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Gene expression pattern

Expression of DLX3 in chick embryos

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Abstract

Higher vertebrates appear to possess six genes encoding a homeodomain of the distal-less type. We report the cloning and expression pattern of the chicken DLX3 gene, a homeobox gene highly related to the DLX5 gene with regard to both the encoded protein structure and the expression pattern. DLX3 RNA was observed during the development of the olfactory and otic placodes, in the distal portion of the first and second visceral arch mesenchyme, in the growing limb buds, and in the tail tip. No expression occurs in the central nervous system.

Keywords: Cloning and expression pattern; Homeobox gene; Chicken; DLX3

1. Introduction

The family of homeobox genes comprises an estimated 200–300 members in vertebrates, and can be subdivided into sub-groups based on sequence comparisons of the homeodomains (Stein et al., 1996). Many homeobox genes appear to be present in highly related pairs, a fact which has been one argument in favour of a general genome duplication having occurred in a putative common ancestor of the vertebrates, but not in the chordate Branchiostoma (Holland et al., 1994). Mice and humans seem to have six genes related to the *Drosophila* homeobox gene distal-less, which are organized in three convergently transcribed pairs, namely Dlx1-Dlx2, Dlx3-Dlx4, and Dlx5-Dlx6, each closely linked to one of the four Hox clusters (Simeone et al., 1994; McGuinness et al., 1996; Stein et al., 1996; Liu et al., 1997; Quinn et al., 1997). We have previously analyzed the chicken DLX5 gene to study ectodermal patterning in the avian embryo (Pera et al., 1999). DLX3 is most similar to DLX5 in terms of protein sequence. Its inactivation in mice resulted in placental failure, so that embryonic phenotypes could not be investigated (Morasso et al., 1999). A human DLX3 gene mutation is responsible for the tricho-dento-osseous (TDO) syndrome which is characterized by abnormal hair, teeth, and bone (Price et al., 1998a,b). Expression of Dlx3 in the mouse and other vertebrates was observed in the skin, branchial arches and the developing inner ear (Ekker et al., 1992; Beauchemin and Savard, 1992; Papalopulu and Kintner, 1993; Dirksen et al., 1994; Robinson and Mahon, 1994). Here, we describe the cloning and expression of the DLX3 gene from chicken.

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ggdlx3  MSGSFDKKLSSILTDLSGSLSCHASSKDSPTLPESSVTDL
mmdlx3  ......R..........S......G..........T....

ggdlx3  GYYS-GQHDYYPGQSYGQPVAHYPY-PQFNLNAIGAGGNY
mmdlx3  ....AP.....S..P...T.NP.T.HH.....GLAGT.A.

ggdlx3  SPKSDYSYSPSYRQYGHFRDQQLPAQDAVSVKEEPEPEVR
mmdlx3  ....E.T.GG......AY.E.P.....P........A...

ggdlx3  MVNGKPKKIRKPRTIYSSYQLAALQRRFQKAQYLALPERA
mmdlx3  ........V.............................

ggdlx3  ELAAQLGLTQTQVKIWFQNRRSKFKKLYKNGEVPLEHSPN
mmdlx3  ..........................................

ggdlx3  NSDSMACNSPPSPAVWDSATHGS-APGRTPLPQPLPYSPS
mmdlx3  ..............L..TSS.STP..A.N...P.....A.

ggdlx3  PAFLEEHS-PWYHPQSLAAPH------QPAAMHHTSPGPP
mmdlx3  .NY.DDPTNS...T.N.SG..LQQQPP...TL..A...

ggdlx3  PNPGAVY
mmdlx3  .......
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Fig. 1. Deduced amino acid sequence of the chicken DLX3 protein. The murine Dlx3 sequence (Robinson and Mahon, 1994) is given for comparison. The homeodomain is boxed, gaps introduced for maximal similarity are indicated by a hyphen, identical amino acids in the murine sequence are indicated by a dot. The EMBL Nucleotide Sequence Database Accession number for chick DLX3 is AJ 243432.

