Published in final edited form as:
Gene Expr Patterns. 2008 May ; 8(5): 291–296. doi:10.1016/j.gep.2008.02.002.

Expression of *trpC1* and *trpC6* orthologs in zebrafish

Clemens C. Möller[1], **Steve Mangos**[1], **Iain A. Drummond**[1], and **Jochen Reiser**[1,*]

1 *Nephrology Division and Program in Glomerular Disease, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02129, USA*

Abstract

Transient receptor potential (*TRP*) genes encode subunits that form cation-selective ion channels in a variety of organisms and cell types. TRP channels serve diverse functions ranging from thermal, tactile, taste, and osmolar sensing to fluid flow sensing. TRPC1 and TRPC6 belong to the TRPC subfamily, members of which are thought to contribute to several cellular events such as regulated migration of neuronal dendrites, contractile responses of smooth muscle cells and maintenance of the structural integrity of kidney podocytes. Pathogenic roles have been suggested for TRPC1 in asthma and chronic obstructive pulmonary disease, and TRPC6 dysfunction was recently linked to proteinuric kidney disease. To explore the potential roles for TRPC channels in zebrafish organ function, we cloned zebrafish *trpC1* and *trpC6* cDNAs, and investigated their expression during zebrafish development. We detected *trpC1* expression in the head, in cells surrounding the outflow tract of the heart, and in the ganglion cells as well as the inner nuclear layer of the eye. *trpC6* expression was detected in the head, pectoral fins, aortic endothelial cells, and gastrointestinal smooth muscle cells. Our results point to roles of TRPC channels in several tissues during zebrafish development, and suggest that the zebrafish may be a suitable model system to study the pathophysiology of TRPC1 and TRPC6 in specific cell types.

Keywords

Transient receptor potential; ion channel; smooth muscle; in situ hybridization

1. Results and Discussion

Transient receptor potential (*TRP*) genes are widely expressed in a number of organs and cell types throughout the species (Ramsey et al., 2006). Since the discovery of the first TRP channel in *Drosophila* (Montell et al., 1985), encoded by the gene *trp*, 27 structurally related TRP proteins in humans and more than 60 orthologs in other species including flies, worms, and mice have been identified. Together they form the TRP superfamily, which is subdivided into the TRPC, TRPV, TRPM, TRPP, TRPN, and TRPML subfamilies (Montell, 2005).

All TRP proteins have six predicted transmembrane segments, intracellular N- and C-termini, and share highly conserved motifs within and downstream of the putative channel pore domain. TRP channels have been shown to mediate receptor-operated calcium entry and are also candidate channels for store-operated calcium entry. They contribute to signaling pathways

* Correspondence: Jochen Reiser, M.D., Ph.D., Massachusetts General Hospital, Nephrology Division and Program in Glomerular, Disease, Suite 8214, CNY 149 13th St, Boston, MA 02129, Email: jreiser@partners.org, Phone: +1.617.726.9363, Fax: +1.617.726.5669.

involved in sensing and responding to environmental stimuli which include mechanical/physical stimuli (such as temperature, light, and pressure), or chemical stimuli (including phorbol esters, hormone ligands, and metabolites of arachadonic acid) (Clapham, 2003).

In zebrafish, five TRP channel genes have thus far been described. *trpM7* was identified as the gene defective in the mutant touchtone/nutria (Elizondo et al., 2005) which exhibits altered skeletal structure, a diminished response to touch, and kidney stones. *trpM7* is expressed in pronephric and mesonephric kidney tubules, corpuscles of Stannius, and the liver (Liu et al., 2007). *trpA1* and *trpN* (also known as NOMPC) have been shown to contribute to ear and lateral line hair cell function (Sidi et al., 2003; Corey et al., 2004). *trpC2*, which is a pseudogene in humans, is expressed in the adult olfactory epithelium superficial layer (Sato et al., 2005). Recently, the osmosensory channel *trpV4* has been detected in multiple developing organs in zebrafish (Mangos et al., 2007).

