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Expression of *cadherin10*, a type II classic cadherin gene, in the nervous system of the embryonic zebrafish

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Abstract

Cadherins are cell surface adhesion molecules that play important roles in development of tissues and organs. In this study we analyzed expression pattern of cadherin10, a member of the type II classic cadherin subfamily, in the embryonic zebrafish using in situ hybridization methods. *cadherin10* message (*cdh10*) is first and transiently expressed by the notochord. In the developing nervous system, *cdh10* was first detected in a subset of the cranial ganglia, then in restricted brain regions and neural retina. As development proceeds, *cdh10* expression domain and/or expression levels increased in the embryonic nervous system. Our results show that *cdh10* expression in the zebrafish developing nervous system is both spatially and temporally regulated.

Keywords

zebrafish; development; cell adhesion molecules; brain; cranial and lateral line ganglia

1. Results and Discussion

Zebrafish has emerged as an important model system to study animal development, especially neural development, due to its numerous experimental advantages including external embryonic development, transparency of the embryos, and its demonstrated utility as a genetic model. The formation of the zebrafish central nervous system (CNS) is similar to other vertebrates in that it involves the partitioning of the anterior portion of the neural tube into three vesicles called the forebrain, midbrain and hindbrain, and subsequent development further divides these vesicles into smaller units, the telencephalon, diencephalon, midbrain and hindbrain (reviewed by Kimmel, 1993).

The zebrafish cranial ganglia originate from neurogenic neural crest precursors and cranial epidermal thickenings called placodes (Metcalf 1985; Andermann et al., 2002). The cranial and lateral line ganglia, situated ventrolateral to the hindbrain and around the otic placode/vesicle, include the trigeminal, facial, statoacoustic, glossopharyngeal, and vagal ganglia, anteriodorsal, anteroventral, medial and posterior lateral line ganglia (Metcalf, 1985; 1989; Raible and Kruse, 2000). The molecular mechanisms underlying the regionalization of the CNS have been under intense investigation, while the molecular mechanisms underlying the formation of the cranial and lateral line ganglia are largely unknown. Specific regulatory molecules (Puelles and Rubenstein, 1993; Macdonald et al., 1994; Mastick et al., 1997; Hauptmann and Gerster, 2000; Andermann and Weinberg, 2001; Andermann et al., 2002) and

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cell adhesion molecules including members of the cadherin family (Inoue et al., 1997, 1998, 2001; Redies, 2000; Kerstetter et al., 2004) have been implicated in the formation of these structures.

The cadherins are Ca^{2+} -dependent cell adhesion molecules that play important roles in animal development. Type-I classic cadherins have a conserved His-Ala-Val (HAV) sequence in their extracellular domain one (EC1), and have a high degree of protein sequence similarity to E-cadherin (Cdh1), while type-II classic cadherins do not have the HAV sequence in their EC1, and they are more similar in protein sequence to cadherin11 (reviewed by Nollet et al., 2000). Classic cadherins are integral membrane proteins that mediate cell adhesion mainly through homotypic interactions (Takeichi, 1991; Gumbiner, 1996). Cadherin expression is spatially and temporally regulated, and functional studies of several classic cadherins (e.g. cadherin2 and cadherin7) have shown that these molecules play essential roles in the development of the vertebrate nervous system (Nakagawa and Takeichi, 1995; Radice et al., 1997; Lele et al., 2002; Malicki et al., 2003; Masai et al., 2003). Cadherin10 (Cdh10) is a member of the type-II cadherin subfamily (Redies, 1995; Nollet et al., 2000). Information on Cdh10/*cdh10* expression in the vertebrate is relatively scarce compared to most other classic cadherins. So far Cdh10/*cdh10* distribution pattern has been examined in chicken central nervous system (Fushimi et al., 1997), in rat limbic system (Bekirov et al., 2002) and in human total RNA from various tissue samples (Kools et al., 1999). To the best of our knowledge, there is no information on Cdh10/*cdh10* expression in the vertebrate peripheral system. Zebrafish *cdh10* cDNA sequence and complete amino acid sequence (predicted by automated computational analysis) were recently published in GenBank (accession number: **XM691696**), but there is no published report on *cdh10* expression in developing zebrafish.

