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EXPRESSION NOTE

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Xnkx-2.1: a homeobox gene expressed during early forebrain, lung and thyroid development in *Xenopus laevis*

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Abstract *Nkx-2.1* is a member of the vertebrate Nkx family of homeobox genes; it was originally identified as a tissue-specific regulator of thyroglobulin and thyroperoxidase gene transcription. Here we report on the embryonic expression of *Xnkx-2.1*, which is expressed in the presumptive forebrain from early neurulation onwards. In tadpole stage embryos *Xnkx-2.1* transcripts are primarily detected in ventral forebrain, lung buds and thyroid anlage. Therefore, *Xnkx-2.1* may be part of the genetic network that controls the early development of these organs.

Key words *nkx* · Forebrain · Lung · Thyroid ·*Xenopus laevis*

Xnkx-2.1 is a member of the NK-2 family of homeobox containing transcription factors that are most homologous to the Drosophila NK-2 (*vnd*) gene (Kim and Nirenberg 1989; Jimenez et al. 1995). In addition to *Nkx-2.1*, nine other family members have been identified in vertebrates and, recently, one other NK-2 related gene in *Drosophila*, called *Scarecrow* (*scro*) has been described (accession number AAF26436; Zaffran et al., unpublished observations). NK-2 family members are known to be expressed in derivatives of all three germ layers, e.g. the central nervous system, cardiac mesoderm and foregut endoderm, where they presumably control cell specification and morphogenetic processes during organ formation (Lyons et al. 1995; Minoo et al. 1995). Whereas *Nkx-2.5* function is required for normal heart development (Lyons et al. 1995; Schott et al. 1998), mice lacking *Nkx-2.2* are arrested in pancreatic cell differentiation (Sussel et al. 1998). Furthermore, it has been demonstrated in mice that *Nkx-2.1* function is essential for the development of the organs where it is expressed, the ventral forebrain, lung and thyroid gland (Kimura et al. 1996; Sussel et al. 1999). In humans, low expression levels of Nkx-2.1 are correlated with a defect in thyroglobulin synthesis (Acebron et al. 1995).

A cDNA fragment including the complete open reading frame which is homologous to human, mouse and chicken *Nkx-2.1* genes was amplified from a *Xenopus* tailbud stage total RNA preparation by RT-PCR. Preparation of total RNA from embryos was done as described previously (Hollemann et al. 1996). RT-PCR was carried out using the Gene Amp RNA PCR kit from Perkin-Elmer. The following primers were used to isolate the complete coding region: nkx-2.1deg-forward (F) 5′-ATG TCG ATG AGY CCW AAG CAY ACG ACT CC-3′ and reverse (R) 5′-TCA CCA GGT CCK ACC RTA WAG CAA GG-3′.

Xenopus Nkx-2.1 is highly conserved in comparison with other vertebrate *Nkx-2.1* genes, with 74%, 75% and 86% overall amino acid identity compared with human, mouse and chicken *Nkx-2.1*, respectively (Fig. 1A). In a comparison with *Xenopus Nkx-2.2*, *Nkx-2.5 and Nkx-2.10*, the overall amino acid sequence identity of *Xnkx-2.1* is only 37%, 28 and 30%, respectively (not shown). Within the homeodomain, vertebrate Nkx-2.1 amino acid sequences are identical. We therefore refer to the described *Xenopus* protein as Xnkx-2.1. Interestingly, a new gene isolated from *Drosophila melanogaster* called *scro* encodes the most similar homeodomain compared with *Nkx-2.1*. On the other hand, the homeodomain of the *Drosophila* gene *vnd* is more similar to *Nkx-2.2*. Therefore, the Nkx-2 class appears to be the first one containing two different *Drosophila* genes (Fig. 1B).

Expression of *Xnkx-2.1* was studied by whole mount in situ hybridisation in early *Xenopus* embryos (NF stages 14–36; Nieuwkoop and Faber 1967). Whole mount in situ hybridisation and vibratome sections of embryos were done as described previously (Hollemann et al. 1996). The first, low level expression of *Xnkx-2.1* was observed as a small, crescent-shaped domain in the sensorial layer of the neuroectoderm in the anterior neural plate, located adjacent to the underlying SHH-secreting axial mesoderm in stage 14 embryos (Fig. 2A) (Hausen and Riebesell 1991; Shimamura et al. 1995). This observation is in