Within the TRP superfamily, the TRPC subfamily shares the highest homology with the original TRP channel discovered in the fly. TRPCs have been implicated in a wide range of diseases, including asthma, chronic obstructive pulmonary disorder, and defective immune responses involving both B cells and T cells (Nilius et al., 2007). Furthermore, it was recently shown that TRPC6 plays a role in genetic and acquired proteinuric kidney diseases (Winn et al., 2005, Reiser et al., 2005, Moller et al., 2007). In mice and humans, TRPC6 protein is expressed in kidney podocytes in close proximity to the filtration slits of the glomerular filter. Recent studies support the notion that TRPC6-mediated calcium signaling contributes to the maintenance of the glomerular slit diaphragm and the regulation of glomerular permselectivity (Huber et al., 2006). *TRPC6* knockout mice display defective vasomotor control and a sensitized myogenic response, suggesting an important role in smooth muscle contractility (Dietrich et al. 2005, Weissmann et al., 2006). Based on these observations, which implicate TRPC channels in the function of a number of cell types, in particular smooth muscle cells and kidney podocytes, we analyzed the developmental expression pattern of *trpC1* and *trpC6* in zebrafish.

1.1 Cloning and bioinformatic analysis of trpC1 and trpC6 in zebrafish

A tBLASTn search of the Ensembl zebrafish cDNA database, using the protein sequences for human TRPC1 and TRPC6 as queries, revealed *trpC1* and *trpC6* orthologs on chromosomes 24 and 21, respectively. Based on hypothetical sequence information available in the National Center for Biotechnology Information (NCBI) CoreNucleotide database (Accession Number XM_694363), we designed 5′ and 3′ primers to amplify full-length zebrafish *trpC1* cDNA by RT-PCR. The zebrafish trp*C1* gene is composed of 13 exons and encodes a predicted protein of 783 amino acids. The *trpC1* amino acid sequence is highly conserved throughout the species (Fig. 1 A,C) and zebrafish *trpC1* shares 81% sequence identity with human TRPC1. For zebrafish *trpC6*, provisional sequence information is available in the NCBI CoreNucleotide database (Accession Number NM_001030282); the zebrafish *trpC6* gene is composed of 11 exons and encodes a 855 amino acid protein sharing sequence similarity with orthologs described in human, mouse, rat, and guinea pig (Fig. 1 B,D). The sequence identity of zebrafish *trpC6* to human TRPC6 is 71%.

1.2 Expression of trpC1 in embryonic and early laval development

The expression of zebrafish *trpC1* was studied by whole mount in situ hybridization and histological analysis of zebrafish embryos. *trpC1* expression was ubiquitous up to 24 hours post-fertilization (hpf) (Fig. 2 A). At 56 hpf, expression was restricted to the head with no detectable expression in the trunk (Fig. 2 B), and strong head expression of *trpC1* persisted until 72 hpf (Fig. 2 C). At 72 hpf, expression of *trpC1* was also detected in cells surrounding the outflow tract of the heart (Fig. 2 C,E,F). *trpC1* expression associated with the outflow tract

of the heart is consistent with the published work on TRPC1 expression in the mammalian heart (Dietrich et al., 2007). *trpC1* expression was also detected in the ganglion cell layer and the inner nuclear layer of the eye in 72 hpf embryos (Fig. 2 D). These structures contain neuronal cells appearing early on the second day post-fertilization (Hu and Easter, 1999). This is consistent with the general role of TRPC channels in sensory physiology (Montell, 1997), and the previously reported immunolocalization of TRPC1 in the chicken retina (Crousillac et al., 2003).