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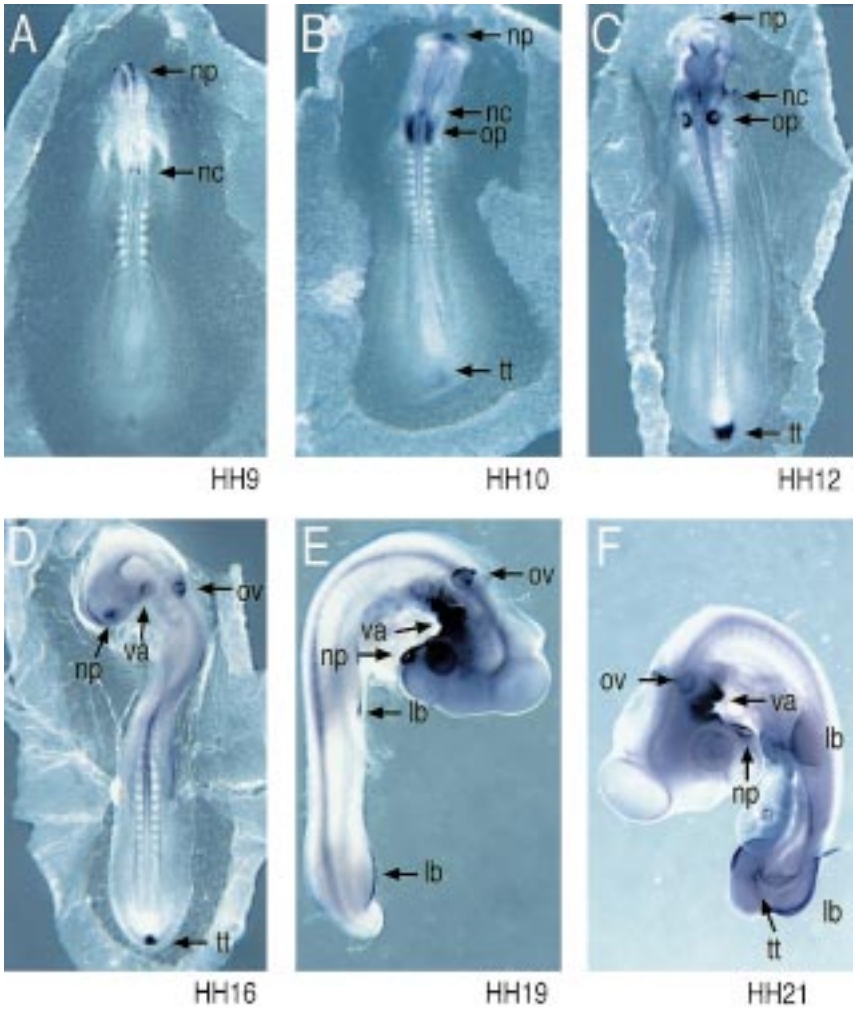

Fig. 2. Expression of DLX3 in chick embryos. Whole-mounted embryos hybridized to an anti-sense DLX3 probe containing the homeobox and the 3′ region (750 bases) are depicted in dorsal views (A–C). After turning, the posterior part of the embryos in (D) and (F) gives a ventral, in (E) a dorsal view. The slight blue staining in the forebrain visible in (C) is an artifact not due to expression. A full account of the expression patterns is given and discussed in the text. lb, limb bud; nc, neural crest; np, nasal placode (A–E) or nasal pit (F); op, otic placode; ov, otic vesicle; tt, tailtip; va, visceral arches.

2. Results

2.1. Isolation of chicken DLX3 cDNA

Overlapping DLX cDNA clones were isolated by low stringency screening from a Hamburger-Hamilton stage 9 (HH9; Hamburger and Hamilton, 1951) head library using a chick DLX5 probe (Pera and Kessel, 1997; Pera et al., 1999). The sequence predicts a homeodomain identical or with only one conservative change if compared to the domains encoded by the zebrafish dlx3 gene, the newt NvHBox-4 gene, the two *Xenopus laevis* Xldll2 alleles and the murine Dlx3 gene (Fig. 1; Ekker et al., 1992; Beauchemin and Savard, 1992; Papalopulu and Kintner, 1993; Dirksen et al., 1994; Robinson and Mahon, 1994). The sequence is deposited in the EMBL Nucleotide Sequence Database under accession number AJ 243432. The chicken DLX3 cDNA encodes a protein of 277 amino acids. The protein is highly related to the murine DLX3, as well as other DLX proteins, in particular DLX5 (Fig. 1).