To confirm the predicted GenBank zebrafish *cdh10* cRNA sequence, we performed reversed transcriptase-polymerase chain reaction (RT-PCR) and 5'-rapid amplification of cDNA ends (5'RACE) using total RNA from 50 hpf zebrafish embryos and zebrafish *cdh10* specific primers (see Experimental procedures). The resulting open reading frame produces a protein of 785 amino acid residues containing a putative hydrophobic signal sequence, presequence, extracellular domains, transmembrane and cytoplasmic domains (Fig. 1). An alignment of related cadherin sequences shows that the zebrafish *cdh10* is most similar to human, mouse, chicken *cdh10* and *cadherin6* (*cdh6*) at the amino acid level, while showing little similarity to *cdh1*, a type I cadherin (Fig. 2A and B). Phylogenetic analysis (Fig. 3) reveals that the zebrafish *cdh10* and *cdh6* sequences are divergent from their *cdh10* and *cdh6* counterparts in mouse, human and chicken with amino acid similarities from EC1 through the end of the cytoplasmic domain of only 63 to 67 for *cdh6* and about 67 for *cdh10*. Though genetically divergent from other evolutionarily distant *cdh10* sequences (Fig. 3) the zebrafish *cdh10* sequence is likely orthologous to the mammalian *cdh10* as the expression profile reported below suggests.

Using whole mount in situ hybridization methods, we analyzed expression of *cdh10* in embryonic zebrafish from 12 hours post fertilization (hpf) to 52 hpf. There was no *cdh10* detected in zebrafish embryos at 12-14 hpf (Fig. 4A). The earliest expression of *cdh10* was found in 18 hpf embryos. At this stage *cdh10* was expressed in the notochord of the trunk region, but not in the tail region (Fig. 4B and C). *cdh6*, a closed related type II cadherin, was expressed in the spinal cord and mesenchymal tissue below the notochord, but not in the notochord (Liu et al., 2006; Fig. 4F). There was no *cdh10* expression in the notochord of older embryos (24 to 52 hpf, Figs. 4G and K, 6A). Except the brief expression in the notochord and later (48-52 hpf) in the first pharyngeal arch (Fig. 6C), *cdh10* was confined to the nervous system in the embryonic zebrafish (see below).

1.1. *cdh10* expression in the cranial and lateral line ganglia

In the zebrafish peripheral nervous system, *cdh10* was first detected at 18 hpf in two regions: the anterior lateral line placode area, located anterior to the otic placode, and the posterior lateral line placode/ganglion, situated caudal to the otic placode (Fig. 4D). Compared to *cdh6* expression in these structures (Fig. 4E), *cdh10* expression domains in these two regions were smaller (Fig. 4D), and *cdh10* was expressed at a later stage (18 hpf) in these two regions than *cdh6* (14 hpf) (Liu et al., 2006). Part of the anterior lateral line placode area becomes the statoacoustic ganglion at about 24 hpf (Andermann et al., 2002). This ganglion was intensely labeled with the *cdh10* cRNA probe (Fig. 4J), while the posterior lateral line ganglion continued expressing *cdh10* message (Fig. 4J).

At 34 hpf, the anterodorsal and anteroventral lateral line ganglia, the other two derivatives of the anterior lateral line placode area, become distinguishable (Andermann et al., 2002), *cdh10* was expressed in the anteroventral lateral line ganglion, not in the anterodorsal lateral line ganglion (Fig. 5A). At this stage the medial lateral line ganglion and the vagal placode/ganglion also became *cdh10* positive, while the statoacoustic ganglion and the posterior lateral line ganglion continued expressing *cdh10* (Fig. 5A). This expression pattern was similar to *cdh6* expression at this stage (Fig. 5B; Liu et al., 2006), except that *cdh10* expression domains were smaller than *cdh6*. By 52 hpf, the statoacoustic ganglion continued expressing *cdh10* (Fig. 6C and F). *cdh10* expression was also detected in the posterior lateral line ganglion, while its expression in the anteroventral lateral line ganglion (fused with the facial ganglion) became undetectable (Fig. 6F).

