Prostaglandin E Synthases in Zebrafish

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Objective—Prostaglandin E synthases (PGESs) are being explored as antiinflammtory drug targets as alternatives to cyclooxygenase (COX)-2. Located downstream of the cyclooxygenases, PGESs catalyze PGE_2 formation, and deletion of microsomal (m)-PGES-1 abrogates inflammation. We sought to characterize the developmental expression of COX and PGES in zebrafish.

Methods and Results—We cloned zebrafish cytosolic (c) and m-PGES orthologs and mapped them to syntenic regions of chromosomes 23 and 5. cPGES was widely expressed during development and was coordinately regulated with zCOX-1 in the inner ear, the pronephros, and intestine. COX-2 and mPGES-1 exhibited restricted expression, dominantly in the vasculature of the aortic arch. However, the enzymes were anatomically segregated within the vessel wall. Experiments with antisense morpholinos and with nonsteroidal antiinflammatory drugs suggest that these genes may not be critical for development.

Conclusions—mPGES-1 is developmentally coregulated with COX-2 in vasculature. Given the high fecundidity and translucency of the zebrafish, this model may afford a high throughput system for characterization of novel PGES inhibitors. (***Arterioscler Thromb Vasc Biol.* 2005;25:315-320.)**

Key Words: prostaglandin E synthase ■ cyclooxygenase ■ zebrafish ■ Danio rerio ■ vascular biology

Nonsteroidal antiinflammatory drugs (NSAIDs) have proven effective in ameliorating symptoms in patients with arthritis.[1] These drugs inhibit coincidentally cyclooxygenase (COX)-1 and COX-2, two forms of the prostaglandin (PG) G/H synthase,[2] which catalyze the biotransformation of arachidonic acid into PGG_2 and PGH_2. These, in turn, are acted on by isomerases and synthases to form PGs.[3] NSAID use is commonly associated with gastric upset and may result in bleeding from both the upper and lower gastrointestinal tract.[4]

COX-1 is expressed constitutively in most cells, whereas COX-2 is most commonly induced by inflammatory cytokines and mitogens.[5] Selective inhibitors of COX-2 were proposed to retain the efficacy of traditional NSAIDs while avoiding the gastrointestinal adverse effects, as this was attributed to inhibition of cytoprotection by products of COX-1 in gastric epithelium.[6] This has been borne out in two clinical outcome trials, Vioxx Gastrointestinal Outcomes Research Study (VIGOR)[7] and Therapeutic Arthritis Research and Gastrointestinal Event Trial (TARGET),[8] but not in a third, Celebrex Long-Term Arthritis Safety Study (CLASS).[9] Given the cardiovascular adverse effect profile associated with selective inhibition of COX-2,[10] interest has shifted to enzymes downstream of the COXs as potential targets of novel antiinflammatory interventions, and small molecule inhibitors of PGE synthases are under development.

PGE synthases (PGES) transform PGH_2 to PGE_2. These enzymes exist in microsomal (mPGES-1 and mPGES-2)[11,12] and cytosolic (cPGES)[13] forms. Two cytosolic gluthathione S-transferases, $\mu 2$ and $\mu 3$, also have the capacity to form PGE_2.[14] There is some evidence that COX-2 and mPGESs may be coordinately regulated in expression systems,[15] whereas COX-1 and cPGES (which is identical to heat shock protein 90 associated p23) are coupled functionally.[13] Indeed, mPGES-1 and COX-2 are overexpressed together in human cancer,[16] and the two enzymes are induced coincidentally in vivo in a rodent model of arthritis.[17] Although mPGES-1 colocalizes with COX-1 and COX-2 in kidney,[18] deletion of mPGES-1 is as effective as NSAID treatment in experimental inflammation in rodents.[19]

We have previously characterized the zebrafish homologs of COX-1 and COX-2.[20] Their dominant product of arachidonic acid is PGE_2, which is depressed by NSAIDs. Furthermore, coincidental inhibition of COX-1 and COX-2, but not COX-2 alone, prolongs bleeding time and inhibits thrombocyte aggregation ex vivo, analogous to the experience in humans.[21] Given the high fecundity and translucency of the zebrafish, this model system (www.zf-models.org) may afford a high throughput system for characterization of novel PGES inhibitors and their cardiovascular biology.