1.3 Expression of trpC6 in embryonic and early laval development

In mammals TRPC6 channels are expressed in cells responding to changes in hydrostatic pressure such as vascular smooth muscle cells. It is abundant in the pulmonary system and in vascular tissues, and contributes to membrane polarization and subsequent vasoconstriction induced by elevated intravascular pressure, which represents the important myogenic constriction response in arteries also known as the Bayliss effect (Welsh et al., 2002). In zebrafish embryos, *trpC6* mRNA was ubiquitously expressed up to 24 hpf (Fig. 3 A). At 48 hpf, expression became restricted to the head, pectoral fins, and the posterior extension of the gut (Fig. 3 B). In the gut, *trpC6* expression persisted to 72 hpf, where expression in the most posterior region of the gut remained high while more proximal regions of the gut showed diminished expression (Fig. 3 C). Histological examination revealed that *trpC6* was highly expressed in cells that surround and encapsulate the gut at 72 hpf (Fig. 3 F). The first detectable smooth muscle cell markers are detected in the vicinity of the gut at approximately 48 hpf (Georgijevic et al., 2007) in cells similar to *trpC6*-expressing cells. From this we conclude that *trpC6* is expressed in gastrointestinal smooth muscle cells that contribute to the stability, contractility and elasticity of the zebrafish gut (Holmberg et al., 2004). *trpC6* expression was also detected in cells lining the aorta (Fig. 3 E). Recent reports indicate important roles of *trpC6* in the mammalian cardiopulmonary vasculature (reviewed in Dietrich et al., 2007), which would be consistent with *trpC6* expression in the zebrafish aorta. In histological sections, *trpC6* expression in the pectoral fins appeared to be strongest on the dorsal surface (Fig. 3 D). The pectoral fin is composed of two simple muscles, the abductor and adductor (Thorsen and Hale, 2005), as well as large dorsal and ventral nerve branches (Thorsen and Hale, 2007). Our sections indicate that *trpC6* is primarily restricted to the dorsal dermal layer of the fin and excluded from muscle and nerve. Despite the published role for TRPC6 in the pathopyhsiology of glomerular kidney disease in the human and in rodents, *trpC6* expression was not detected in the glomeruli of 3 days post-fertilization (dpf) larvae (Fig. 3 G). Even though all glomerular cell types are present at this stage of development and the zebrafish pronephros is required to function as an osmoregulatory kidney, it has been shown that podocyte foot processes are not fully mature yet, and slit-diaphragms between foot processes are rarely observed (Kramer-Zucker et al., 2005). In contrast, at 4 dpf, the filtration apparatus appears mature with podocyte foot processes present as fine, evenly spaced cell processes separated by slit-diaphragm cell–cell junctions (Kramer-Zucker et al., 2005). This is why we studied *trpC6* expression in glomeruli also in 5 dpf larvae (Fig. 3 H) and in adult zebrafish kidney (Fig. 3 I). At neither of these later stages were we able to observe a specific labeling pattern of *trpC6* in podocytes.

1.4 Conclusion

In the zebrafish, *trpC1* and *trpC6* are expressed in cell types that respond to physiological mechanical signals including neurons, smooth muscle cells, and endothelial cells. Notably, despite the published role for TRPC6 in the pathopyhsiology of glomerular kidney disease, *trpC6* expression was not detected in pronephric podocytes. This could be due to low abundance of *trpC6* channels in these cells or expression only under pathophysiological conditions. Equally possible is the notion that *trpC6* does not play the same role in glomerular filtration in the human and fish. Albeit zebrafish and higher vertebrate share a high degree of similarity of organ cell types and tissue substructures, obviously fish organ shape and size is

different from the human and other mammalian model systems, with fish e.g. lacking collecting and complex nephron systems. Further studies of *trpC6* expression in genetic and inducible zebrafish models of glomerular injury will be informative about the role it plays in physiological regulation.

2. Experimental Procedures

2.1 Zebrafish embryos

Wild-type TL or TÜAB zebrafish lines were maintained and raised as previously described (Westerfield, 1995). Embryos were reared at 28.5 °C in E3 solution with 0.003% PTU (1-Phenyl-2-thiourea, Sigma) added to retard pigment formation. Embryonic staging was performed as previously described (Westerfield, 1995). All animal studies were approved by the Subcommittee on Research Animal Care of the Massachusetts General Hospital.