To the best of our knowledge, this is the first report of *cdh10* expression in the vertebrate peripheral nervous system. *cdh10* expression in the cranial and lateral line ganglia was distinct from *cdh2* (*n-cadherin*) and *cdh4* (*r-cadherin*) (Liu et al., 2003), and similar (spatially, see below) to *cdh6*. Similar to human *cdh10* (Kools et al., 1999), the zebrafish *cdh10* shows a higher degree of similarity at the amino acid level to *cdh6* than any other known cadherins. The similar expression patterns of these two cadherins in the developing zebrafish cranial and lateral line ganglia suggest possible cross-hybridization of their cRNA probes. This is unlikely due to the following reasons. First, despite their similar expression patterns in the cranial and lateral line ganglia in space, their temporal expression was distinct (e.g. the onset of *cdh10* expression is about 4 hours later than *cdh6* in the peripheral nervous system). Second, these two probes label distinct sets of structures in the central nervous system and non-neural tissues. Examples include their labeling or lack of labeling in the spinal cord and notochord at 18 hpf (Fig. 4), their different labeling patterns in the brain (see below), head mesenchymal and nephric tissues (Fig. 4; Liu et al., 2005). Third, the *cdh10* and *cdh6* cDNA templates used to generate their respective cRNA probes share only 52% identity.

1.2. *cdh10* expression in the central nervous system

There was no *cdh10* expression in the central nervous system (CNS) of zebrafish at 18 hpf and younger (Fig. 4). By 24 hpf, viewing laterally, *cdh10* was detected in three thin strips, all somewhat parallel to the optic recess, in the forebrain (Fig. 4H). These strips became more distinct at 34 hpf (Fig. 5). The first stripe consisted of *cdh10* positive cells located in the lateral regions of the central telencephalon, occupying a similar position as the anterior commissure viewing laterally (Figs. 4H and I, 5F and H). *cdh10* positive cells in the second strip were found in the lateral regions of the central thalamus (Fig. 4H, 5F), appearing to be situated in the same position as the postoptic commissure (Figs. 4H and I, 5F and H). The *cdh10* expression domain in the hypothalamus (the third strip) extended the entire width of this region (Fig. 5F and K). This hypothalamic expression pattern was similar to *cdh6* expression in the hypothalamus

except that the *cdh10* positive strip was thinner than the *cdh6* expressing strip (Fig. 5F and G; Liu et al., 2006).

cdh10 expression in other brain regions were different from *cdh6*. There was no obvious *cdh6* expression in the central telencephalon and thalamus at this stage (34 hpf). But *cdh6* was expressed in the pineal organ, cerebellum and dorsal hindbrain where there was no *cdh10* expression (Fig. 5F and G). *cdh10* expression was also detected in bilaterally located three small clusters of cells in the ventrolateral hindbrain (Fig. 5A, D and E). The first, second and third clusters appeared to be located within the rhombomere 1, rhombomere 2 and rhombomere 4, respectively (Fig. 5A, C, D and E). *cdh4*, a member of the type I classic cadherins, also exhibits segmental expression pattern in the zebrafish hindbrain, but *cdh4* is expressed by larger clusters of cells in all rhombomeres at this stage (Liu et al., 2001). The neural retina (Fig. 5I-K) and spinal cord contained no *cdh10* at this stage (data not shown).

By 52 hpf, *cdh10* expression in the brain greatly increased (Fig. 6). In the telencephalon, *cdh10* positive cells were located mainly in two small clusters in the dorsal region and in the posterior telencephalon immediately anterior to the optic recess (Fig. 6B). In the diencephalon, *cdh10* expressing cells were found mainly in the dorsal thalamus including the pretectal area (Fig. 6D), while in the hypothalamus, *cdh10* expression became confined to two clusters in the central hypothalamus (Fig. 6B and D). The midbrain had become intensely labeled with the *cdh10* cRNA probe (Fig. 6C, D and F). Both the optic tectum and the tegmentum contained many *cdh10* positive cells. The cerebellum began to express *cdh10* (Fig. 6F). In the hindbrain, *cdh10* was strongly expressed by the lateral central portion of the anterior hindbrain dorsal to the statoacoustic ganglion (Fig. 6C, F and G). In the retina, *cdh10* was detected in the retinal ganglion cell layer and the inner most region of the inner nuclear layer (Fig. 6E).

cdh10 expression in the embryonic zebrafish is similar to the situation in the chicken and humans in that *cdh10* is mainly expressed by the nervous tissues, it reflects the neuromeric organization of the early chicken brain, it exhibits restricted spatial expression at all developmental stages, its expression in all major brain regions and by a subset of neural circuits such as the auditory circuit (e.g. the statoacoustic ganglion and the hindbrain area immediately dorsal to the ganglion) and visual circuit (e.g. the retinal ganglion cells and their major projecting target the optic tectum) (Fushimi et al., 1997; Kools et al., 1999). Moreover, similar to the chicken (Fushimi et al., 1997) and rat (Bekirov et al., 2002) *cdh10* expression in the zebrafish CNS is distinct from expression patterns of other classic cadherins such as *cdh1* (Babb et al., 2001), *cdh2* (Bitzur et al., 1994), *cdh4* (Liu et al., 1999) and *cdh6* (Liu et al., 2006).

