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Gene expression pattern

Embryonic expression of a $P2X_3$ receptor encoding gene in zebrafish

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Abstract

From studies performed primarily in mammals, it is thought that the $P2X_3$ purinoreceptor is involved in mediating sensory and nociceptive signals in adult tissues. However, little is known concerning the expression or function of P2X family genes during early development. Here we describe the expression of a gene (*p2x3*) encoding a $P2X_3$ receptor during zebrafish development. We find that zebrafish *p2x3* is expressed in the anlage of the trigeminal ganglion from very early stages of development, most likely in neural crest derived trigeminal cells as opposed to placode derived cells. *p2x3* is also expressed in the spinal sensory Rohon-Beard cells and in the putative posterior lateral line ganglion.

Keywords: ATP gated ion channel; Rohon-Beard cells; Trigeminal; HNK-1; Posterior lateral line ganglion

1. Results

The $P2X_3$ receptor belongs to a group of ATP gated ion channels (purinoceptors) that are characterized by two transmembrane domains with a large extracellular loop and intracellular C and N terminals. $P2X_3$ has been extensively studied with respect to function and pharmacology in adult neural tissues (Burnstock, 2000; Garcia-Guzman et al., 1997). The adult expression of $P2X_3$, as studied in mouse, rat, and human, is restricted to specific groups of neural cells (Bradbury et al., 1998; Eriksson et al., 1998; Xiang et al., 1998) that include the nociceptive neurones of the trigeminal and dorsal root ganglia (DRG), and a subset of axon terminals in the dorsal spinal cord. Immunohistochemical studies suggest that $P2X_3$ expression in the trigeminal ganglion may be specific to neural crest derived neuronal subtypes (Chen et al., 1995).

Little has been done to characterize P2X genes during development and in the present study we describe the embryonic expression pattern of a gene encoding $P2X_3$ in zebrafish. An EST clone (Genbank accession numbers AI588308 and AI588766) was obtained from the Washington University zebrafish EST project (W.U. Zebrafish Genome Research database: www.wustl.wuzgr.edu). DNA sequence analysis shows that the clone is 1724 bp long and contains an open reading frame of 416 amino acids, encoding for a putative full length cDNA. A similarity tree based on the amino acid sequences of various P2X genes confirms that the zebrafish gene encodes for a purinoceptor. The highest homology was seen to both the human and rat $P2X_3$ receptors (Fig. 1). The pharmacological properties of this protein have recently been investigated in cell culture (Egan et al., 2000).

Expression of the zebrafish *p2x3* gene starts around 6 somite stage in two lateral stripes adjacent to the dorsal neural keel and caudal to the eye (Fig. 2A,B). This position is consistent with expressing cells being cranial neural crest. A pair of bilateral spots of stronger expression at the posterior end of the stripes (between the developing eye and ear) persists throughout development (Fig. 2A,C,F,I). At around 12 somite stage (Fig. 2C–H), weak and transient expression is visible in developing cells of the rostral diencephalon and telencephalon (Fig. 2G), and in scattered cells within the spinal cord (Fig. 2E,H). Both sites of expression disappear shortly after this stage, although it is possible that either all, or a subset of the cells in the trunk are early Rohon-Beard cells.

During late somitogenesis, expression in sensory Rohon-Beard neurones in the spinal cord becomes apparent (Fig. 2I). Rohon-Beard neurones are easily recognizable due to their prominent size and position in the dorsal neural tube (Grunwald et al., 1988; Metcalfe et al., 1990). *p2x3* expression at this site decreases over time consistent with apopto-

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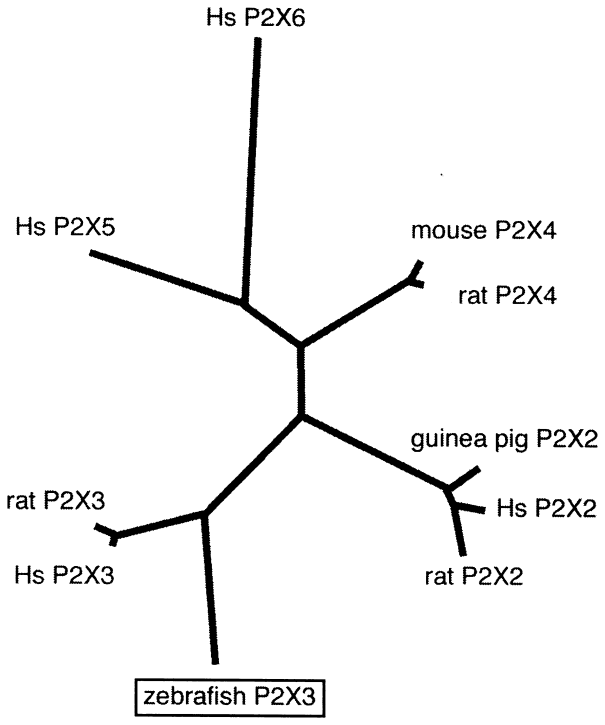

