Dev Genes Evol (1998) 208:168–171

EXPRESSION NOTE

Edgar M. Pera · Michael Kessel

Demarcation of ventral territories by the homeobox gene *NKX2.1* during early chick development

Received: 3 February 1998/Accepted: 26 February 1998

Abstract Members of the *NK-2* homeobox gene family are expressed in distinct parts of the central nervous system and in other non-neural territories not only in the fruitfly *Drosophila melanogaster*, but also in vertebrates. The murine *Nkx2.1* (*TTF-1*, *T/ebp*) gene was previously shown to be active and indispensable in the developing forebrain, hypophysis, thyroid and lung. Here we report the early transcript distribution of the chick *NKX2.1* gene. By whole-mount in situ hybridization we detect a novel transient expression domain in the early epiblast. Further expression occurs in the ventral medial endoderm, which becomes restricted to the anlage fields of the thyroid and lung, in the ventral diencephalon and telencephalon. These findings suggest that *NKX2.1* is part of a *Nkx* code which specifies ventral territories in the vertebrate embryo.

Key words *NKX2.1* · *TTF-1*· *T/ebp* · *Nkx* code

Results and discussion

A randomly primed Lambda-ExCell (Pharmacia) cDNA library prepared from the head region of HH9 chicken embryos was screened under low stringency conditions with a probe derived from a 122-bp AvaII/PvuII-homeobox fragment of the chick *DLX5* cDNA (Ferrari et al. 1995). A 1784-bp clone in the pExCell vector was obtained, which represented a complete cDNA for the chicken *NKX2.1* gene, an ortholog of the murine *Nkx2.1* (*TTF-1*, *T/ebp*) gene. The predicted chick NKX2.1 protein consists of 346 amino acids. Its primary structure is very similar to the homologous proteins of rat, mouse, dog and of humans, including 100% identity within the homeodomain (Guazzi et al. 1990; Oguchi et al. 1995; Saiardi et al. 1995; Van Renterghem et al. 1995). The sequence was deposited in the EMBL Nucleotide Sequence Database under accession number AJ223618.

The expression of *NKX2.1* was studied by whole-mount in situ hybridization in young chick embryos (HH3-21; Hamburger and Hamilton 1951) probing with the 1784-bp BamHI cDNA fragment. In situ hybridization and histology of whole embryos was performed as described, except that specimens from all stages were treated with proteinase K (Pera and Kessel 1997; Stein and Kessel 1995; Lemaire et al. 1997). The first, low transcript levels were detected in the ectoderm of primitive streak embryos in two symmetrical, wing-shaped domains extending from the middle of the streak towards the anterior pole (HH4; Figs. 1A, 2 A), which are maintained up to the head process stage (HH5; Fig. 1B). The transient ectodermal *NKX2.1* domain is reminiscent of the initial expression of the *Drosophila NK-2/vnd* gene, which is expressed in two longitudinal stripes of the blastoderm flanking the prospective mesoderm (Jimenez et al. 1995; Mellerick and Nirenberg 1995). Both *NKX2.1* and *NK-2* demarcate cells of the early ectoderm, which do not gastrulate.

Shortly before head fold formation a strong expression domain appears in the medial endoderm anterior to the prechordal mesendoderm (HH5; Figs. 1B, 2B). It passes over to the anterior intestinal portal and the ventral midline of the foregut (Figs. 1C–G, 2D). Later it is focused in two derivatives of the ventral foregut, first of all in the anlage of the thyroid (Fig. 1I, 3D) and then more posteriorly in the developing trachea and lung (Fig. 3A,E,F). While this expression is suggestive of a cell lineage relationship, this connection is not proven at the present time.