2.2 Cloning

The protein sequences for human TRPC1 (NCBI accession: NP_003295) and TRPC6 (NP_004621) were used as queries for a tBLASTn search of the Ensembl zebrafish cDNA database (www.ensembl.org/Danio_rerio), and sequences coding for the putative zebrafish orthologs were identified for *trpC1* on chromosome 24 and for *trpC6* on chromosome 21. We isolated total RNA from 2-day old zebrafish embryos using Trizol reagent (Invitrogen) and performed reverse transcription with olido dT-Primers. Based on hypothetical sequence information for *trpC1* available in the NCBI CoreNucleotide database (Accession Number XM_694363), we designed 5′ (5′-ATGGCTGCTCTATATCAGGGC-3′) and 3′ (5′-TTAGCTTCTGGGGTAGAACATG-3′) primers to amplify the actual full-length zebrafish *trpC1* ortholog. *trpC1* was then subcloned into the pCRII-TOPO vector (Invitrogen) and four different clones containing the *trpC1* open reading frame were sequenced using T7 forward and SP6 reverse primers. For zebrafish *trpC6*, provisional sequence information recently became available (Accession Number NM_001030282). Based on this information, we designed 5′ (5′-ATTGGCCAGTCCGGCTTACC-3′) and 3′ (5′-CCTTGGGACCAGATCTCCTT-3′) primers to amplify a sequence region spanning 711 base pairs specific for zebrafish *trpC6* (bases 796-1506). The amplified *trpC6* cDNA fragment was then subcloned into the pCRII-TOPO vector (Invitrogen) for antisense riboprobe generation. Sequencing of the fragment revealed identity to the provisional sequence in 708 of 711 base pairs. There were also three base pair mismatches (C1018 vs. T1018; C1144 vs. T1144; A1162 vs. C1162), all of which represented synonymous single nucleotide polymorphisms. Multiple sequence alignments were performed using the ClustalW algorithm, Version 1.83 (Thompson et al., 1994). Phylogenetic trees were generated in the Phylip type.

2.3 In situ hybridization and histology

Whole-mount in situ hybridization was performed as previously described (Thisse and Thisse, 1999). For *trpC1* and *trpC6* antisense probes, the templates (pCRII-TOPO-*trpC1* and pCRII-TOPO-*trpC6*) were linearized with *Not*I (New England Biolabs) and antisense riboprobes were transcribed using SP6 RNA polymerase (Ambion). Embryos were hybridized with digoxigenin-labeled riboprobes at 65 °C. Anti-DIG-AP (1:5,000) and the NBT/BCIP substrate (Roche Diagnostics) were used to detect the probe. After the color reaction was stopped, embryos were washed with methanol and equilibrated in clearing solution (1/3 benzoyl-alcohol and 2/3 benzoyl-benzoate) and photographed using a Leica MZ12 dissecting microscope (Leica). Histological analysis on embryos after in situ hybridization analysis was carried out after stained embryos were fixed in 4% paraformaldehyde then dehydrated through a series of methanol/PBST washes of 25%/75%, 50%/50%, 75%/25%, and finally 100% methanol for 10 min each followed by embedding in JB-4 (Polysciences). A Riechert–Jung Supercut 2065 (Leica) microtome was used to generate 10 μm sections. A Nikon E800 microscope equipped

with a Spot Image digital camera was used for photography (Nikon). In situ hybridizations were carried out 3 different times using between 12 and 24 embryos each time with consistent results. Sense probes did not produce a detectable background signal when applied to otherwise equally treated embryos [Mangos et al., 2007].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by NIH grants R01 DK73495-01 to J.R. and DK54711 to I.A.D. C.C.M. was funded by a pre-doctoral scholarship from the Deutscher Akademischer Austausch Dienst (DAAD). S.M. was funded by post-doctoral fellowship 126a2f from the Polycystic Kidney Disease Foundation. We thank Mélanie Becker for technical assistance.