Fig. 1. Similarity tree showing the relationship between $P2X_3$ and other members of the P2X family of ATP gated ion channels. The accession numbers are: $P2X_3$ zebrafish AF239292, $P2X_3$ human AB016608, $P2X_3$ rat AF084975, $P2X_2$ rat AF020756, $P2X_2$ human AF190822, $P2X_2$ guinea pig AF05332, $P2X_4$ rat U47031, $P2X_4$ mouse AF089752, $P2X_6$ human AF065385, $P2X_5$ human AF016709. The tree is based on an alignment of amino acid sequences performed with CLUSTAL W. The tree has been generated using the maximum likelihood method/puzzle (Strimmer and von Haeseler, 1996). Abbreviations: Hs, human.

tic depletion of Rohon-Beard neurones during the second day of development (see Williams et al., 2000). A double staining with Islet -1 antibody, which is known to label both Rohon-Beard neurones and primary motorneurones, confirms this identity (Fig. 2J,K).

The domain of *p2x3* expression between the eyes and ears elongates slightly at 20 hpf, frequently fragments into two groups of cells around 24 hpf (Fig. 2I; arrows) and coalesces again to a single domain by around 30 hpf (Fig. 3A). By 50 hpf (Fig. 3B,C; arrows), the rostral domains of expression are adjacent to the eye and a second pair of bilateral expression domains appears at the anterior border of the first somite, just caudal to the ear. This is the position occupied by the posterior lateral line ganglion (Metcalfe et al., 1985) suggesting late *p2x3* expression may be within sensory neurones of this cranial ganglion.

To determine the identity of cells expressing *p2x3* between the eye and the otic vesicle, we combined in situ hybridization with both HNK1 and Islet -1 antibody staining (Metcalfe et al., 1990; Korzh and Thor, 1993). HNK1 antibody labels developing neurones – initially trigeminal ganglion neurones, later Rohon-Beard cells and eventually many CNS and PNS neurones. Double staining with HNK1 reveals that the bilateral sites of *p2x3* expression are cells of the trigeminal ganglion (Fig. 3D), but mostly not the differentiated HNK1 expressing neurones themselves (Fig. 3E,F).

Islet -1 immunostaining labels the nuclei of primary neurones (Korzh and Thor, 1993). Double staining with anti Islet -1 antibody shows that a subset of Islet -1 positive trigeminal cells coexpress *p2x3* and this is consistent with HNK1 double staining (Fig. 3G). It is known that the trigeminal ganglion is composed of both placodal and neural crest derived cells (reviewed for chick by Noden, 1993; for mammals by Verwoed and van Oostrom, 1979). The localization of p2x3 transcripts to HNK1 negative cells is consistent with expression being restricted to the neural crest derived component of the trigeminal ganglion, as early differentiating neurones derive from the placodal lineage, whereas the neural crest give rise to later differentiating neurones and glia (Moody et al., 1989; D'Amico-Martel and Noden, 1983). The neural crest origin of *p2x3* expressing trigeminal cells is also supported by the early lateral stripes of expression which are in the right place to be migratory neural crest that could include the cells that later express $P2X_3$ in the trigeminal ganglion.

We further analyzed *p2x3* expression in a zebrafish mutant, *narrowminded* (Artinger et al., 1999). The mutation leads to a reduced number of early neural crest cells and eliminates all Rohon-Beard neurones. Embryos (24 hpf) show little response to touch, reduced pigmentation and a reduction in the size of the trigeminal ganglion as only placode derived cells are present (Artinger et al., 1999). In situ hybridization on 24 hpf *narrowminded* mutants shows a lack of *p2x3* expression in both the trigeminal anlage and Rohon-Beard neurones (Fig. 3H,I), confirming the identity of *p2x3* expressing trigeminal cells as neural crest derivatives.