Methods

Acquaculture and Isolation of Zebrafish (z) cPGES and mPGES

Zebrafish embryos were obtained from pair-wise mating of wild-type AB strain fish raised in E3 media as described.[22]

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Zebrafish expressed sequence tag (EST) clones with high homology to human cPGES, (accession no. AI722807, clone fc31h04.y1) and mPGES (accession no. BQ261637, clone fz67c11.y1) were identified from GenBank (National Center for Biotechnology Information). The EST clone fc31h04.y1 (www.rzpd.de) was sequenced and spanned the entire length of the putative zcPGES cDNA (AY724692). Clone fz67c11.y1, which contained 409 bp of the putative zmPGES cDNA and a small portion of the 3′ untranslated region (UTR), was used to generate polymerase chain reaction (PCR) primers. Full-length zmPGES cDNA (AY724692) was obtained by performing RT-PCR (30 cycles at 56°C, primers: 5′ CAT TAT TGC CAT CAT CAC G 3′ and 5′ GTC TTC ACC AGT CTC AGA GC 3′) and 5′ anchored PCR (Invitrogen Corp) using total RNA from adult zebrafish. Promoter sequence prediction from genomic DNA sequence was performed using PROMOTER SCAN Version 1.7 (http://bimas.cit.nih.gov/molbio/proscan/). Phylogenetic reconstruction was performed by alignment of protein sequences using the ClustalW algorithm and Fitch-Margoliash method (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Eicosanoids were measured as described.[20]

Chromosomal Mapping

zcPGES and zmPGES were physically mapped by using a zebrafish radiation hybrid panel (Invitrogen).[23] The RT PCR for zcPGES mapping was performed with the primers 5′ CAG AAC CTG GCA AGT CTT GG 3′ and 5′ CTC TTC TTC ATC TGC ACC ATC C 3′; the RT-PCR for zmPGES was performed with 5′ GCA CTA AAA GCA CCG ACA CG 3′ and 5′ ACT GGG TCA TGC GAA TGA GG 3′ (30 cycles at 55.5°C).

RNA Localization

Whole-mount in situ hybridizations were performed on zebrafish embryos fixed in paraformaldehyde (4%) overnight at 4°C. The zcPGES riboprobe was generated from *Bgl*II-cut EST clone fc31h04.y1 and contained the 3′UTR (SP6 mMessage mMachine kit, Ambion Inc). The zmPGES full-length coding sequence cDNA was subcloned into the mammalian expression vector pcDNA3.1/V5-His-TOPO (Invitrogen). The zmPGES probe was made from the *Spe*I-linearized construct vector with T7 mMessage mMachine kit (Ambion). In situ hybridizations were performed on embryos from at least 3 separate matings as described.[24]

Protein Expression

zcPGES and zmPGES proteins were detected by using polyclonal antibodies raised against human specific peptides (cPGES and mPGES-1 polyclonal antibodies, Cayman Chemical). The homology between the human and zebrafish cPGES peptides was ≈85%, whereas for the mPGES peptides it was 75%. Fixed embryos were stored at −20°C in methanol. The embryos were then rehydrated in PBS, treated with collagenase 0.1% in PBS plus 0.1% tween (PBST) for 35 minutes at room temperature, and fixed again in 4% paraformaldehyde. Embryos were incubated with cPGES or mPGES-1 antibodies (1:100) in 5% goat serum in PBST overnight at 4°C. Immunostaining was performed after three washes with PBST by using a fluorescein isothiocyanate (FITC) labeled secondary antibody (Jackson ImmunoResearch Laboratories Inc; 1:200 in 5% goat serum in PBST), which was also used alone as negative control. Samples were embedded in Vectashield (Vector Laboratories) and visualized on a confocal microscope (Radiance 2000-MP, Bio-Rad Laboratories).

Data Deposition

The sequences reported in this article have been deposited in the GenBank database (accession numbers AY724691 and AY724692).

Results

Zebrafish cPGES and mPGES Sequences

PGE_2 was the predominant PG in adult zebrafish tissue (Figure I, available online at http://atvb.ahajournals.org). Derivatives of eicosapentaenoic acid were formed in minor abundance in fish fed brine shrimp, themselves capable of synthesizing ω-3 fatty acids.[25] We identified two EST clone sequences in GenBank that showed high homology with human cPGES and mPGES. Sequence analysis of the clone fc31h04.y1 revealed a 444-bp open reading frame of the putative zcPGES, coding for a protein of 148 aa in length, which showed >75% sequence identity with the human protein (Figure IIA, available online at http://atvb.ahajournals.org). Furthermore, a residue, Tyr9, thought to act as glutathione binding site in the catalytic center of the human enzyme and in human hematopoietic PGD synthase[26] was conserved in the zebrafish sequence. BLAST search of the cDNA sequence against the Ensemble zebrafish genomic database (http://www.ensembl.org/Multi/blastview/) identified the putative gene (Unigene Dr.5031, contig Zv4_scaffold1916.1) with two exons of length 116 to 184 bp spanning 2 kb on chromosome 23 of the zebrafish genome (position 32570012 bp). Computational analysis of the region up to 5 kbp upstream of the start codon resulted in a weak prediction of a TATA box binding protein (TBP) binding site at position −530 on the reverse strand. As no promoter studies of human cPGES have been reported, we performed a sequence analysis of the 5 kbp upstream the human cPGES start codon. It resulted in a weak prediction of a promoter region on the forward strand between −1694 and −1444, with the following sequence elements: −1664 JCV repeated sequence; −1590 major late transcription factor (MLTF); −1578 Albumin US2; −1444 NFMHCS_II-B. None of these binding sites were found in the zebrafish sequence.