2. Experimental procedures

Zebrafish embryos, obtained from in house breeding, were maintained at 28°C as described in the Zebrafish Book (Westerfield, 1995). Embryos for whole mount in situ hybridization were raised in PTU (1-phenyl-2-thiourea, 0.003%) and fixed in phosphate buffered 4% paraformaldehyde.

Reverse transcriptase-polymerase chain reaction (RT-PCR) was performed using total RNA isolated from 50 hpf whole zebrafish embryos. The following zebrafish *cdh10* specific primers (designed according to the predicted zebrafish *cdh10* sequence in GenBank, accession number: **XM 691696**) were used for PCR for verification of the predicted *cdh10* sequence, generation of cDNA templates for *in vitro* synthesis of *cdh10* cRNA probes and/or 5'RACE: cdh10Fd (5'-CAGATCCAAAAGGGACTGGA-3'), cdh10Fd1 (5'-CTCGACGATTTTACCCAGGA-3'), cdh10Fd2 (5'-TGGACTCGGAAAAGTCTCT-3'), cdh10Fd3 (5'-TTTCCCAAGAGGGTGTCTATC-3'), cdh10Fd4 (5'-GAGGAGTTGACGCTCCTGTC-3'),

cdh10Fd5 (5'- TGTAGTAGCTCACGCGGATG-3'), cdh10Fd6 (5'- AAGGAGGATCAAAGGCGATT-3'), cdh10RV (5'- AGGGCCCAGATGAAAGAAAT-3'), cdh10RV2 (5'- ACTGCCTCCACATCCAGTC-3'), cdh10RV3 (5'- CTAGTTGGCGATTCTGTATC-3'), and cdh10RV4 (5'- AGCGTAGACAGCCCTGGTAA-3'). To confirm the predicted GenBank zebrafish *cdh10* cDNA sequence from the beginning of EC1 to the end of the cytoplasmic domain, we performed reverse transcriptase-polymerase chain reaction (RT-PCR) using *cdh10* specific primers (cdh10Fd and cdh10RV, cdh10Fd3 and cdh10RV4). Two PCR products were obtained, and sequencing of the products revealed that these two partially overlapping sequences were nearly identical to the predicted GenBank zebrafish *cdh10* sequence. Our sequence differed from the predicted zebrafish *cdh10* sequence at eight nucleotide positions, but these differences resulted in no change in corresponding amino acids. To confirm the predicted 5' end sequence (signal and presequence) of the zebrafish *cdh10*, we performed RT-PCR using primers designed according to the predicted 5' end of the zebrafish *cdh10* cDNA sequence in GenBank (for the forward primers, cdh10Fd1, cdh10Fd2, cdh10Fd4, cdh10Fd5, or cdh10Fd6), or according to the confirmed zebrafish *cdh10* sequence (for the reverse primers, cdh10RV or cdh10RV2). But the experiments resulted in no amplified product, suggesting that at least part of the predicted signal and presequence for the zebrafish *cdh10* gene in GenBank is incorrect. To obtain the sequence of the missing 5' end of the zebrafish *cdh10*, 5'-rapid amplification of cDNA ends (5'RACE) was performed using a 5'RACE kit (Invitrogen, Carlsbad, CA) and zebrafish *cdh10* specific primers (cdh10RV, cdh10RV2 and cdh10RV4). This experiment yielded a nucleotide sequence that could be aligned with the GenBank sequence only from nucleotide 937 forward. The sequence suggested a likely start codon much closer to the extracellular domain than previously suggested, equivalent to base position 1079 of the **XM 691696** GenBank accession. The imputed start codon was identified because (1) it was the first methionine codon following a 397-nucleotide 5'-untranslated sequence; (2) a canonical signal sequence follows this methionine codon; (3) The presequence (including the signal sequence) found in our clone is similar in length (about 50 amino acids) to presequences from other Cdh10 and type-II cadherin sequences (from *Xenopus*, mouse, chicken and human), while presequences in type-I cadherins (e.g. Cdh1, Cdh2 and Cdh4) are much longer (about 150 amino acids). The complete coding sequence of 785 amino acids plus 5' sequence for the zebrafish *cdh10* gene was deposited in GenBank (accession number: **DQ 303467**)