As soon as the neural tube fuses anteriorly, a strong *NKX2.1* domain arises in the primordium of the ventral forebrain (HH8+; Figs. 1F,G, 2C). We recently demonstrated that this expression is dependent on the presence of the prechordal plate (Pera and Kessel 1997). Caudal-

Edited by R. Balling

E.M. Pera (✉) · M. Kessel
Max-Planck-Institut für biophysikalische Chemie,
D-37077 Göttingen, Germany

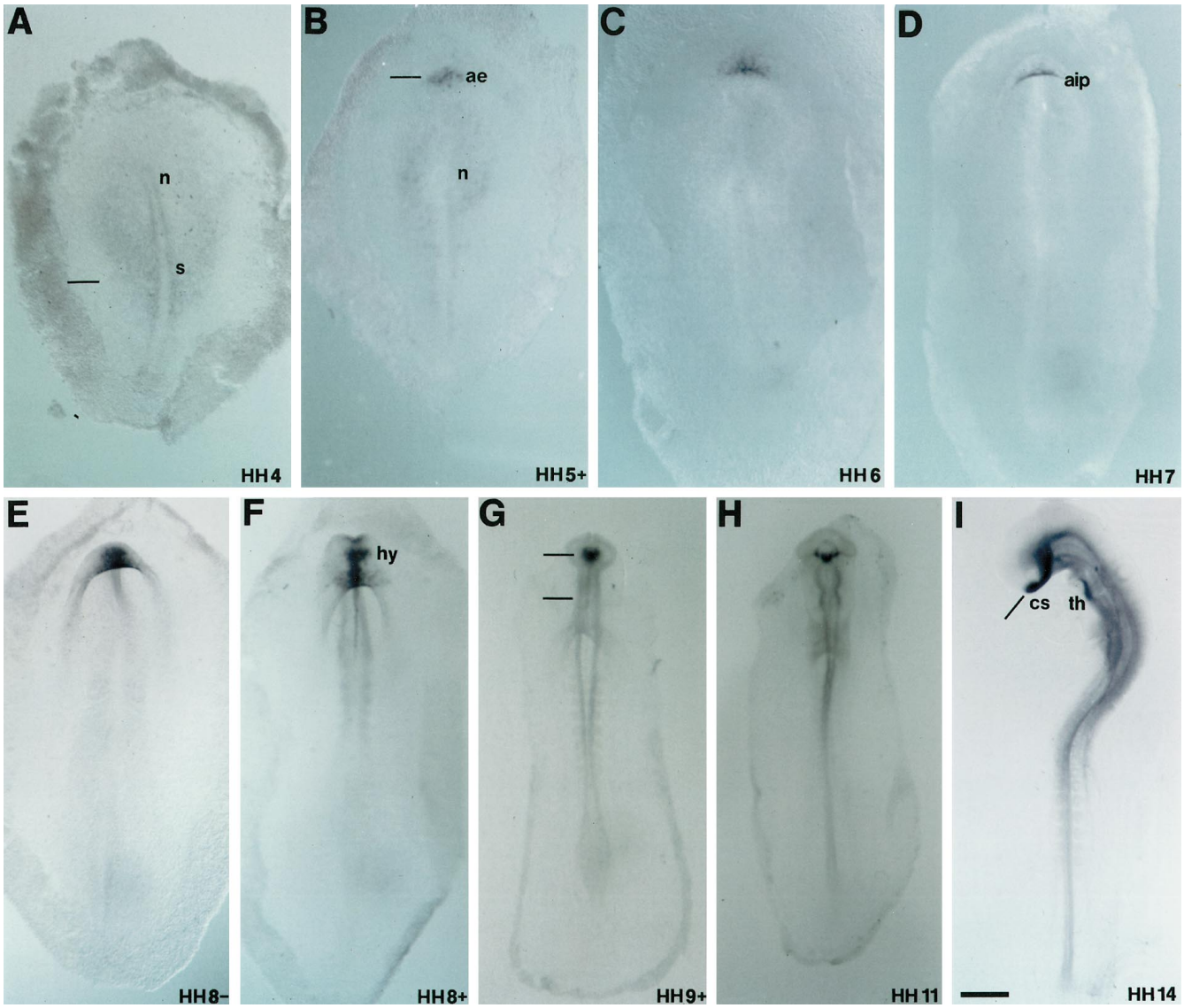

Fig. 1A–I Early expression of *NKX2.1* mRNA in chick embryos at the indicated stages (HH4-14; Hamburger and Hamilton 1951). *Lines* indicate the levels of sections depicted in Fig. 2 (*ae* medial anterior endoderm, *aip* anterior intestinal portal, *cs* anlage of corpus striatum, *hy* prospective hypothalamus, *n* node, *s* streak, *th* primordium of the thyroid). *Bar* represents 80 µm in **A–F** and 100 µm in **G–I**