Sequence analysis of the amplification products from adult fish total RNA resulted in a 438-bp cDNA product of the putative zmPGES. BLAST search against zebrafish genomic sequence revealed a ≈120-kbp sequence contig (BX005029) on zebrafish chromosome 5 (position 34959468 bp) that contained the complete sequence. The zmPGES gene (Ensemble gene: ENSDARG00000023544) spans 7 kbp and like the human homolog is divided into three exons (83 to 247 bp). Sequence analysis of the 5 kb upstream of the start of the coding region for transcription factor binding sites predicted a TBP recognition site on the reverse strand at position −117. In contrast, the human mPGES promoter contains consensus binding motifs for AP-1, C/EBPα and β, two progesterone receptor and three glucocorticoid receptor elements two tandem GC boxes, and no TATA box.[27]

Translation of the zmPGES cDNA resulted in a protein sequence of 146 aa in length (Figure IIB). Alignment with the human mPGES-1 sequences revealed ≈63% identity. Domain analysis for zmPGES revealed the conservation of a MAPEG (membrane associated proteins in eicosanoid and glutathione metabolism) domain, characteristic of the family, as well as Arg104, thought to bind the cofactor glutathione (Figure IIB). Members of the human MAPEG family, 5-lipoxygenase activating protein (FLAP), leukotriene C4 synthase (LTC4) and mPGES-1, all contain a consensus sequence (XERXXXAXXNXXD/E) that may convey their sensitivity to indole inhibitors.[28] This was conserved in zmPGES (Figure IIB) and in zebrafish sequences similar to FLAP (BG737558), and LTC4 synthase (NM_200061). A zebrafish protein with high homology to MGST1 (NP_001002215) also contained a similar sequence. Compar-

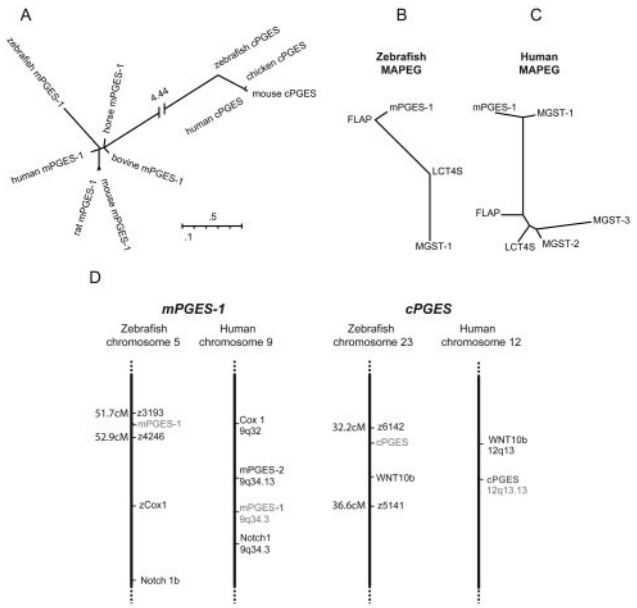

Figure 1. A, Unrooted dendrogram of vertebrate cPGES and mPGES-1 proteins. Accession numbers of analyzed sequences: human cPGES (AAA18537), chicken cPGES (B56211), mouse cPGES (NP_062740), human mPGES-1 (NP_004869), bovine mPGES-1 (NP_776868), horse mPGES-1 (AAL18255), mouse mPGES-1 (NP_071860), rat mPGES-1 (AAG24803). B and C, Phylogenetic analysis of cloned zebrafish (B) and human (C) MAPEG (membrane associated proteins in eicosanoid and glutathione metabolism) families plotted as unrooted dendrograms. Accession numbers of analyzed sequences. Zebrafish: FLAP (BG737558), LTC4 synthase (NM_200061), MGST1 (NP_001002215). Human: FLAP (=ALOX5AP; NM_001629), MGST-1 (NM_020300), mPGES-1 (=MST-L1; NM_004878), MGST-2 (NM_002413), MGST-3 (NM_004528), LTC4S (NM_000897). D, Composite maps of syntenic regions of the mPGES-1 and cPGES loci in the human and zebrafish genomes are based on the Tübingen Map (http://wwwmap.tuebingen.mpg.de/). Human maps are based on genome sequence and G4 radiation hybrid maps. The human cPGES locus (12q13.13) is derived from the UCSC Genome Browser version hg16 (http://genome.ucsc.edu) where hcPGES is annotated as the unactive progesterone receptor, 23kD protein (nucleotide position 55368156 to 55343649 on the chromosome 12 sequence).