In summary, embryonic zebrafish $P2X_3$ expression in putative neural crest derived trigeminal cells correlates well with expression in the adult trigeminal ganglia in mammals (Chen et al., 1995). Expression in Rohon-Beard neurones is consistent with a role for $P2X_3$ in many primary sensory neurones. Rohon-Beard neurones are not present in higher vertebrates but in embryonic and larval fish and frogs these CNS cells mediate sensory responses prior to the differentiation of the dorsal root ganglia (Clarke et al., 1984).

2. Experimental procedures

In situ hybridizations were performed according to standard procedures (MacDonald et al., 1997). The HNK1 antibody (Sigma) was used as described in Metcalfe et al. (1990). Combined *p2x3* and HNK1 staining was carried out sequentially with a short fixation step after the in situ hybridization. The *narrowminded* mutant allele is m805 (Artinger et al., 1999).

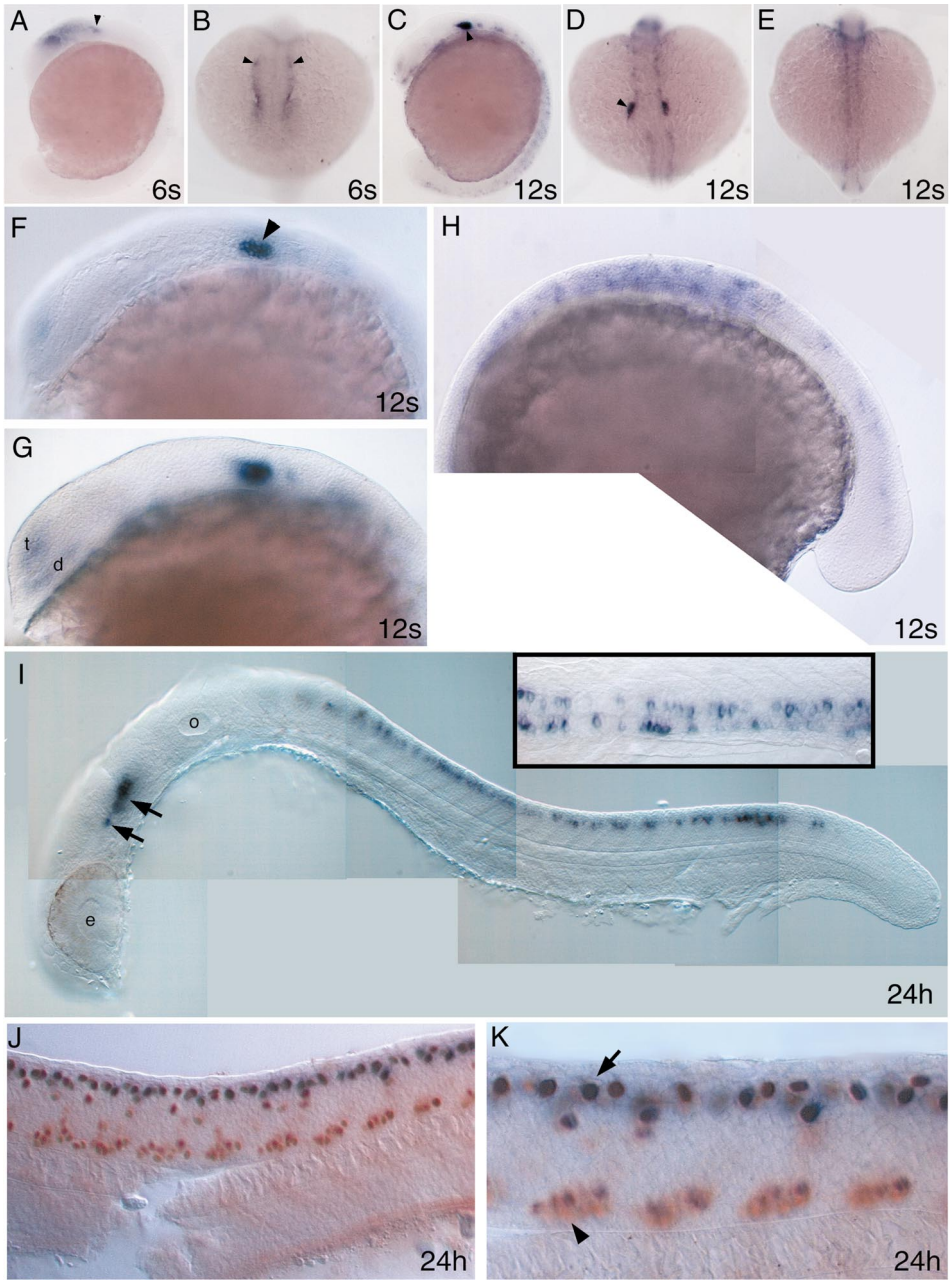

Fig. 2. Early expression of *p2x3* in putative central and peripheral neural cells. Lateral (A,C,F–K) and dorsal (B,D,E, with anterior down) views of embryos showing *p2x3* expression at the stages indicated. (A,B) Expression in dorsal cells adjacent to the midbrain and anterior hindbrain. The arrowheads indicate spots of higher expression. (C–H) Twelve somite stage (12 s) embryos showing expression in putative trigeminal ganglion cells (arrowheads, C,D,F) in the forebrain (G) and in various spinal cord cells (E,H). (I) Twenty-four hour embryo in which expression in the putative trigeminal ganglia cells has condensed to two spots (arrows) and in which expression