2.2. DLX3 in the olfactory placode and pit

We analyzed DLX3 expression by whole mount in situ hybridization in chick embryos up to stage HH21. The first DLX3 transcripts were detected on the ridges of the anterior neural folds, which are elevated and fusing at stage HH9 (Fig. 2A). These areas are fated to become the olfactory placodes as demonstrated previously by chick-quail chimeras (Couly and Le Douarin, 1987). The two separated areas approach each other at HH10, when the neural pore is about to close, but demarcate two more lateral positions of surface ectoderm at HH16 and 19 (Figs. 2B–E and 3B,C). Histology reveals the significant thickening of the DLX3 expressing cells already at HH10, indicating the beginning of nasal placode formation (Fig. 3A). By HH21, the placodes are invaginating to form the nasal pits, which

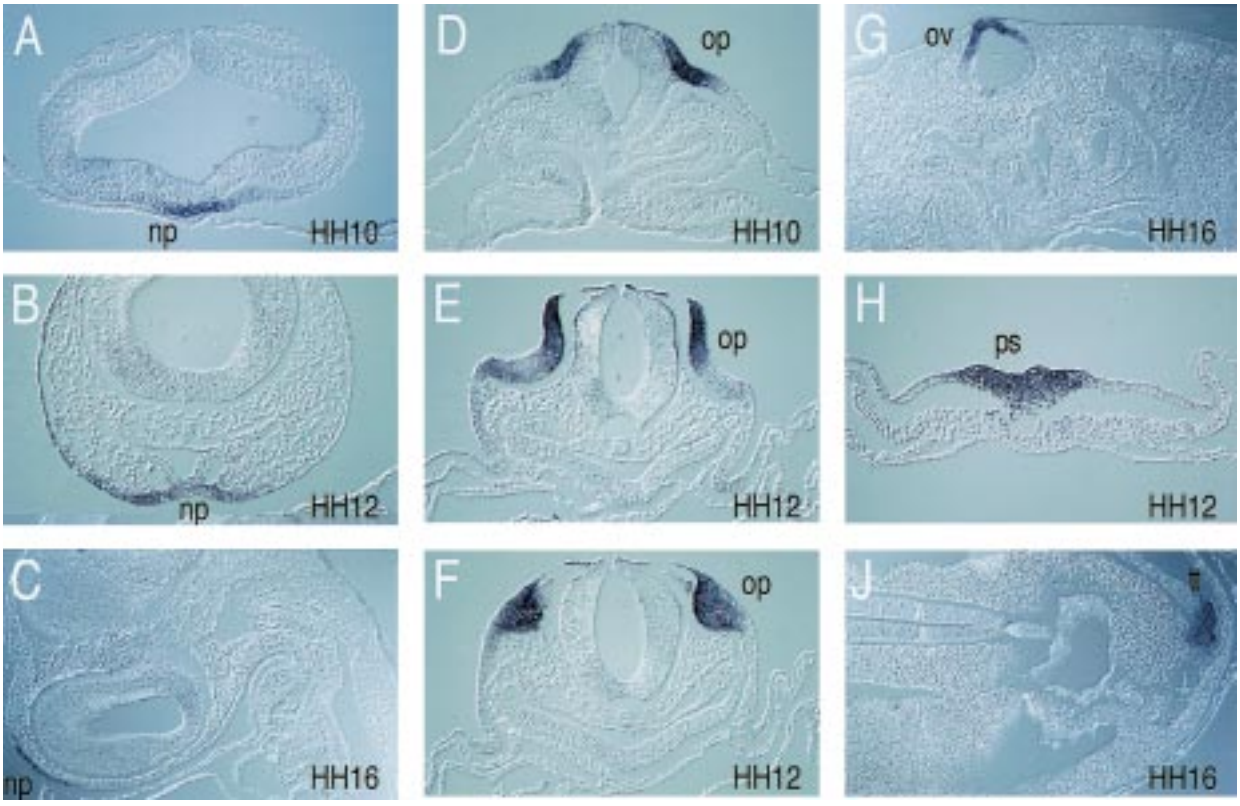

Fig. 3. Histology of the DLX3 expression domains. The development of the olfactory placode is illustrated in (A–C), and of the otic placode in (D–G). The posterior domain is shown in (H) and (J). np, nasal placode; op, otic placode; ov, otic vesicle; ps, primitive streak, tt, tailtip.