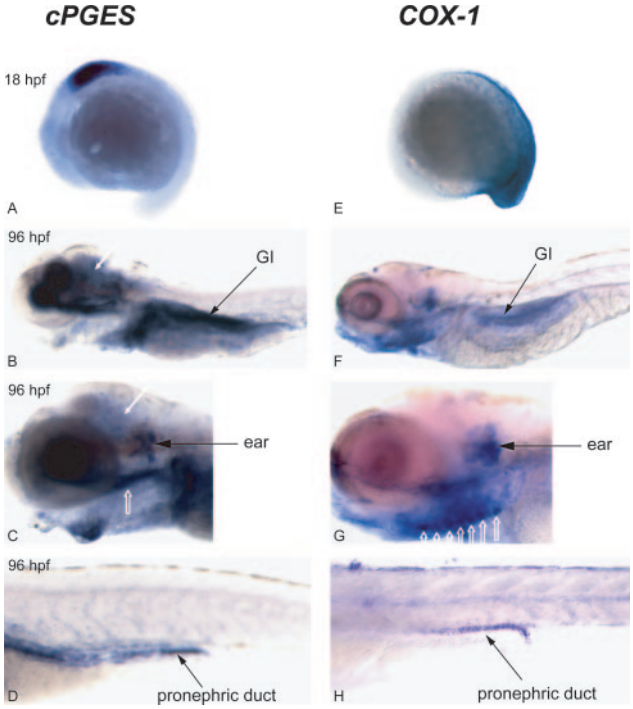

Figure 2. Comparison of zcPGES (A through D) and zCOX-1 (E through H) transcript expression by whole mount in situ hybridization. A and E, Lateral views of 18-somite stage embryos show endodermal zcPGES (A) expression, whereas zCOX-1 (E) is projecting onto the paraxial mesoderm of the tailbud. B and F, Lateral views of embryos at 96 hpf reveal cPGES (B) expression in mid and hindbrain (white arrow) and in the gastrointestinal (GI) tract. zCOX-1 (F) expression in the GI tract is more restricted. C and G, zCPGES is expressed in inner ear (black arrow), brain (white arrow), and pharynx (open arrow). zcPGES expression colocalizes with zCOX-1 (G) in the ear (black arrow) but is distinct the pharyngeal region where only zCOX-1 is expressed in the aortic arches (open arrows). D and H, Expression of both genes coincides in the pronephric duct.

ison between the amino acidic sequences of the zmPGES and the recently identified rat mPGES-2 showed little similarity with <20 homologous amino acids.

The genetic distances between the zebrafish PGESs and other known PGESs calculated from the amino acid sequences are shown in a dendrogram in Figure 1A. The zebrafish sequences located unambiguously to the respective arms of the vertebrate cPGES and mPGES enzymes. Interestingly, the genetic distances within the zebrafish and the human MAPEG families were distinct (Figure 1B and 1C).

Chromosomal Assignment of Zebrafish cPGES and mPGES-1

The zmPGES-1 gene mapped onto zebrafish chromosome (linkage group) 5 between the markers Z3193 and Z4246 (51.7 cM and 52.9 cM from the telomeric end; Figure 1D). The syntenic relationships between zmPGES and two other genes, COX-1 and NOTCH1β, were conserved between human chromosome 9 and zebrafish chromosome 5. zcPGES was assigned to chromosome 23, between the markers Z6142 and Z5141 (32.2 cM and 36.6 cM from the chromosomal telomeric end, respectively; Figure 1D). Again, the syntenic relationships were conserved in this region, as cPGES mapped close to WNT10b both on zebrafish chromosome 23 and human chromosome 12.

