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Gene expression pattern

The expression of chick *EphA7* during segmentation of the central and peripheral nervous system

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Abstract

We have isolated a novel chick Eph-related receptor that corresponds to the *EphA7* gene. Within the nervous system, *EphA7* expression is restricted to prosomeres 1 and 2 in the diencephalon and all the rhombomeres in the hindbrain during segmentation stages. Later on, a superimposed pattern appears that correlates with the formation of several axonal tracts. In the somitic mesoderm, the expression correlates with segmentation and the guidance of both neural crest and motor axons through the sclerotomes. © 1997 Elsevier Science Ireland Ltd.

Keywords: Chick embryo; Eph; EphA7; Cek11; Neural plate; Neural tube; Forebrain; Diencephalon; Midbrain; Hindbrain; Prosomeres; Rhombomeres; Segmentation; Axon guidance; Neural crest migration; Somite; Somitomere; Sclerotome; Limb patterning; Mesenchyme; Apical ectodermal ridge; Digit; Joint

1. Introduction

The members of the Eph family of receptor tyrosine kinases and their ligands have been shown to play pivotal roles during embryonic development in vertebrates (for review see Nieto, 1996). We have identified a new member of this family that we have named *EphA7* according to the new proposed nomenclature (Eph Nomenclature Committee, 1997). Overlapping clones isolated from a 2-day-old (E2) chick embryo cDNA library contain the full-length open reading frame of the *EphA7* gene (Genebank accession number #Y14271). The sequence shows a very high degree of similarity to the mouse *MDK-1* (84%) (Ciossek et al., 1995; Ellis et al., 1995), the rat *Ehk-3* (85%) (Valenzuela et al., 1995), the human *HEK-11* (88%) (Fox et al., 1995) and the zebrafish *ZDK1* (Taneja et al., 1996) genes, as well as a similarity of around 60% with other members of the chick Eph family.

Analysis of the expression pattern by in situ hybridization showed that before neural tube closure, *EphA7* is expressed at the edges of the neural plate all along the anteroposterior axis (Fig. 1A,B), with higher levels at the presumptive forebrain and hindbrain regions. Expression becomes restricted to the prosencephalic and rhombencephalic regions before the morphological appearance of segments. During segmentation stages, *EphA7* transcripts are located at the anterior neuropore, the diencephalon and the hindbrain (Fig. 1C,D) and become restricted to the dorsal alar plate of prosomeres 1 and 2 (Puelles and Rubenstein, 1993) in the diencephalon and to the alar plate of all the rhombomeres in the hindbrain (Fig. 1E–G). The segmental expression appears prior to the morphological appearance of the segments, suggesting its implication in this patterning process as has been shown for *EphA4* (Sek1) in the *Xenopus* and zebrafish hindbrain (Xu et al., 1995).

At later stages of development a refinement of the expression pattern occurs. There is a superimposed pattern that could be related to the formation of several axonal tracts. In the diencephalon (Figs. 1F,G and 2A), the transcripts are observed in the proposed location for the posterior commissure (dorsal and caudal half of p1), the tectal commissure (rostral midbrain, arrow in Fig. 1F) and at the region of the p2/p3 boundary (according to Puelles and Rubenstein, 1993; D1/D2 boundary according to Figdor and Stern, 1993). The

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latter contains the tract of the zona limitans intrathalamica, which is penetrated by axons ascending from the dorsal thalamus (p2) to the telencephalon.

In the hindbrain, *EphA7* expression is downregulated in the rhombomeric boundaries at stage 13–14, just before the transversely orientated reticular axons preferentially develop their tracts at these boundaries between the segments (Lumsden and Keynes, 1990) (Fig. 2C–E). At stage 18, transcripts are detected both in the alar and in the basal plates drawing longitudinal columns (Fig. 2D), adjacent to the position of neuron-specific β-tubulin positive columns (Fig. 2E). These columns correspond to the lateral (llf) and medial (mlf) longitudinal tracts (Clarke and Lumsden, 1993).

