Supplemental Results

Cloning and Sequence Analysis of Zebrafish PGLYRPs

PGLYRP-2 is located on chromosome 8 and the coding region of this gene is organized into 5 exons (Fig. S1) and codes for a protein with 458 amino acids. Our clone for PGLYRP-2 has 6 single nucleotide mismatches, 3 single nucleotide deletions, one 105 bp insertion, and an additional 386 bp at the 3' end compared to the predicted sequence XM691972. Our clone also differs from the EST clone CK029852 in the 105 bp insertion. This insertion is at a spice junction, which could indicate alternate splicing. However, despite repeated attempts we were unable to clone a full-length cDNA exactly matching this EST clone. The entire PGLYRP-2 cDNA is located on the genomic clone NW4947 and is identical in sequence to the genomic clone except for 13 single or double bp mismatches. PGLYRP-5 is located on chromosome 18 and the coding region of this gene is organized into 4 exons (Fig. S1) and codes for a 238-amino acid protein. Our clone has 4 bp differences and an additional 218 bp at the 3' end compared to the predicted sequence XM687301. The complete PGLYRP-5 cDNA is located on the genomic clone NW633877 and the sequence is identical except for 3 single nucleotide mismatches. PGLYRP-6, so far, is not mapped to a chromosome. The coding region of this gene is organized into 5 exons (Fig. S1) and codes for a protein with 496 amino acids. Our clone has 15 single bp mismatches, a 3 bp insertion, and an additional 61 bp at the 3' end compared to the predicted sequence XM695448. The complete PGLYRP-6 cDNA is located on the genomic clone NW652509. The sequence of our clone differs from the genomic sequence by 14 single or double bp mismatches or deletions.

PGRP domains in almost all vertebrate and invertebrate PGRPs have at least one disulfide bond between conserved Cys labeled 3 and 4 in Fig. S2. Mammalian PGRP domains have additional one or two disulfides between Cys labeled 1 and 6 and 2 and 5 in Fig.S2, but these last two disulfides are not present in insect PGRPs. Zebrafish PGLYRP-2 and PGLYRP-6 have four of these conserved Cys, and thus likely have two disulfides, between Cys 1 and 6 and between Cys 3 and 4, similar to human PGLYRP-2. By contrast, zebrafish PGLYRP-5 has only one of these conserved Cys, and thus likely does not have any of these three disulfides. Unlike human PGLYRP proteins, zebrafish PGLYRP proteins do not form disulfide-linked dimers, and accordingly, do not have the Cys corresponding to the Cys involved in the formation of dimers in human PGLYRPs (Fig. S2). One disulfide bond between cysteines in the PGRP domain is believed to be required for the proper three-dimensional structure and for the amidase function (Mellroth et al., 2003; Wang et al., 2003). However, this may not be absolutely required because bacteriophage amidases and PGLYRP-5 do not have these Cys and are still active (this study).

Zebrafish PGLYRP-2 and PGLYRP-6 were predicted to be DAP-specific as they have the required Gly and Trp in the peptidoglycan-binding groove (Fig. S2), that correspond to Gly(G)68 and Trp(W)69 in human PGLYRP-1 (Swaminathan et al., 2006) and the essential Arg (Fig. 2) corresponding to Arg(R)254 in *Drosophila* PGRP-LE (Lim et al., 2006). PGLYRP-5 has the required Gly and Arg but lacks Trp that is also considered essential (Swaminathan et al., 2006).

Supplemental Figure Legends

Figure S1. Genomic organization of three-zebrafish *pglyrp* genes. Exons coding for the proteins and intervening introns are shown.

Figure S2. The C-terminal regions of zebrafish and human PGLYRP proteins are conserved. Alignment of amino acid sequences of PGRP domains of zebrafish (Dr) and human (Hs) PGLYRPs. Zn^{2+}-binding amino acids required for amidase activity, and conserved cysteines forming internal disulfides and dimers are boxed. Identical amino acids are in bold and shaded dark and similar amino acids are shaded light. The Gly (G), Trp (W), and Arg (R) that are believed (Lim et al., 2006; Swaminathan et al., 2006) to determine preferential DAP specificity are indicated by arrows.

Figure S3. Zebrafish PGLYRP proteins, purity and reactivity with antibodies. PGLYRP proteins were purified from supernatants of stable S2 cell transfectants and analyzed by Coomassie blue staining (5 μg/lane) or Western blots (WB, 0.5 μg/lane). Proteins were detected on Western blots using anti-PGLYRP-2, anti-PGLYRP-5, anti-PGLYRP-6, or anti-V5 antibody. Size markers are shown on the left.