Primers cdh10Fd and cdh10RV, and cdh10Fd3 and cdh10RV3 were used to obtain cDNA fragments corresponding to the nucleotides 1179-2061 and 1941-3336, respectively, of the predicted zebrafish *cdh10* (GenBank). Both the shorter (882 bp) and longer (1395 bp) cDNA fragments were separately cloned into the PCRII-TOPO vector (Invitrogen, Carlsbad, CA) and verified using restriction mapping and sequencing. Each *cdh10* cDNA was used as a template for the synthesis of digoxigenin-labeled zebrafish *cdh10* cRNA sense or antisense probes for in situ hybridization. Detailed procedures for the cRNA probe synthesis and whole mount in situ hybridization were described previously (Liu et al., 1999). *cdh10* expression pattern in zebrafish embryos (12-50 hpf) using either of the shorter or longer antisense *cdh10* cRNA probes was indistinguishable. The imaging data shown in this study was from experiments using the shorter cRNA probe. There was no staining in zebrafish embryos from 14-50 hpf using the sense *cdh10* probes (data not shown).

Anti-acetylated tubulin antibody (Sigma, St Louis, MO) was used at 1:6000, while zn12 (a monoclonal antibody recognizing L2/HNK-1 tetrasaccharide, Zebrafish International Resource Center, Eugene, OR) was used at 1:1500. The secondary antibody was biotinylated anti-mouse IgG (Vector laboratories, Burlingame, CA). Detailed procedures for whole-mount immunocytochemistry were reported previously (Liu et al., 2001).

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Sig	
<u>MIMNHFLWLLPLVFPHLSSPVILLERNDGNLFHNWSYQMGDKMVLHRSKR</u>	51
EC1	
DWMWRQFFLSEEYMGNNNQYVGKLRSKDRGDGTARYVLSGEGAGTVFRIDEKSGDLHAT	
QRLDREEKSSYTLOAQAFNRITGLPLEPATEFIVKIHNDINDNEPKF	157
EC2	
TRAVYAASVPMSDVGTFTVIEVNATDADDATYGNSAKLVYSILQGQPYFSVEPETGIVRI	
ALPNMDREVRKDYQVVIQAKDMAGQMGGLSGTTMVNVTLTDVNDSPPRF	266
EC3	
AYSSYHLSTYESAEIGSTVGRIKANDGDEGENAEMKYRMSDGDGKEYLDIITDEISQEGV	
IVIKKKLDYETKRVYTVKVEVSNTHQDPNFFHLGPFSDTAIVKVTARDVDEPPVF	381
EC4	
SRTQYVFEVNEDTPKGTVIGTVTARDPDATDYPILYAIDQHTDPERLYHIDPKNGSITIL	
KSLDREVSQWHNISVLASEMNNPHQSRVPVLIKVLVDVNDNAPEF	486
EC5	
AMİYETFCENFKAGELIQTISAIDTDDPLVGHKFVFSLSLNPNTIFDNEDNTARILT	
RRSGFNRREMSVYYLPVVISDSYPIQSSTSTLTIRVCSDATGTMRSCSVD	598
TM	
ALLLSAGLSTGALVAILLCILILLMIVVLFSAI	631
Cyto	
KKQRRKEPLIIISKEDVRDNVVSYNDEGGGEEDTQAFDIGTLRHPEVIESNKLRRDIIPEM	
LFPYHRPSPMKECADVRDFIISRLQENDTDPFAPPYDSLATYAYEGNGSIAESLSSLESS	
LTEGDHEYDYLNSWGPQFKKLADMYIGKDTGIAN	785

Figure 1.

Deduced amino acid sequence of zebrafish Cdh10. The putative hydrophobic signal sequence (Sig) is underlined. Other abbreviations: cyto, cytoplasmic domain; EC1-EC5, extracellular domains 1-5; TM, transmembrane domain.

Figure 2A.