in dorsal Rohon-Beard neurons is prominent (the inset shows a dorsal view of the spinal cord). (J,K) Twenty-four hour embryos showing *p2x3* expression (in blue) and Islet -1 antibody stain (in brown). Ventral brown cells are primary motorneurones (arrowhead), and dorsal black cells show double labelled Rohon-Beard cells (arrow). Abbreviations: e, eye; d, ventral diencephalon; o, otic vesicle; t, telencaphalon.

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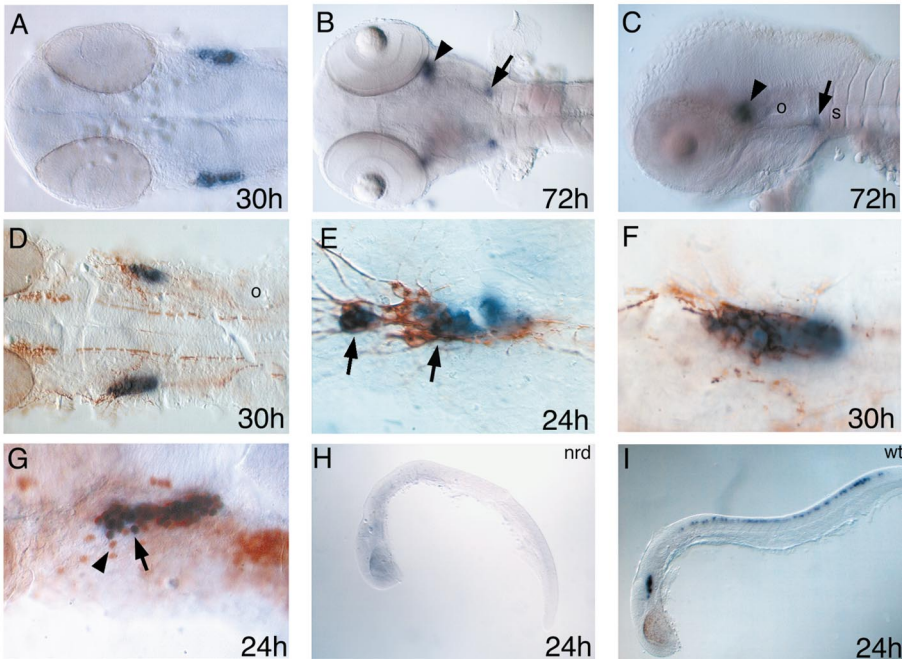

Fig. 3. *p2x3* is expressed in trigeminal cells and in putative posterior lateral ganglion cells. Dorsal (A,B,D–G with anterior to the left) and lateral (C,H,I) views of embryos showing *p2x3* expression (in blue) and HNK1 antibody labelling (in brown) at the stages indicated. A–C) Expression in the trigeminal ganglia (A) and the posterior lateral line ganglia (B,C). The arrowheads indicate the trigeminal ganglia and the arrows the posterior ganglia. (D–F) Double labelling of *p2x3* and HNK1 in the trigeminal ganglia. Arrows show the split domains of *p2x3* expression in the trigeminal ganglia (E). (G) Double labelling of *p2x3* and Islet -1 in the trigeminal ganglion. An Arrowhead indicates a putative placode derived cell that only expresses Islet -1 and the arrow points to a double labelled putative neural crest derived cell. (H,I) Lateral views of *p2x3* expression in both a *narrowminded* mutant (H) in which no staining can be seen and a wildtype sibling (I) which shows normal expression. Abbreviations: o, otic vesicle; s, first somite.

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