Table S1. Oligonucleotides used in this study

Name		Sequence 5′ → 3′
Oligonucleotides used for cloning		
PGLYRP-2	5′ RACE	CTTGTCTGTGTCCGAGAATGGTGAAATCC
PGLYRP-2	3′ RACE	GGACGCAATAACGTGGGGTATGG
PGLYRP-2	Full length	ATGGCTGGAATATGCATGCAGAGCTCAGCT
PGLYRP-5	5′ RACE	TTGCCCATGAAGGCGATGCCAACAGA
PGLYRP-5	3′ RACE	GGCAGAGGATGGGGGATTGTAGGA
PGLYRP-5	Full length	ATGCAGCACAGTTTCTTCATCTTCCTG
PGLYRP-6	5′ RACE	GCTACTCAGAGACTCCTCAGGATGGTCAGA
PGLYRP-6	3′ RACE	CTCGTCTGACCATCCTGAGGAGTCT
PGLYRP-6	Full length	ATGGGAAGGCAGTTTATCAGTGTGTGC
Oligonucleotides used for subcloning into pGEM-T vector		
PGLYRP-2F		ATGGCTGGAATATGCATGCAGAGCTCAGCTG
PGLYRP-2R		GCAGCTGTTATAAAATGATGAAGTGCTTGGCTG
PGLYRP-5F		ATGCAGCACAGTTTCTTCATCTTCCTG
PGLYRP-5R		CCGCTCCTGCATGTGCATGCGCTGAATGTG
PGLYRP-6F		ATGGGAAGGCAGTTTATCAGTGTGTGC
PGLYRP-6R		CCGTGATATCCGACACATGGTGATTGATGAG
Oligonucleotides used for subcloning into pMT/BiP/V5-His vector		
PGLYRP-2F		CGAGATCTGCTGGAATATGCATGCAGAGCTCA
PGLYRP-2R		GCTCTAGACTTCAAAGGATCCTTATCTTT
PGLYRP-5F		GCAGATCTCAGCACAGTTTCTTCATCTTCCTG
PGLYRP-5R		GCTCTAGATGCTTGCAAAAGTTCATTGTTTTGC
PGLYRP-6F		GCAGATCTGGAAGGCAGTTTATCAGTGTGTGC
PGLYRP-6R		GCTCTAGAAGGCAGATAACTCTGGTATCGCTC
Oligonucleotides used for generating hybridization probes		
PGLYRP-2F		ATGGCTGGAATATGCATGCAGAGCTCAGCTG
PGLYRP-2R		GCAGCTGTTATAAAATGATGAAGTGCTTGGCTG
PGLYRP-5F		ATGTTGGAGCATACAGGGAACGAAAAG
PGLYRP-5R		CCGCTCCTGCATGTGCATGCGCTGAATGTG
PGLYRP-6F		ATGATGGATCTACGGCTGTTTTCCCTGCTCA
PGLYRP-6R		CCGTGATATCCGACACATGGTGATTGATGAG

[F] Supplemental Figure 1

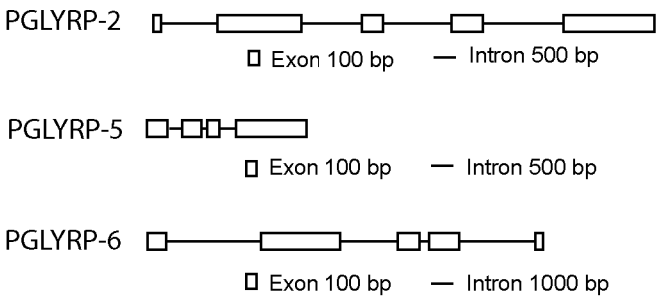

Figure S1

[F] Supplemental Figure 2

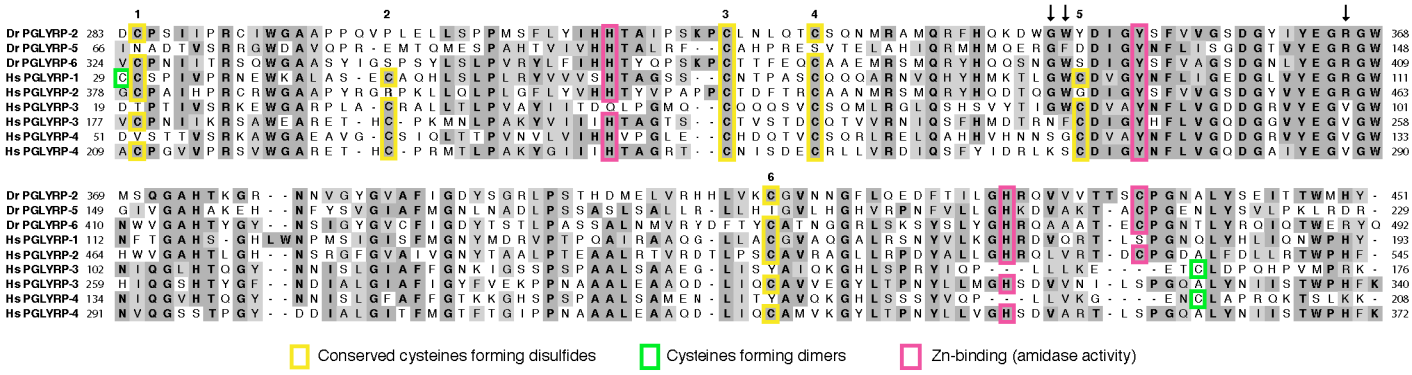

Figure S2

[F] Supplemental Figure 3

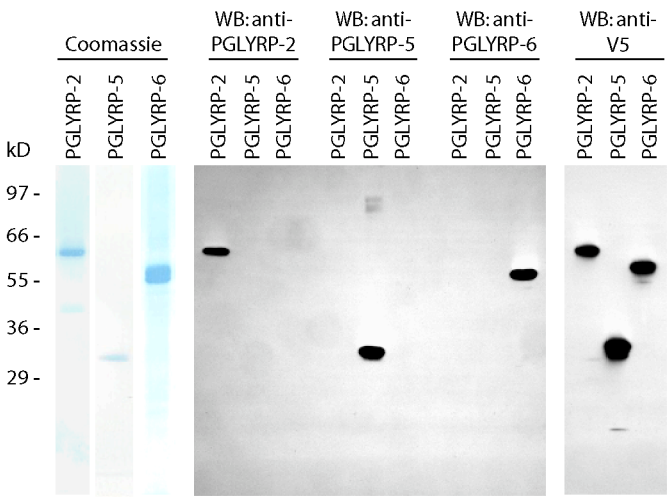

Figure S3