Amino acid sequence comparison between the deduced zebrafish Cdh10 amino acid sequence (Zcdh10), chicken Cdh10 (Ccdh10), human Cdh10 (Hcdh10), mouse Cdh10 (Mcdh10), zebrafish Cdh6 (Zcdh6), and zebrafish Cdh1 (Zcdh1). Comparisons were between published sequences from the EC1 to the end of the coding sequences. Sequences highlighted by yellow boxes indicate residues that are common to all six sequences, and sequences highlighted by blue boxes indicate amino acids that are identical to at least half of the sequences. Figure 2B shows sequence identity percentages for pairwise comparisons between all six sequences shown in the alignment. Diagonal shaded boxes indicate sequence comparisons between the same sequences, and therefore, represent 100% identity. Sequence comparisons were

performed using Align X (InforMax Inc., North Bethesda, MD). Abbreviations the same as in figure 1.

Figure 3.

Phylogram resulting from neighbor-joining distance analysis of EC1 through the carboxy-terminal protein sequence alignment. The tree was rooted with the zebrafish Cdh1 amino acid sequence. GenBank accession numbers follow the sample names. The sequence generated as part of this study is shown in bold.

Figure 4.

cdh10 expression in 12-24 hpf zebrafish embryos. All panels show lateral views of whole mount embryos labeled with *cdh10* cRNA (panels A-D, G, H, J and L), *cdh6* cRNA (panels E and F), anti-acetylated tubulin (anti-tub, panel I) or *zn12* (panel K) antibodies. Panels D, E, H, I, J and K are higher magnifications of the head region (anterior to the left and dorsal up), while panels C, F and L are higher magnifications of the posterior trunk and tail region (due to the bend between the trunk and tail, these two regions have different orientations: for panels C and F, anterior down and dorsal to the left for the posterior trunk region, while anterior to the left and dorsal up for the tail region; for panel L, anterior to the left and dorsal up for the trunk region, while anterior to the left upper corner and dorsal to the right upper corner). The otic placode is outlined with dashed lines. Each of three arrowheads in panel H points to a *cdh10* expression strip in the forebrain.

Abbreviations: a, anterior lateral line placode area; ac, anterior commissure; c, cerebellum; di, diencephalon; dlc, dorsal longitudinal tract; ey, eye; he, head region; h, hindbrain; nc, notochord; or, optic recess; ot, optic tectum; ov, otic vesicle; p, posterolateral line placode/ganglion; poc, postoptic commissure; sa, statoacoustic ganglion; sc, spinal cord; sot, supraoptic tract; sp, spinal cord; te, telencephalon; tpc, tract of posterior commissure; tpoc, tract of postoptic commissure.

Figure 5.

cdh10 expression in 34 hpf embryos (panels A, D-F, and I-K) compared to *cdh6* expression (panels B and G) and anti-acetylated tubulin staining (panels C and H). Panels A-C, and F-H are lateral views of whole mount embryo heads with anterior to the left and dorsal up. Panel D is a dorsal view (anterior to the left) of a deconvoluted embryo. Panel E is a parasagittal section of the hindbrain (anterior to the left and dorsal up). Panels I-K are frontal views (dorsal up) focusing on planes indicated by three correspondingly labeled arrows in panel F. The arrowhead, long and short arrows in panels A, D and E point to the same *cdh10* expressing cell clusters. Abbreviations: ad, anterodorsal lateral line ganglion; av, anteroventral lateral line ganglion; hy, hypothalamus; m/v, medial lateral line/vagus ganglion, r1-r6, rhombomeres 1-6; t, trigeminal ganglion. The remaining abbreviations are the same as in figure 4.

Figure 6.

cdh10 expression in 52 hpf embryos. Panels A-C are lateral views (anterior to the left and dorsal up) of whole mount embryos. Labeled arrows in panel A indicate levels of cross sections in respective panels. Panels B and C are higher magnifications of the head region of the embryo in panel A, focusing on *cdh10* expression domains in more medial and lateral regions, respectively. Panels E and F are parasagittal sections of the eye and the posterior half of the brain, respectively with anterior to the left and dorsal up. The asterisk in panel C indicates *cdh10* expression in the first pharyngeal arch medial to the lower jaw. The dashed line in panel D indicates the border between the thalamus and optic tectum. The dashed line in panel F indicate the boundary between the mid- and hindbrains. The hindbrain border in panel G is outlined by the dashed line. Abbreviations: f/av, facial and anteroventral lateral line ganglion; gcl, retinal ganglion cell layer; inl, inner nuclear layer; onl, outer nuclear layer; pt, pretectal area; teg, tegmentum; t/ad, trigeminal and anterodorsal lateral line ganglion. The remaining abbreviations are the same as in the previous figures.