Both in the diencephalon and in the hindbrain, EphA7 transcripts appear at the location of the axonal tracts long before these can be detected with anti-acetylated tubulin antibodies (compare Fig. 2A,B and D,E), as would be expected for a molecule involved in axon guidance. Other members of the Eph family and their ligands have been implicated in axon guidance in the central nervous system, both in the formation of topographic maps in the retinotectal and the septohippocampal systems (Cheng et al., 1995; Drescher et al., 1995; Nakamoto et al., 1996; Zhang et al., 1996; Monschau et al., 1997) and in the formation of brain commissures (Henkemeyer et al., 1996; Orioli et al., 1996).

Outside of the nervous system there is a very dynamic expression pattern in the somitic mesoderm. During early somitogenesis, bands of expression can be detected in the dorsal part of the newly formed somites and the first somitomere (Fig. 3A–C). These bands of expression are reminiscent of those of *EphA4* (Sek-1) in the forming somites (Nieto et al., 1992) suggesting again the involvement of this gene family in the process of segmentation of both the ectoderm and the mesoderm. In more mature somites, expression of *EphA7* is observed in the caudal half of the sclerotome and at very low levels in the dermamyotome (Fig. 3D–F). Double labelling with the HNK-1 antibody (a marker for migratory neural crest cells, Fig. 3D–F) and with the neuron-specific β-tubulin antibody (not shown) delineates the domain of *EphA7* expression as complementary to the region of neural crest migration and motor axons outgrowth. Several ligands of Eph receptors have been implicated in the migration of the branchial and trunk neural crest and in the guidance of the spinal motor axons through the anterior half of the sclerotomes (Krull et al., 1997; Smith et al., 1997; Wang and Anderson, 1997).

Another site of prominent expression is the limb bud, where transcripts appear at stage 18 in the apical ectodermal ridge (open arrowheads in Fig. 3A) and in the dorso-proximal mesenchyme of the limb bud from stage 23 onwards, advancing distally during limb outgrowth (Fig. 4A,B). Later on in development, transcripts can be observed in the joints between the bones (Fig. 4C–D).

Other sites of expression include the developing retina (Sefton et al., 1997), the olfactory and branchial placodes (dFig. 1F), the mesenchyme around the spinal cord and the brain and the mesonephric kidney (data not shown). It is also worth noting that *Eph A7* expression is dorsally restricted in different tissues, such as the mesenchyme of the limb, the dorsal part of condensing somites, the dorsal retina and the dorsal neural tube.

2. Methods

2.1. PCR screening and cDNA isolation

We utilized degenerate primers corresponding to conserved sequences within the catalytic domain of receptor tyrosine kinases to amplify fragments of cDNA reverse transcribed from mRNA isolated from embryos ranging from stages 12 to 15. All embryos were staged according to Hamburger and Hamilton (1951). The sequences of the primers used were as follows: TK1, 5′ GCGGGATCC-CGC$^{A}/_{G}$TNCA$^{C}/_{T}^{A}/_{C}$GNGA$^{C}/_{T}^{C}/_{T}$T 3′ and TK2, 5′ GCG-CTGCAGCGCCC$^{A}/_{G}^{A}/_{T}$AN$^{G}/_{C}^{A}/_{T}$CCANAC$^{A}/_{G}$TC 3′.

The amplified cDNAs, fragments between 210 and 240 bp in length, were cloned into the pGEM T-vector (Promega) and sequenced. The *EphA7* fragment was used to screen a 2-day-old chick embryo cDNA library to obtain the full-length cDNA.