Developmental Expression of zcPGES and zmPGES

Whole mount in situ hybridization of the optically clear embryo revealed highly localized expression of zcPGES in the frontal endoderm at 18 hours post fertilization (hpf) (Figure 2A). As the three germ layers develop in zebrafish, endodermal progenitors involute and migrate to the midline of the embryo during gastrulation. The endoderm of 18-hpf embryos lacks apparent histological organization. However, beginning at 21 hpf, cells differentiate to form the embryonic gut tube. Associated gastrointestinal organs, such as the liver and the exocrine pancreas, are formed from common endodermal precursor cells in a parallel process in zebrafish.[29] Indeed, cPGES mRNA is expressed in the developing gut tube at 24 hpf (not shown) and was localized in pharynx, esophagus, intestine, and liver by 96 hpf (Figure 2B). High expression of zcPGES was also evident in the

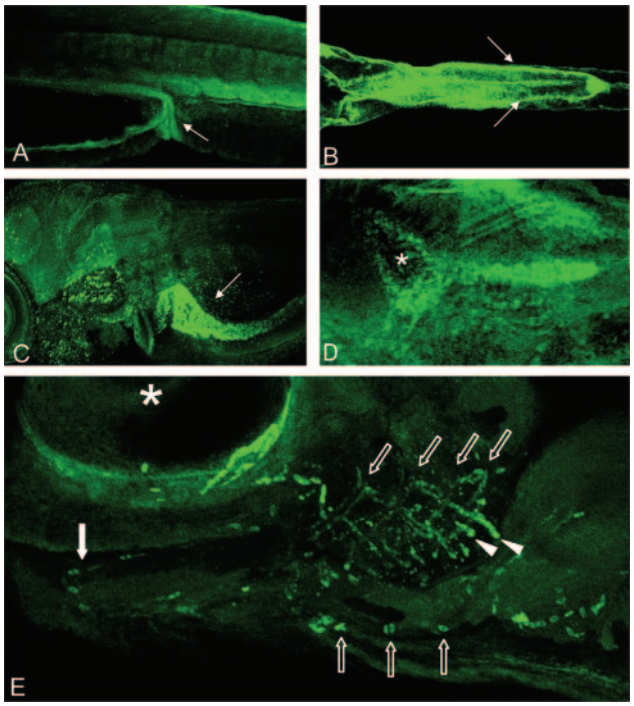

Figure 3. Expression of zPGESs by whole mount immunostaining in zebrafish larvae. A and B, Expression of zcPGES in the pronephric ducts at 2 days post fertilization (dpf) (A, arrow) and at 5 dpf (B, arrows). C, cPGES is highly expressed in the exocrine pancreas (C, arrow). D, The single islet forming the endocrine pancreas (asterisk) is spared from zcPGES expression. E, zmPGES-1 immunostaining of endothelial cells of the gill vasculature at 5 dpf. The embryo is tilted slightly from a straight left lateral to a ventro-lateral view to show a longitudinal confocal section of the left aortic arch vasculature. The asterisk marks the center of the eye, of which only the lower half is visible. Four open arrows pointing diagonally downward indicate the endothelial lining of aortic arch vessels (arteries and veins) with their loop forming protrusions (two protrusions are highlighted with white arrow heads as an example). These sprouting protrusions will develop into the finely branched gill vasculature. The upward pointed arrows indicate cross sections through the counter lateral aortic arch vessels. The arrow on the left side of the image (downward) points to the endothelial cells of the first aortic arch, the hypobranchial artery.

exocrine pancreas (Figure 3C), whereas the endocrine pancreas, a single islet in zebrafish, was deficient in zcPGES immunostaining (Figure 3D).

zcPGES was also detectable in the developing brain and in the semicircular canals of the inner ear (Figure 2C). However, we did not detect zcPGES in the early otic placode, suggesting that its expression is induced after mid-somitogenesis.[30] Indeed, low levels of zcPGES mRNA and protein were detectable in most tissues thereafter, consistent with a "housekeeping" function of the enzyme.

PGE_2 plays an essential role in regulation of blood flow and electrolyte homeostasis in the mammalian kidney.[31] zcPGES was expressed in the pronephric ducts, beginning toward the end of day 1 after fertilization and persisting until the beginning of the larval stage. At 96 hpf, zcPGES mRNA (Figure 2D) and corresponding protein expression (Figure 3A and 3B) was apparent in the in the bilateral pronephric ducts which conjoin before they open into the cloaca.