ly the expression extends weakly into the floorplate of the prospective midbrain, hindbrain and anterior spinal cord. From HH10 onwards, this neuroectodermal expression is confined to the developing hypothalamus in the ventral diencephalon (Figs. 1H,I, 2E, 3B,C,D). When the anterior brain adopts a cranial and cervical flexure (HH13-14) an additional domain forms in the anlage of the medial corpus striatum in the ventral telencephalon (Figs. 1I, 2E, 3B,E). In vitro studies suggest that this telencephalic expression is induced by planar signals from the ventral diencephalon (Ericson et al. 1995).

In accordance with recent descriptions of the expression and function of *Nkx2.1* homologs in amniotes, *NKX2.1* gene activity specifies distinct ventral territories within the foregut and forebrain (Lazzaro et al. 1991; Price et al. 1992; Ericson et al. 1995; Shimamura et al. 1995; Kimura et al. 1996; Pera and Kessel 1997). In this respect, *NKX2.1* is reminiscent of other vertebrate members of the *NK2*-family such as *Nkx2.2*, *2.3*, *2.5*, *2.7* or *2.8*. These genes are expressed in overlapping, non-identical expression domains in ventral territories, from mostly medial regions, such as *NKX2.1*, to more ventrolateral regions, such as *NKX2.8*. These patterns suggests the existence of a *Nkx*-code specifying regional differentiation in ventral parts of the vertebrate embryo (Boettger et al. 1997; Lemaire and Kessel 1997).

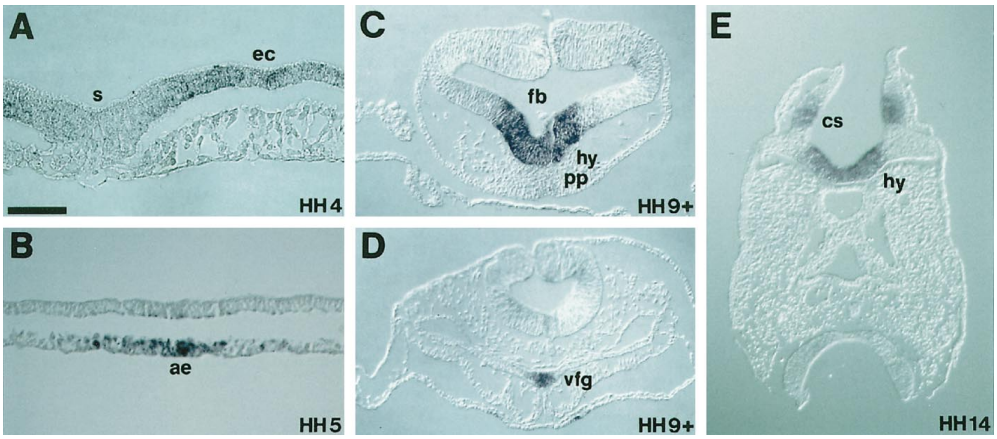

Fig. 2A–E *NKX2.1* expression in transversal sections of the stained embryos shown in Fig. 1. *Bar* represents 100 µm in **A**, 120 µm in **B**, 200 µm in **C** and **D** and 1 mm in **E**. **A** Section through a HH4 primitive streak (see Fig. 1A). Note expression in the ectoderm (*ec*). **B** Section anterior of a HH5 neural plate (see Fig. 1B). Note expression in the medial anterior endoderm (*ae*). **C** Section through a HH9$^+$ forebrain (see Fig. 1G). Note expression in the hypothalamus anlage (*hy*) above the prechordal plate (*pp*). **D** Section through a HH9$^+$ head at the hindbrain level (see Fig. 1G). Note expression in the medial ventral foregut (*vfg*). **E** Section through a HH14 head (see Fig. 1I). Note expression in the primordia of the hypothalamus (*hy*) and the medial corpus striatum (*cs*)