2.2. In situ hybridization and immunohistochemistry

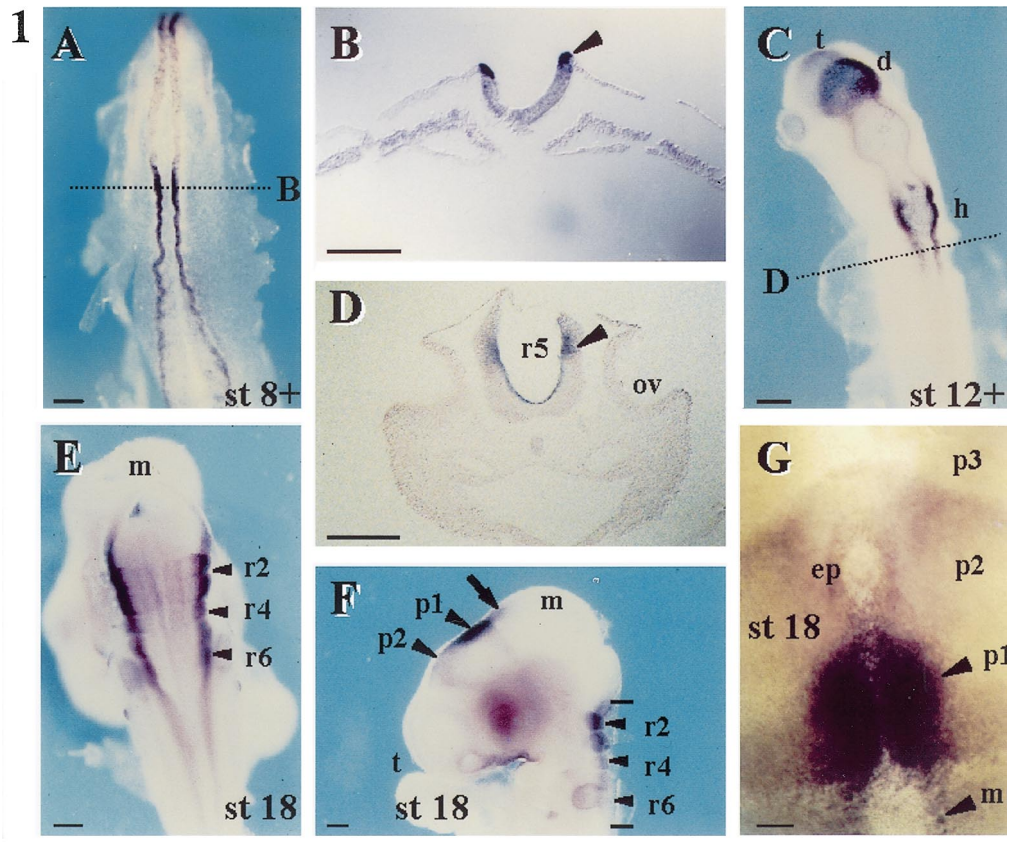
Both single and double labelling protocols were carried out in whole mount as previously described (Nieto et al., 1996). The digoxigenin labelled probe was synthesized from a 716 bp fragment of the complete cDNA (nucleotides 2757–3473). The anti-acetylated tubulin antibody (1:500) used corresponds to the TuJ1 antibody described by Moody et al. (1987). In some cases, following hybridization, the embryos were embedded in fibrowax and sectioned at 15 mm.