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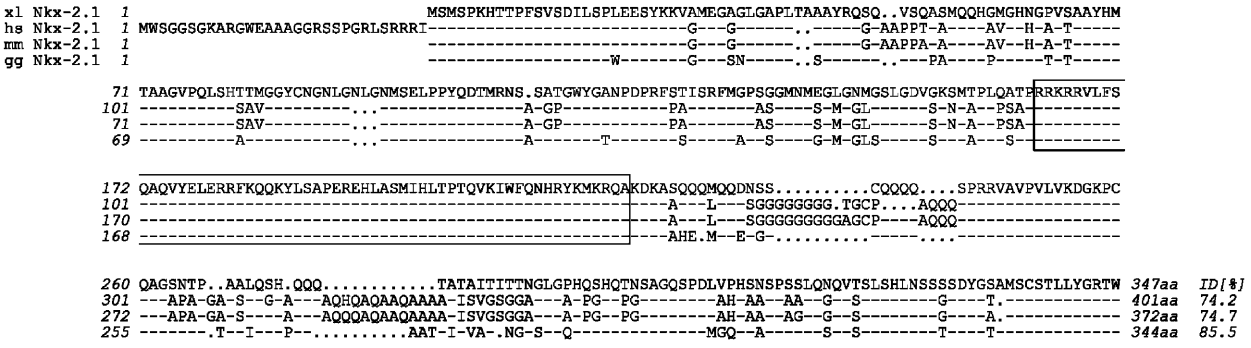
A)



B)

Nkx-2.1	xl,hs,mm,gg	RRKRRVLFSQAQVYELERRFKQQKYLSAPEREHLASMIHLTPTQVKIWFQNHRYKMKRQA	ID[%]
	dm (scro)	--------T--------------R-----------L-K---------------C----	92
Nkx-2.2	xl,mm,dr	K--------K--T-------R--R-----------L-R-----------------AR	85
	dm (vnd)	K-------TK---T------R--R-----------L-R---------------T--AQ	82
Nkx-2.3	hs,mm,dr	---P---------F---------R----------SLK--S---------R---C---R	83
Nkx-2.4	mm	----------	100
Nkx-2.5	xl,gg,dr	---P-----------------------D---NVLK--S---------R---C---R	83
	hs,mm	---P-----------------------DQ---VLK--S---------R---C---R	83
Nkx-2.6	mm	Q--S---------LA--------R--T---------ALQ--S---------R---S-S-R	77
Nkx-2.7	dr	---P------T--F---------R-------D---LALK--S---------R---C---R	78
Nkx-2.8	gg	---P------T--L--------------L------NVLQ--S---------R---C---R	80
Nkx-2.9	mm	---------K--TL------R--R--------Q--RLLR----------------L--GR	78
Nkx-2.10	xl	---P------M--FQ-------R--------Q--LALK--S---------R---C---K	77

Fig. 1 **A** Comparison of the predicted amino acid sequences of *Xenopus* Nkx-2.1 and those from other species. The DDBJ/ EMBL/ GenBank accession number for *Xnkx-2.1* is AF250347. *Dots* represent gaps introduced into the amino acid sequence in order to obtain optimal sequence homology. Identical amino acids are represented by *hyphens*, the homeodomain is *boxed*. The percentage of amino acid identity (ID) of Xnkx-2.1 in comparison with human, mouse and chicken sequences shown here is indicated at the end of each individual sequence. **B** Homeodomain amino acid sequence alignment of Nkx related proteins. With the exception of mammalian Nkx-2.5, the vertebrate sequences in each subclass are identical to non-mammalian variants; the two primary sequence deviations in the Nkx-2.5 subclass are shown in *bold*. The homeobox sequences of *Drosophila* scro and *Drosophila* vnd are also highly conserved (92% and 82% identity in comparison to Nkx-2.1, respectively), although vnd is even more closely related to Nkx-2.2 (93% identity compared with Nkx-2.2). Outside the homeobox, the vertebrate and invertebrate Nkx related proteins are only moderately conserved (comparison not shown). Abbreviations: *ce, Caenorhabditis elegans*; *dm, Drosophila melanogaster*; *dr, Danio rerio*; *gg, Gallus gallus*; *hs, Homo sapiens*; *mm, Mus musculus*; *rn, Rattus norvegicus*; *xl, Xenopus laevis*

good agreement with the finding that *sonic hedgehog* is able to induce *Nkx-2.1* (Shimamura and Rubenstein 1997). This anterior *Nkx-2.1* expression domain defines the ventral archencephalon after neural fold closure at stage 18 (Fig. 2B, C) which will develop into the prospective hypothalamus at later stages (Fig. 2D–G) (Eagleson et al. 1995). A similar pattern of expression in the ventral forebrain was described for chicken *Nkx2–1*, mouse *Nkx-2.1* (Pera and Kessel 1998; Lazzaro et al. 1991) and *Xenopus Nkx-2.2*, although *Xnkx-2.2* is expressed in more ventro-lateral regions and, in contrast to *Xnkx-2.1*, is expressed in the developing spinal cord (Saha et al. 1993). In addition to the expression in the ventral forebrain (telencephalon and diencephalon), *Xnkx-2.1* transcripts are also found in two derivatives of the ventral foregut, the thyroid and the lung buds at tadpole stages (Fig. 2E, F, H, H'). In *Xenopus*, two other homeobox genes are known to be expressed in the thyroid, namely *Pax2* and *Xhex* (Heller and Brändli 1999; Newman et al. 1997), but *Xnkx-2.1* is the first gene described which shows expression in the primary lung buds.