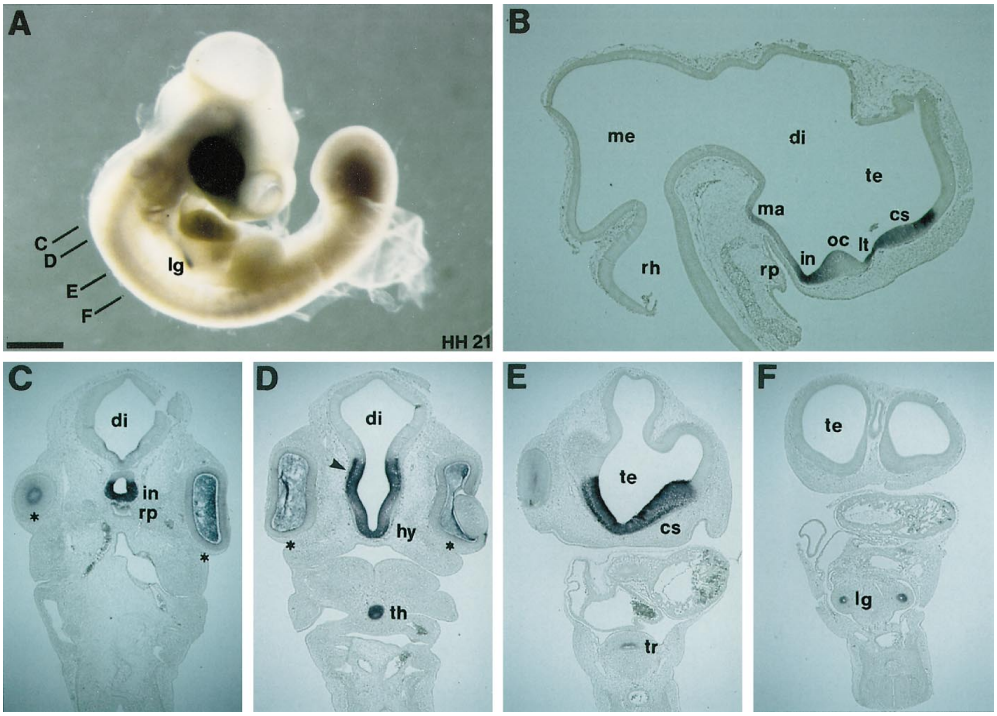

Fig. 3A–F Localization of *NKX2.1* transcripts in a HH21 embryo. **A** Lateral view of the whole embryo. *Lines* indicate the levels of transversal sections in **C–F**. The *asterisks* indicate non-specific staining in the optic vesicles, the dark regions in the heart and tailbud do not represent specific staining (*cs* medial corpus striatum, *di* diencephalon, *hy* hypothalamus, *in* infundibulum, *lt* lamina terminalis, *lg* lung, *ma* mammillary body, *me* mesencephalon, *oc* optic chiasma, *rh* rhombencephalon, *rp* Rathke's pouch, *te* telencephalon, *th* thyroid, *tr* trachea). *Bar* represents 1.2 mm in **A**, 300 µm in **B** and 400 µm in **C–F**. **B** Mid-saggital section through the head. Note expression in the mammillary body, infundibulum, lamina terminalis and medial corpus striatum. **C** Expression in the infundibulum. **D** Expression in the hypothalamus and thyroid. Note the strongly stained postmitotic neurons in the hypothalamus (*arrowhead*). **E** Expression in the corpus striatum and trachea. **F** Expression in the lung

Acknowledgements We thank S. Stein for the *DLX5* cDNA and W. Behrens for excellent technical assistance. The authors are supported by the Max-Planck-Gesellschaft and SFB 271.