References

Clapham DE. TRP channels as cellular sensors. Nature 2003;426:517–524. [PubMed: 14654832]

Crousillac S, LeRouge M, Rankin M, Gleason E. Immunolocalization of TRPC channel subunits 1 and 4 in the chicken retina. Vis Neurosci 2003;20:453–463. [PubMed: 14658773]

Corey DP, Garcia-Anoveros J, Holt JR, Kwan KY, Lin SY, Vollrath MA, Amaltano A, Cheung EL, DerXer BH, Duggan A, Geleoc GS, Gray PA, HoVman MP, Rehm HL, Tamasauskas D, Zhang DS. TRPA1 is a candidate for the mechanosensitive transduction channel of vertebrate hair cells. Nature 2004;432:723–730. [PubMed: 15483558]

Dietrich A, Mederos y Schnitzler M, Gollasch M, Gross V, Storch U, Dubrovska G, Obst M, Yildirim E, Salanova B, Kalwa H, Essin K, Pinkenburg O, Luft FC, Gudermann T, Birnbaumer L. Increased vascular smooth muscle contractility in TRPC6−/− mice. Mol Cell Biol 2005;25:6980–6989. [PubMed: 16055711]

Dietrich A, Kalwa H, Fuchs B, Grimminger F, Weissmann N, Gudermann T. In vivo TRPC functions in the cardiopulmonary vasculature. Cell Calcium 2007;42:233–244. [PubMed: 17433435]

Elizondo MR, Arduini BL, Paulsen J, MacDonald EL, Sabel JL, Henion PD, Cornell RA, Parichy DM. Defective skeletogenesis with kidney stone formation in dwarf zebraWsh mutant for trpm7. Curr Biol 2005;15:667–671. [PubMed: 15823540]

Georgijevic S, Subramanian Y, Rollins EL, Starovic-Subota O, Tang AC, Childs SJ. Spatiotemporal expression of smooth muscle markers in developing zebrafish gut. Dev Dyn 2007;236:1623–1632. [PubMed: 17474123]

Holmberg A, Schwerte T, Pelster B, Holmgren S. Ontogeny of the gut motility control system in zebrafish Danio rerio embryos and larvae. J Exp Biol 2004;207:4085–4094. [PubMed: 15498954]

Hu M, Easter SS. Retinal neurogenesis: the formation of the initial central patch of postmitotic cells. Dev Biol 1999;207:309–321. [PubMed: 10068465]

Huber TB, Schermer B, Muller RU, Hohne M, Bartram M, Calixto A, Hagmann H, Reinhardt C, Koos F, Kunzelmann K, Shirokova E, Krautwurst D, Harteneck C, Simons M, Pavenstadt H, Kerjaschki D, Thiele C, Walz G, Chalfie M, Benzing T. Podocin and MEC-2 bind cholesterol to regulate the activity of associated ion channels. Proc Natl Acad Sci U S A 2006;103:17079–17086. [PubMed: 17079490]

Kramer-Zucker AG, Wiessner S, Jensen AM, Drummond IA. Organization of the pronephric filtration apparatus in zebrafish requires Nephrin, Podocin and the FERM domain protein Mosaic eyes. Dev Biol 2005;285:316–329. [PubMed: 16102746]

Liu Y, Pathak N, Kramer-Zucker A, Drummond IA. Notch signaling controls the differentiation of transporting epithelia and multiciliated cells in the zebrafish pronephros. Development 2007;134:1111–1122. [PubMed: 17287248]

Mangos S, Liu Y, Drummond IA. Dynamic expression of the osmosensory channel TRPV4 in multiple developing organs in zebrafish. Gene Expr Patterns 2007;7:480–484. [PubMed: 17161658]

Montell C, Jones K, Hafen E, Rubin G. Rescue of the Drosophila phototransduction mutation trp by germline transformation. Science 1985;230:1040–1043. [PubMed: 3933112]

Montell C. New light on TRP and TRPL. Mol Pharmacol 1997;52:755–763. [PubMed: 9351965]

Montell C. The TRP superfamily of cation channels. Sci STKE 2005 2005:re3.