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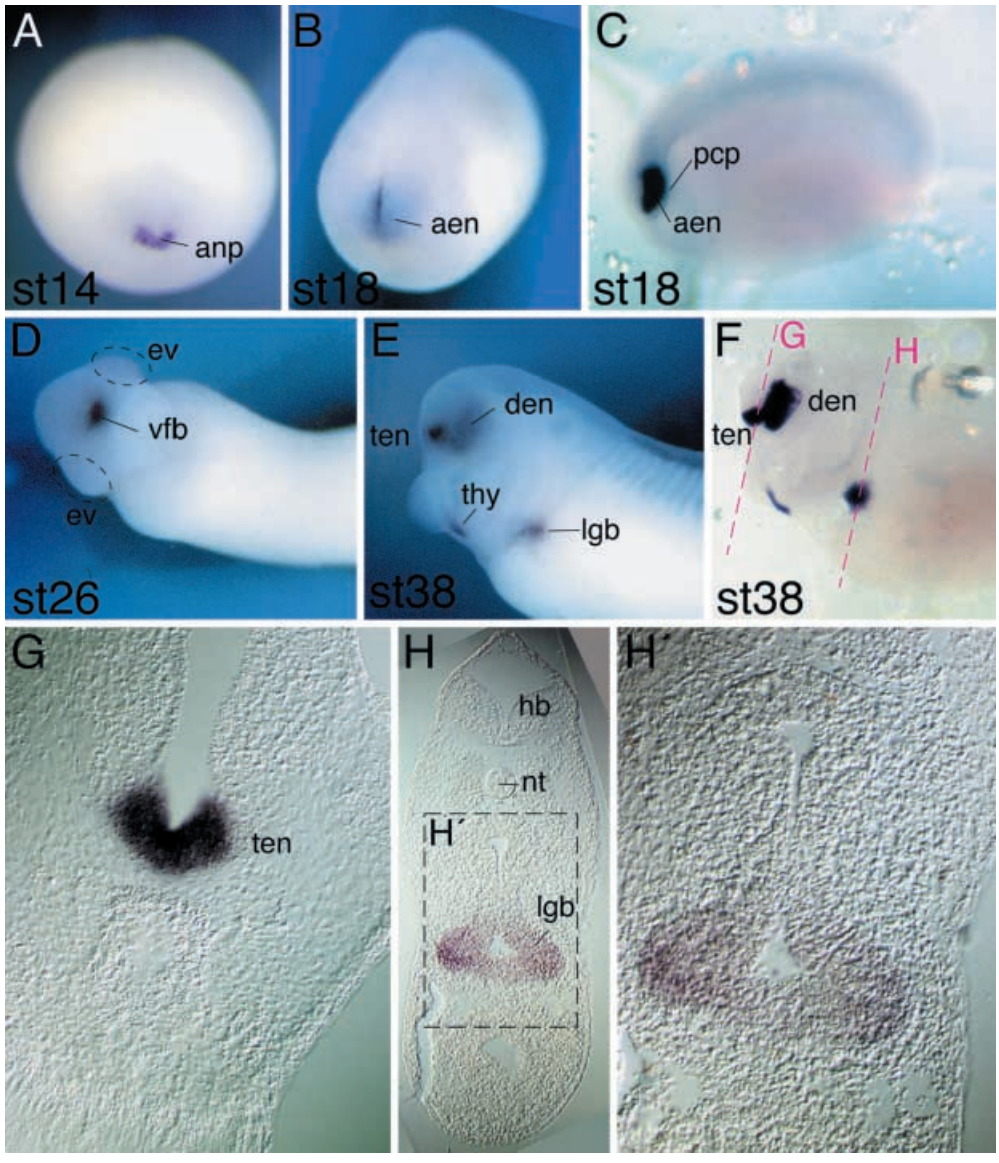

Fig. 2A–H Expression of *Xnkx-2.1* during *Xenopus* development. Whole mount in situ hybridisation was performed using *Xnkx-2.1* antisense RNA and staged *Xenopus* embryos. Nieuwkoop-Faber stages of embryogenesis are indicated. **A, B** Anterior view. **C, E, F** lateral view. **D** Ventral view. To improve resolution, cleared embryos of **B** and **E** are shown in **C** and **F**, respectively. **A** *Xnkx-2.1* is expressed in the anterior open neural plate at stage 14. **B, C** Expression of *Xnkx-2.1* marks the archencephalon after neural tube closure. **D–F** At later stages *Xnkx-2.1* is expressed in the ventral forebrain (telencephalon and diencephalon) and in two derivatives of the foregut endoderm, the primary lung buds and the thyroid gland. **G, H, H'** The position of frontal vibratome sections as indicated by the *red dashed line* across the embryos in **F**. Details of **H** as shown in **H'** are indicated by the *broken lines*. Abbreviations: *aen* archencephalon; *anp* anterior neural plate; *den* diencephalon; *ev* eye vesicle; *hb* hind brain; *lgb* lung buds; *nt* notochord; *pcp* prechordal plate; *ten* telencephalon; *thy* thyroid; *vfb* ventral forebrain

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