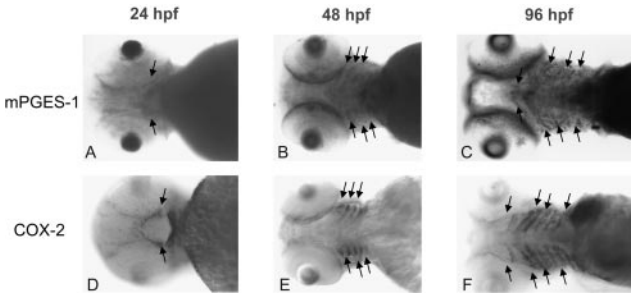

Figure 4. Comparison of zmPGES-1 (A through C) and zCOX-2 (D through F) transcript expression by whole mount in situ hybridization. Ventral views show developmental expression of zmPGES and zCOX-2 in the pharyngeal arches (arrows) at 24 hpf (A and D), 48 hpf (B and E), and 96 hpf (C and F). Although zCOX-2 is expressed in the vessel wall of the aortic arch arteries,[20] zmPGES staining projects onto the endothelial lining of the vasculature (also see Figure 3).

Zebrafish COX-1 expression was quite distinct from zcPGES expression at 18 hpf (Figure 2E). zCOX-1 was prominently expressed in the tail bud in an area projecting onto the intermediate mesoderm that is destined to become the pronephric tubule and ducts. However, at later stages, beginning at 24 hpf, COX-1 and cPGES were coexpressed in the intestine (Figure 2F), the inner ear (Figure 2G) and the pronephric ducts (Figure 2H).

Expression of zmPGES was more restricted than zcPGES. Beginning at 24 hpf, zmPGES expression appeared in the developing pharyngeal arches where it colocalized with zCOX-2 (Figure 4A and 4D). The developing pharyngeal arches are dynamic structures formed by migrating streams of neural crest cells which develop into cartilage, pigment, neuronal, and glial cells, whereas adjacent mesoderm becomes fated to develop into muscle and endothelium. The cellular localization of zCOX-2 and zmPGES were distinct at 48 hpf. Periluminal zmPGES contrasted with zCOX-2 in the aortic arch wall (Figure 4B and 4E), where it remained evident at 96 hpf (Figure 4F). zmPGES was exclusively expressed in the endothelial cells of the pharyngeal arch vasculature, which includes loops of sprouting gill arteries (Figure 4C). At this stage the vessels of the caudal arches have already separated into efferent and afferent arch arteries. However, the vascular sprouts which are destined to give rise to the branchial laminar arteries of the gills are still closed loops, not yet connecting the efferent and afferent arch arteries. Immunostaining of a 5-day-old embryo and visualization of the gill vasculature by confocal microscopy (Figure 3E) revealed that the sprouting endothelial loops that express zmPGES have formed interconnections between afferent and efferent vessels of the caudal arches.

Discussion

Deletion of mPGES-1 in mice abrogates the inflammatory response to carigeenan, similar to the effects of a traditional NSAID,[19] and a pathway linking COX-2/mPGES-1 derived PGE_2 activating the EP4 receptor has been suggested relevant to the destablilization of atherosclerotic plaques in humans.[32] Interest in mPGES-1 as a drug target is based on two untested assumptions: that other sources of PGE_2 formation, such as mPGES-2 and cPGES, are relatively unimportant in inflammation, and that cardiovascular complications of COX-2 inhibitors are attributable solely to suppression of PGI_2[10] but

not PGE_2. Given the accessibility of zebrafish vasculature to investigation of mPGES-1 distribution and function and the power of small molecule inhibitor screens in this model,[33] we sought to characterize their PGESs to permit study of their coregulation with COX enzymes in vivo during development.

The homology of the zebrafish and human enzymes was not as close as between the enzymes in humans and mice. However, the gene structure of the zebrafish orthologues was preserved, and all of the residues thought critical to catalytic functions of PGES were retained. Furthermore, the zebrafish genes mapped to regions of conserved synteny on chromosomes 5 and 23 and parsimony analysis assigned them to the predicted branches of other microsomal and cytosolic PGES proteins. Promoter sequence homology was less impressive. For example, computational analysis predicted TATA box binding protein recognition sites in the putative promoter regions of both zebrafish genes, but not in the human genes.

Despite the absence of several promoter motifs in zebrafish mPGES thought critical to gene regulation and the lack of concordance between putative human and zebrafish cPGES promoter sequences, the pattern of expression of the enzymes during development bears a striking similarity to their described distribution in rodents and in human tissues.[13,34] Thus, zcPGES and zmPGES were developmentally regulated and each showed a highly distinct pattern of organ and tissue specific expression. Firstly, zcPGES was quite diffusely expressed. ZmPGES-1 was, by contrast, expressed almost exclusively in endothelial cells of the developing pharyngeal arch vasculature.