Moller CC, Wei C, Altintas MM, Li J, Greka A, Ohse T, Pippin JW, Rastaldi MP, Wawersik S, Schiavi S, Henger A, Kretzler M, Shankland SJ, Reiser J. Induction of TRPC6 channel in acquired forms of proteinuric kidney disease. J Am Soc Nephrol 2007;18:29–36. [PubMed: 17167110]

Nilius B. TRP channels in disease. Biochim Biophys Acta 2007;1772:805–812. [PubMed: 17368864]

Ramsey S, Delling M, Clapham DE. An introduction to TRP channels. Annu Rev Physiol 2006;68:619–647. [PubMed: 16460286]

Reiser J, Polu KR, Möller CC, Kenlan P, Altintas MM, Wei C, Faul C, Herbert S, Villegas I, Avila-Casado C, McGee M, Sugimoto H, Brown D, Kalluri R, Mundel P, Smith PL, Clapham DE, Pollak MR. TRPC6 is a glomerular slit diaphragm-associated channel required for normal renal function. Nat Genet 2005;37:739–744. [PubMed: 15924139]

Sato Y, Miyasaka N, Yoshihara Y. Mutually exclusive glomerular innervation by two distinct types of olfactory sensory neurons revealed in transgenic zebrafish. J Neurosci 2005;25:4889–4897. [PubMed: 15901770]

Sidi S, Friedrich RW, Nicolson T. NompC TRP channel required for vertebrate sensory hair cell mechanotransduction. Science 2003;301:96–99. [PubMed: 12805553]

Thisse C, Thisse B. Antivin, a novel and divergent member of the TGFbeta superfamily, negatively regulates mesoderm induction. Development 1999;126:229–240. [PubMed: 9847237]

Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994;22:4673–4680. [PubMed: 7984417]

Thorsen DH, Hale ME. Development of zebrafish (Danio rerio) pectoral fin musculature. J Morphol 2005;266:241–255. [PubMed: 16163704]

Thorsen DH, Hale ME. Neural development of the zebrafish (Danio rerio) pectoral fin. J Comp Neurol 2007;504:168–184. [PubMed: 17626269]

Weissmann N, Dietrich A, Fuchs B, Kalwa H, Ay M, Dumitrascu R, Olschewski A, Storch U, Mederos y Schnitzler M, Ghofrani HA, Schermuly RT, Pinkenburg O, Seeger W, Grimminger F, Gudermann T. Classical transient receptor potential channel 6 (TRPC6) is essential for hypoxic pulmonary vasoconstriction and alveolar gas exchange. Proc Natl Acad Sci U S A 2006;103:19093–19098. [PubMed: 17142322]

Welsh D, Morielli A, Nelson M, Brayden J. Transient receptor potential channels regulate myogenic tone of resistance arteries. Circ Res 2002;90:248–250. [PubMed: 11861411]

Westerfield, M. The Zebrafish Book. University of Oregon Press: Eugene, Oregon; 1995.

Winn MP, Conlon PJ, Lynn KL, Farrington MK, Creazzo T, Hawkins AF, Daskalakis N, Kwan SY, Ebersviller S, Burchette JL, Pericak-Vance MA, Howell DN, Vance JM, Rosenberg PB. A Mutation in the TRPC6 Cation Channel Causes Familial Focal Segmental Glomerulosclerosis. Science 2005;308:1801–1804. [PubMed: 15879175]

Fig. 1.

Sequence analysis of zebrafish *trpC1* and *trpC6*. (A,B) Alignments of the zebrafish *trpC1* and *trpC6* sequences to known homologs in other species using the ClustalW algorithm. Conserved amino acids are highlighted in yellow. The highly conserved channel pore domain (LFW) and TRP box (EWKFAR) are highlighted in gray. (C,D) Phylogenetic trees representative of evolutionary relationships between the zebrafish *trpC1* and *trpC6* ortholog and cloned full-length TRPC1 and TRPC6 channels of other species. Branch length is proportional to evolutionary distance.