References

Boettger T, Stein S, Kessel M (1997) The chicken *NKX2.8* homeobox gene: a novel member of the NK-2 gene family. Dev Genes Evol 207: 65–70

Ericson J, Muhr J, Placzek M, Lints T, Jessell TM, Edlund T (1995) *Sonic hedgehog* induces the differentiation of ventral forebrain neurons: a common signal for ventral patterning within the neural tube. Cell 81: 747–56

Ferrari D, Sumoy L, Gannon J, Sun H, Brown AM, Upholt WB, Kosher RA (1995) The expression pattern of the *Distal-less* homeobox-containing gene *Dlx-5* in the developing chick limb bud suggests its involvement in apical ectodermal ridge activity, pattern formation, and cartilage differentiation. Mech Dev 52: 257–64

Guazzi S, Price M, De Felice M, Damante G, Mattei MG, Di Lauro R (1990) Thyroid nuclear factor 1 (TTF-1) contains a homeodomain and displays a novel DNA binding specificity. EMBO J 9: 3631–3639

Hamburger V, Hamilton HL (1951) A series of normal stages in the development of the chick embryo. J Morphol 88: 49–92

Jimenez F, Martin-Morris LE, Velasco L, Chu H, Sierra J, Rosen DR, White K (1995) *vnd*, a gene required for early neurogenesis of *Drosophila*, encodes a homeodomain protein. EMBO J 14: 3487–3495

Kimura S, Hara Y, Pineau T, Fernandez-Salguero P, Fox CH, Ward JM, Gonzalez FJ (1996) The T/ebp null mouse: thyroid-specific enhancer-binding protein is essential for the organogenesis of the thyroid, lung, ventral forebrain, and pituitary. Genes Dev 10: 60–69

Lazzaro D, Price M, De Felice M, Di Lauro R (1991) The transcription factor TTF-1 is expressed at the onset of thyroid and lung morphogenesis and in restricted regions of the foetal brain. Development 113: 1093–1104

Lemaire L, Roeser T, Izpisua-Belmonte JC, Kessel M (1997) Segregating expression domains of two *goosecoid* genes during the transition from gastrulation to neurulation in chick embryos. Development 124: 1443–1452

Lemaire L, Kessel M (1997) Gastrulation and homeobox genes in chick embryos. Mech Dev 67: 3–16

Mellerick DM, Nirenberg M (1995) Dorsal-ventral patterning genes restrict NK-2 homeobox gene expression to the ventral half of the central nervous system of *Drosophila* embryos. Dev Biol 171: 306–316

Oguchi H, Pan YT, Kimura S (1995) The complete nucleotide sequence of the mouse thyroid-specific enhancer-binding protein (T/EBP) gene: extensive identity of the deduced amino acid sequence with the human protein. Biochim Biophys Acta 1261: 304–306

Pera EM, Kessel M (1997) Patterning of the chick forebrain anlage by the prechordal plate. Development 124: 4153–4162

Price M, Lazzaro D, Pohl T, Mattei MG, Rüther U, Olivo JC, Duboule D, Di Lauro R (1992) Regional expression of the homeobox gene *Nkx-2.2* in the developing mammalian forebrain. Neuron 8: 241–255

Saiardi A, Tassi V, De Filippis V, Civitareale D (1995) Cloning and sequence analysis of human thyroid transcription factor 1. Biochim Biophys Acta 1261: 307–310

Shimamura K, Hartigan DJ, Martinez S, Puelles L, Rubenstein JL (1995) Longitudinal organization of the anterior neural plate and neural tube. Development 121: 3923–33

Stein S, Kessel M (1995) A homeobox gene involved in node, notochord and neural plate formation of chick embryos. Mech Dev 49: 37–48

Van Renterghem P, Dremier S, Vassart G, Christophe D (1995) Study of *TTF-1* gene expression in dog thyrocytes in primary culture. Mol Cell Endocrinol 112: 83–93