Developmental expression of genes often coincides in kidney and ear, suggesting the linked development of these organs. Both COX-1 and cPGES-1 are coordinately expressed in the developing ear and pronephros of zebrafish. Mutation of such coexpressed genes or their transcription factors may result in oto-renal syndromes.[35] The distribution of cPGES also suggests the possibility of transcriptional integration of otic and renal development within endodermally derived organs, such as liver and pancreas, as observed with the homeobox transcription factor vHnf1.[36]

It has been estimated that only between 1400 and 2400 of >23,000 predicted zebrafish genes (http://www.ensembl.org/Danio-rerio/) are essential for embryonic development and organogenesis.[37,38] Mutation or suppression of these genes would result in a visible or lethal phenotype in the zebrafish embryo. Although we have previously reported a developmental phenotype resulting from knock down of COX-1,[20] injection of antisense morpholino modified oligonucleotides directed against the predicted translational start sites of both zcPGES and zmPGES-1 failed to produce visible morphological phenotypes (data not shown). Similarly, mPGES-1 deficient mice do not show developmental defects,[19] and administration of NSAIDs to zebrafish embryos also fails to result in a visible phenotype (T. Grosser, unpublished observation, 2002). However, among the adverse effects of COX inhibitors are tinnitus, renal impairment, and renovascular hypertension. Thus, despite these observations, the zebrafish enzymes, like zCOX-2, may act as modifiers of development or, indeed, contribute to function in developing organs.

Both COX-2 and mPGES-1 were expressed in a more restricted repertoire of tissues than COX-1 and cPGES-1. Their expression was largely confined to the developing pharyngeal arch vasculature. Closure of the ductus arteriosus, which derives from the sixth pharyngeal arch artery, is predominantly mediated by COX-2 derived PGE_2 at birth, with some modifying contribution of COX-1.[39,40] PGE_2 acts through the EP4 receptor to reduce ductal tone[41] and suppression of PGE_2 in the late gestational phase initiates closure of the ductus arteriosus at birth.[40] Regulated expression of COX-2 and mPGES, but not cPGES, is the source of PGE_2 in the ductus of the perinate.[42]

Although both zmPGES and zCOX-2 were expressed in the vascular structures of all pharyngeal arches in zebrafish, their expression was anatomically segregated. mPGES was expressed in the endothelium, whereas COX-2 was expressed in the wall of the aortic arch. Although it remains to be determined whether these enzymes are biochemically integrated, zmPGES-derived PGE_2 generation may contribute to vascular development. Thus, mPGES-1 appears to be the predominant source[43] of PGE_2 which promotes endothelial sprouting in vitro and in vivo.[44,45] The expression pattern of both COX-2 and mPGES-1 in zebrafish raises the possibility that they may function as modifiers of genes critical to vascular development, such as VEGF.

In summary, both COX-1 and cPGES were widely expressed during zebrafish development. Although their expression was often coordinated, cPGES was expressed without COX-1 in certain tissues and at certain stages in development. COX-2 and mPGES-1, by contrast, were essentially confined to the developing vasculature. Experiments with antisense morpholinos and with NSAIDs suggest that these genes may not be critical for development, but may modify this process and/or contribute to the function of developing organs. The expression of pattern mPGES-1 suggests that the consequences of its inhibition in the mature vasculature merits close attention in the light of the experience with rofecoxib.[10] In the meantime, characterization of the PGES enzymes in zebrafish offers a high throughput model system which may facilitate the detection of novel enzyme inhibitors and complement studies in the rodent in elucidating the role of these enzymes in vivo.

Acknowledgments

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Prostaglandin E Synthases in Zebrafish

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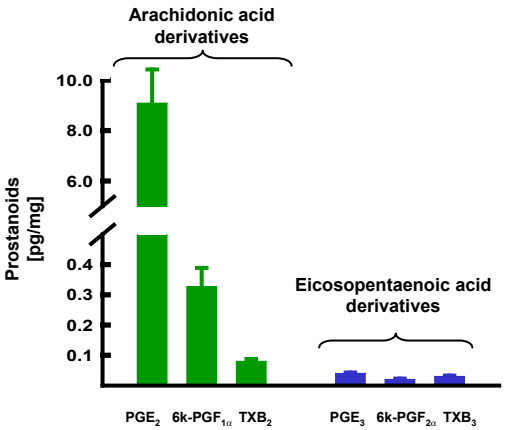
Prostaglandin E synthases in zebrafish

Online Figure I: Tissue concentrations of arachidonic acid derived (PGE_3, 6-keto $PGF_{1\alpha}$, TxB_2) and linoleic acid der PGE_3, 6-keto $PGF_{2\alpha}$, TxB_3) prostanoids were measured mass spectrometrically in homogenized adult zebrafish on a brine shrimp diet.