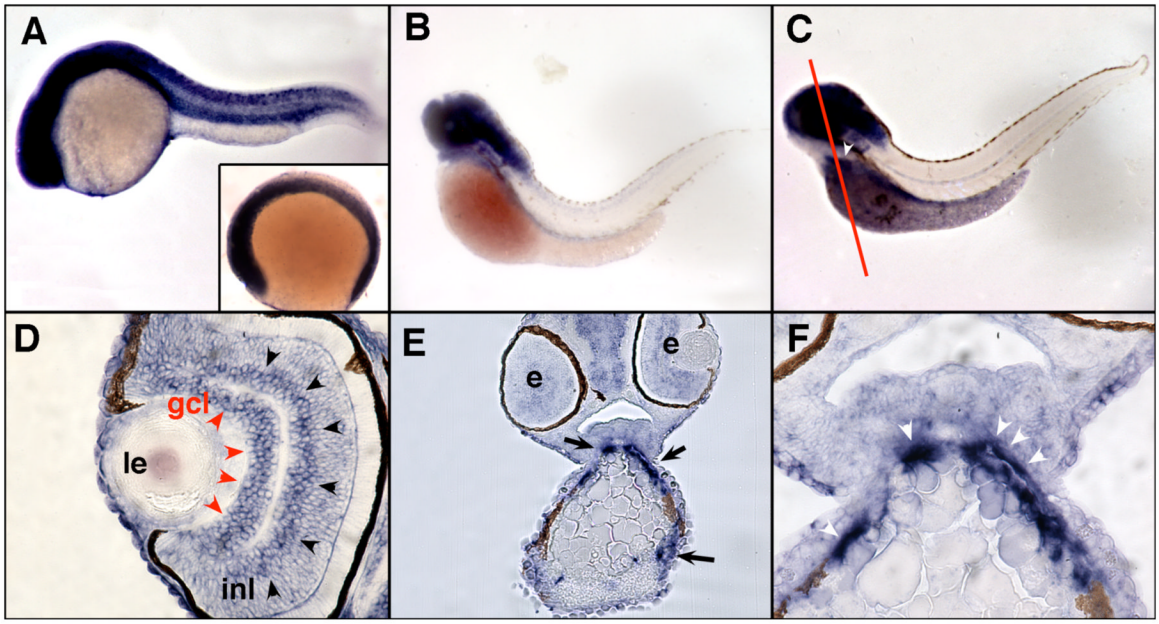

Fig. 2.

Expression of zebrafish *trpC1* by whole mount in situ hybridization and histological analysis. Expression of *trpC1* mRNA is ubiquitous in 6 somite embyos (A; inset) and stages up to and including 24 hpf (A). At 56 hpf, expression is restricted to the head with no detectable expression in the trunk (B). Strong head expression of *trpC1* persists until 72 hpf (C), in addition to expression in the outflow tract of the heart (white arrowhead; white line denotes plane of section in E and F). Histological examination of 72 hpf embryos reveals specific expression of *trpC1* in the ganglion cell layer of the eye (gcl, red arroheads) and in the inner nuclear layer (inl, black arrowheads). Anterior sections of 72 hpf embryos (line in C) confirms expression of *trpC1* in the outflow track (E, black arrows). A magnified view (F) shows a high level of expression in the cells associated with the outflow tract (white arrowheads). Le=lens, e=eye, gcl=ganglion cell layer, inl=inner nuclear layer.