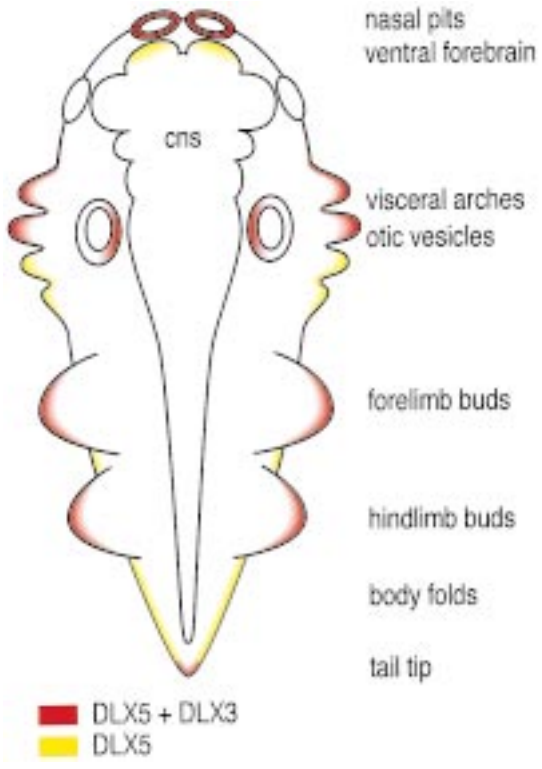

Fig. 4. Schematic view of DLX3 and DLX5 expression. This graphic representation was chosen in order to point out the common principle behind DLX3 and DLX5 gene expression.

maintain expression of DLX3 (Fig. 2F). This aspect of the DLX3 expression pattern resembles that of DLX5, however is spatially much more restricted, and the initiation of DLX5 transcription surrounding the anterior neural plate starts much earlier (Pera et al., 1999). DLX3 is never transcribed in any part of the central nervous system, in contrast to the DLX1,2,5 and 6 genes (Liu et al., 1997).

2.4. DLX3 in the otic placode and pit

The largest and strongest expression domains at HH10 are two paraxial regions of thickened, non-neural ectoderm, the otic placodes (Figs. 2B and 3D). Invagination is restricted to DLX3 expressing cells, which by HH12 form the still wide open auditory pits (Figs. 2C and 3E,F). By HH16, the openings of the auditory pits become constricted. DLX3 expression is maintained also after the formation of auditory vesicles, where transcripts are confined to the dorsal part (Figs. 2D and 3G). In comparison, the DLX5 pattern lacks the early phase of expression in the placodes, however is very similar once the otic vesicles are forming.

2.5. DLX3 in the visceral arches

At HH10, a small, medial/dorsal DLX3 domain lies anterior of the two otic placode domains (Fig. 2A). These are premigratory neural crest cells. They could include the precursors of neural crest cells found later in the anterior

visceral arches (Lumsden et al., 1991). Visceral arches 1 and 2 are clearly distinct by HH16 and show a restricted pattern of DLX3 expression in neural crest derived mesenchyme of the maxillary as well as the mandibular part of the first arch (Fig. 2D). Also, the ectoderm of the maxillary process contains DLX3 transcripts. By HH21, expression is observed both in the first and the second arch (Fig. 2F). In comparison, DLX5 transcription in the dorsal, medial hindbrain is much more extended in the anteroposterior direction, thus demarcating more of the prospective visceral arch neural crest cells (Pera et al., 1999). Consequently, DLX5 cells are found in all four of the branchial arches.

2.6. DLX3 in the tailtip

The most posterior tip of the chick embryo develops a small, narrow zone of DLX3 expression. It is very weak at stage HH10, strong at HH12 and HH16, and still recognizable by HH21 (Fig. 2B–F). It appears to represent the most posterior part of the primitive streak or tailbud, and certainly does not include the chordo-neural hinge, the remnant of Hensen's node at the anterior border of the tailbud (Le Douarin et al., 1998). In comparison, DLX5 labels the posterior primitive streak already from HH5 onwards. By HH16, DLX5 labeling extends into the posterior, lateral body folds.

2.7. Limb bud

DLX3 is strongly expressed in the apical ectodermal ridges and some underlying mesodermal cells of the growing limb buds in a pattern very similar to DLX5 as described by Ferrari and colleagues (Fig. 2E,F; Ferrari et al., 1995).

2.8. Conclusion: common aspects of DLX expression domains

The vertebrate Dlx genes show remarkable similarity to the *Drosophila* Distal-less gene which is required for the developing sensory organs and distal components of the appendages (Cohen and Jürgens, 1989). A common aspect of vertebrate Dlx genes is their expression in structures sticking out from, or peripheric to, the main body axis, including the prospective epidermis around the neural plate, ventral forebrain, the olfactory and otic placodes as well as their derivatives, the branchial arches, the limb buds, the bodyfolds and the posterior tip of the embryo (Fig. 4; Porteus et al., 1994). DLX5 and DLX3, a pair of highly related homeobox genes, are expressed in a nested Russian doll pattern, similar to those found for other subgroups of homeogenes. While DLX3 domains are mostly later and more restricted than DLX5 domains, this is reversed in the case of the otic placodes.

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