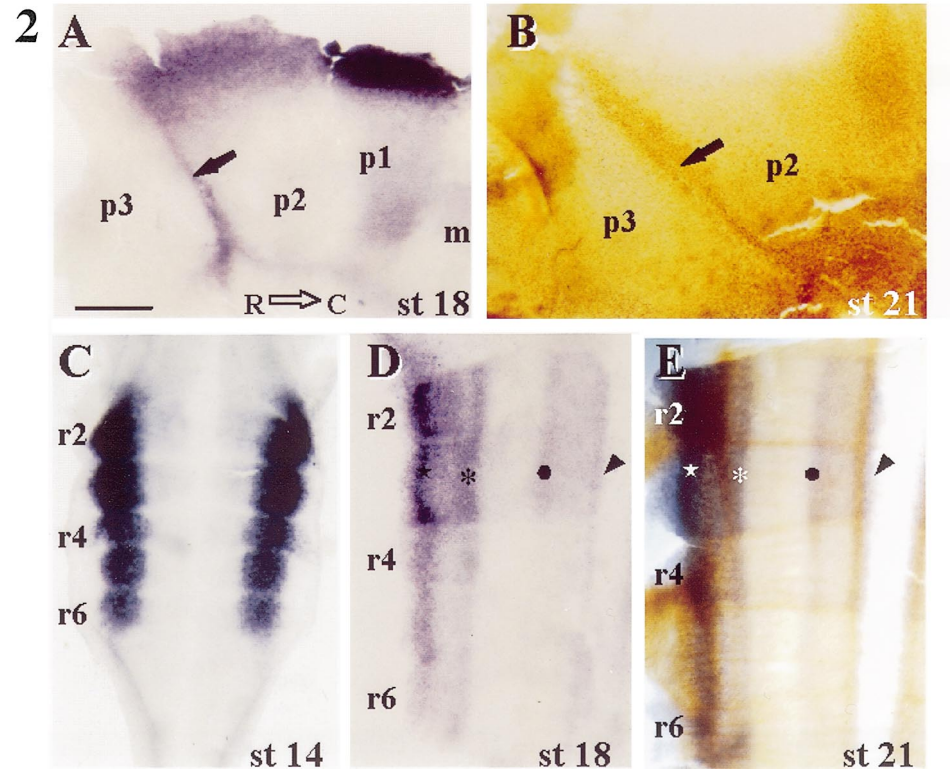
Fig. 1. Expression pattern of *EphA7* in the developing nervous system. Expression is detected at the edges of the neural plate (A,B) and, later on, in the diencephalon and the hindbrain (C–G). Developmental stages are indicated in the photographs. The dashed lines in (A,C) indicate the approximate positions of the sections shown in (B,D), respectively. d, diencephalon; ep, epiphysis; h, hindbrain; m, midbrain; ov, otic vesicle; p, prosomere; r, rhombomere; t, telencephalon. Bar, 250 μm.

Fig. 2. Correlation between *EphA7* expression and the formation of early axonal tracts. Transcripts appear at the presumptive location of several axonal tracts (see text). Arrows in (A,B) indicate the p2/p3 boundary. Symbols in (D,E) indicate the position of the corresponding longitudinal columns of *EphA7* expression. m, midbrain; p, prosomere; r, rhombomere; C, caudal; R, rostral. Bar, 250 μm.

2.3. Additional information

A preliminary description of the *c-EphA7* expression pattern in the central nervous system was presented in a poster at the 1st Congress of the Spanish Society of Developmental Biology and the abstract published in the Int. J. Dev. Biol.





(Araujo and Nieto, 1996). The pattern of expression of *EphA7* together with that of other Eph family members in the developing retina has been reported elsewhere (Sefton et al., 1997). In these reports the gene was referred to as *Cek11*, according to the previous nomenclature for chick embryonic kinases (Pasquale, 1990).