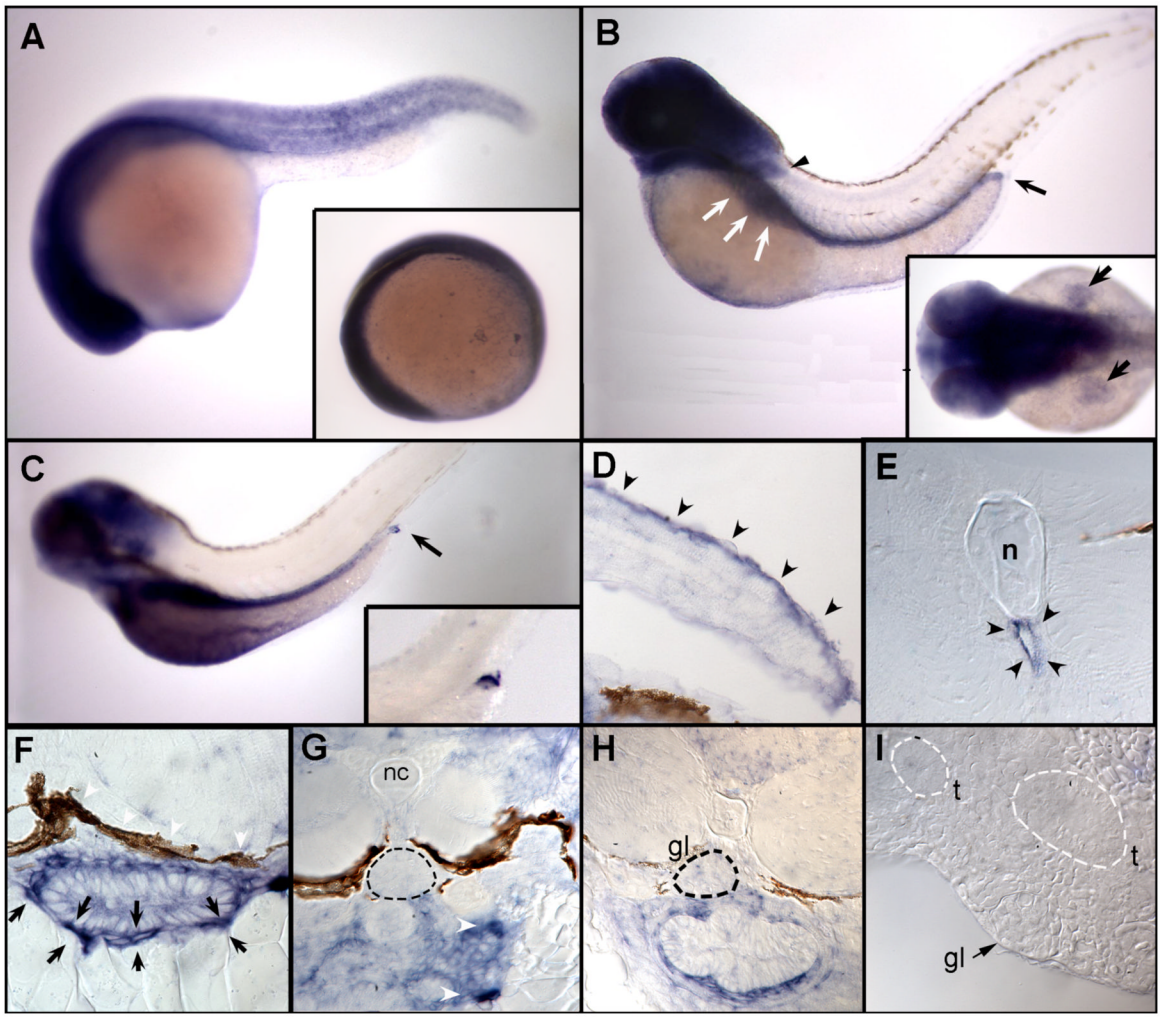

Fig. 3.

Expression of zebrafish *trpC6* by whole mount in situ hybridization and histological analysis. Expression of *trpC6* mRNA is ubiquitous at 6 somites (A, inset) and in all stages tested up to and including 24 hpf (A). (B) At 48 hpf, expression becomes restricted to the head, pectoral fins (black arrowhead), the area of the gut (white arrows) extending to the posterior end (black arrow). Dorsal view of expression in fins at 48 hpf (B, inset). This pattern of *trpC6* expression persists to 72 hpf (C), where expression in the most posterior region of the gut (black arrow and inset) remains high while more proximal regions of the gut show diminished expression. Histological examination reveals that *trpC6* expression in the pectoral fins is restricted to the dorsal surface (D, black arrowheads). Sectioning of the trunk of 72 hpf embryos shows that *trpC6* mRNA is expressed in cells lining the dorsal aorta (E, black arrowheads). A closer examination of the gut reveals that *trpC6* is highly expressed in cells that surround and encapsulate the gut (F, black arrows). (G) Sections through the glomerulus of a 3 dpf larva (G, dashed black circle) demonstrate that *trpC6* RNA is not detectably expressed podocytes, whereas cells encapsulating the anterior gut are positive for *trpC6* (G, white arrowheads). Later stage, 5 dpf (H) and adult glomeruli (I) do not display specific labeling for *trpC6*. nc=notochord, gl=glomerulus, t=tubules.