Online Figure II: Identification of the cDNA sequences and deduced protein sequences for zebrafish cytosolic PGES (A) and zebrafish microsomal PGES-1 (B). The putative binding sites for glutathione tyrosine (Y) and arginine (R) residues of zcPGES and zmPGES-1 are underlined. Conserved protein motifs of the human and zebrafish cPGESs are underlined; Putative N-glycosilation (red), Casein kinase II phosporylation (magenta), Myristate binding (green) and Protein kinase C phosporylation sites (blue) were predicted using OMIGA sequence analysis software (Accelrys, San Diego, CA).

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ATG CAG CCA GCT ACT GCC AAG TGG TAT GAC AGA CGG GAA GCT GTC TTC ATC GAG TTT TGT 60
 M   Q   P   A   T   A   K   W   Y   D   R   R   E   A   V   F   I   E   F   C
ATA GAA GAC AGC AAA GAT GTC CAA GTT AAA TTT GAC AAA ACA AAG CTT GAT TTC AGT TGT 120
 I   E   D   S   K   D   V   Q   V   K   F   D   K   T   K   L   D   F   S   C
GTT GGT GGA ACA GAT AAC ATG AAA CAC CAC AAT GAA GTA GAT CTA TTG GAG GCC ATT GAC 180
 V   G   G   T   D   N   M   K   H   H   N   E   V   D   L   L   E   A   I   D
CCA AAT GAC TCT AAA CAC AAG CGC ACA GAC AGA TCT GTG TTT TGC TGT CTA AAA AAA GCA 240
 P   N   D   S   K   H   K   R   T   D   R   S   V   F   C   C   L   K   K   A
GAA CCT GGC AAG TCT TGG CCA AGG TTA ACA AAA GAG AAA GCA AAG CTT AAC TGG CTT AGT 300
 E   P   G   K   S   W   P   R   L   T   K   E   K   A   K   L   N   W   L   S
GTT GAC TTC AAT AAC TGG AAA GAC TGG GAG GAT GAC TCT GAT GAA GAA TTG TCC AGT TTT 360
 V   D   F   N   N   W   K   D   W   E   D   D   S   D   E   E   L   S   S   F
GAC CGA TTT TCA GAG ATG ATG AAC AAC ATG GGA GGG GAA GAT GAC CTA CCA GAT GTG GAT 420
 D   R   F   S   E   M   M   N   N   M   G   G   E   D   D   L   P   D   V   D
GGT GCA GAT GAA GAA GAG TCT GGA 444
 G   A   D   E   E   E   S   G
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A

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ATG CTC GGG AGC GAC ATA CAG TTG TGC TTC ATC TTC TAC AGC ACG CTT TTA ATC TTG AAG  60
 M   L   G   S   D   I   Q   L   C   F   I   F   Y   S   T   L   L   I   L   K
ATG TAC ATT ATT GCC ATC ATC ACA GGC CAA GTG AGA CTT CGG AAA AAG GCG TTT GCT AAC 120
 M   Y   I   I   A   I   I   T   G   Q   V   R   L   R   K   K   A   F   A   N
CCA GAG GAC GCC GAG AGA CAC GGA GGT GTG CAG TTC TGC CGC ACG GAT CCA TAT GTG GAG 180
 P   E   D   A   E   R   H   G   G   V   Q   F   C   R   T   D   P   Y   V   E
                                                                MAPEG domain
CGC TGT AGG AGA GCA CAG CAG AAT GAC ATG GAG AAC ATT TTG CCC TTT TTA TTT CTT GGA 240
 R   C   R   R   A   Q   Q   N   D   M   E   N   I   L   P   F   L   F   L   G
                                    MAPEG domain
GCG GTC TAC TCC ATG ACA AGC CCA TCA TAT GCA GCA GCA CAA CTT CAT TTC CTC ATC TTC 300
 A   V   Y   S   M   T   S   P   S   Y   A   A   A   Q   L   H   F   L   I   F
                                    MAPEG domain
TTC CTG GGT CGA GTT CTT CAC AGC GTT GCA TAT CTG CTG GCA CTA AAA GCA CCG ACA CGT 360
 F   L   G   R   V   L   H   S   V   A   Y   L   L   A   L   K   A   P   T   R
                                    MAPEG domain
TCA TTG GCC TAT GTC ATC GCT CAG GTG CCT TGC ATT TCA ATG GCC ATA CAG ATA CTC ATG 420
 S   L   A   Y   V   I   A   Q   V   P   C   I   S   M   A   I   Q   I   L   M
                                    MAPEG domain
GAA GTG GCC TCA TTC GCA 438
 E   V   A   S   F   A
   MAPEG domain
```

B