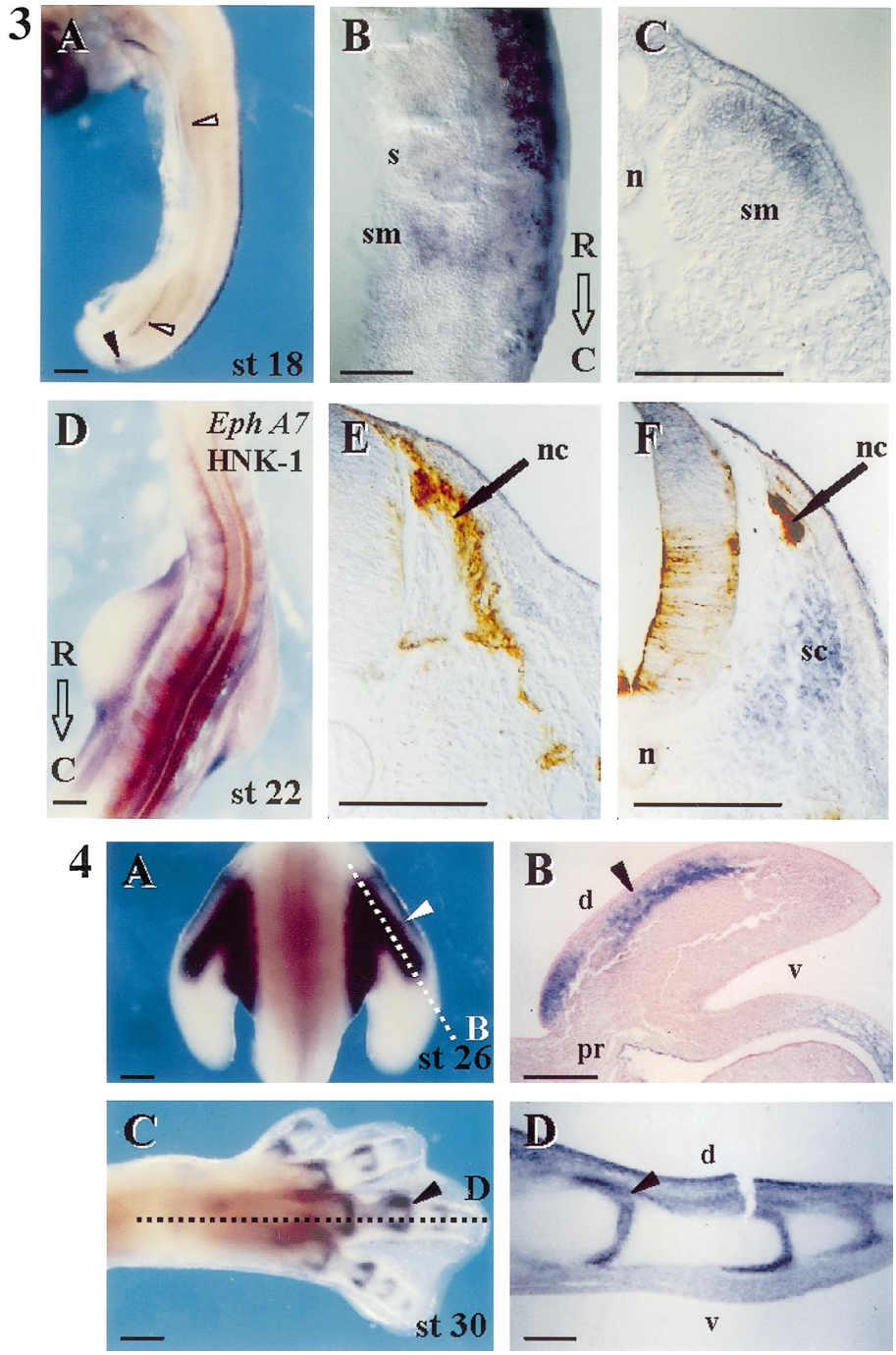


Fig. 3. Expression of *EphA7* in the developing somites. (A) Embryo showing the expression in the forming somites (black arrowhead). (B) Flat mounted tail and (C) transverse section through the first somitomere. (D) Double labelled embryo showing *EphA7* expression (blue) and the migratory trunk crest cells (HNK-1 immunoreactivity, brown). (E,F) Sections taken from the embryo shown in (C) at the anterior (E) or posterior (F) half levels of somites. n, notochord; nc, neural crest; s, somite; sc, sclerotome; sm, somitomere. Bar, 250 μm.

Fig. 4. *EphA7* pattern of expression in the developing limb buds. Whole mounted (A,C) and section preparations (B,D) of the limb buds at stages 26 (A,B) and 30 (C,D). The dashed lines in (A,C) indicate the position of the sections shown in (B,D), respectively. d, dorsal; v, ventral; pr, proximal. Bar, 250 μm.

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