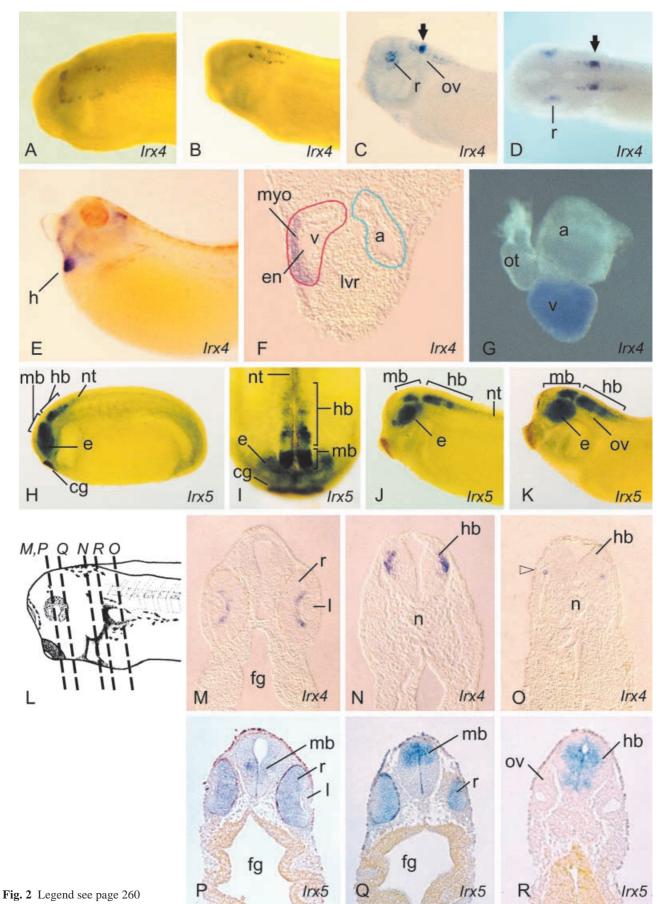


Fig. 2 Developmental expression of *Irx4* and *Irx5* in the *Xenopus* embryo. All embryos were assayed by whole-mount in situ hybridization as described by Gerber et al. (1999). A Dorsal view of stage 21 embryo showing Irx4 expression in discrete cells of the hindbrain. B, C Lateral views of Irx4 stained 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

Acknowledgements We thank Mike King for the *Xenopus* stage 42 tadpole library. This work was supported by the NHLBI, NIH grant HL63926 to P.A.K.. P.A.K. is the Allan C. Hudson and Helen Lovaas Endowed Professor of the Sarver Heart Center.

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