*J Physiol* 592.5 (2014) pp 881–897



The Journal of Physiology

SYMPOSIUM REVIEW

# Teleost fish models in membrane transport research: the PEPT1(SLC15A1) $H^+$–oligopeptide transporter as a case study

Alessandro Romano, Amilcare Barca, Carlo Storelli and Tiziano Verri

*Laboratory of General Physiology, Department of Biological and Environmental Sciences and Technologies, University of Salento, Lecce, Italy*

**Abstract** Human genes for passive, ion-coupled transporters and exchangers are included in the so-called solute carrier (SLC) gene series, to date consisting of 52 families and 398 genes. Teleost fish genes for SLC proteins have also been described in the last two decades, and catalogued in preliminary SLC-like form in 50 families and at least 338 genes after systematic GenBank database mining (December 2010–March 2011). When the kinetic properties of the expressed proteins are studied in detail, teleost fish SLC transporters always reveal extraordinary 'molecular diversity' with respect to the mammalian counterparts, which reflects peculiar adaptation of the protein to the physiology of the species and/or to the environment where the species lives. In the case of the $H^+$–oligopeptide transporter PEPT1(SLC15A1), comparative analysis of diverse teleost fish orthologs has shown that the protein may exhibit very eccentric properties in terms of pH dependence (e.g. the adaptation of zebrafish PEPT1 to alkaline pH), temperature dependence (e.g. the adaptation of icefish PEPT1 to sub-zero temperatures) and/or substrate specificity (e.g. the species-specificity of PEPT1 for the uptake of L-lysine-containing peptides). The revelation of such peculiarities is providing new contributions to the discussion on PEPT1 in both basic (e.g. molecular structure–function analyses) and applied research (e.g. optimizing diets to enhance growth of commercially valuable fish).

(Received 2 June 2013; accepted after revision 21 August 2013; first published online 27 August 2013)

**Corresponding author** T. Verri: Laboratory of General Physiology, Department of Biological and Environmental Sciences and Technologies, University of Salento, Via Provinciale Lecce-Monteroni, I-73100 Lecce, Italy. Email: tiziano.verri@unisalento.it

**Abbreviations** PEPT1, peptide transporter 1; SLC, solute carrier; SLC15A1, solute carrier 15 family member A1.

## The teleost fish model: general features

With about 30,000 species – more than 95% of all living fish species and roughly half of all living vertebrate species – teleost fish represent the largest and most diverse group of vertebrates (Nelson, 2006; see also www.fishbase.org). They exhibit an astonishing level of diversity that is evident by examining their morphology,

**Tiziano Verri** is Associate Professor of Physiology at the University of Salento (formerly University of Lecce), Lecce, Italy. He got his MSc (Biology) from the University of Lecce (1989), and PhD (Physiology) from the 'Federico II' University, Naples, Italy (1995). His education/training/appointments include a doctoral fellowship at the Institute of Physiology, University of Zurich-Irchel, Zurich, Switzerland (1993–1994), a post-doctoral fellowship at the National Research Council Istituto Sperimentale Talassografico 'A. Cerruti', Taranto, Italy (1995–1996), a post-doctoral fellowship at the Laboratory of General Physiology, Department of Biology, University of Lecce (1997–2000), and the post of Assistant Professorship (Physiology) at the University of Lecce (2000–2006). His main research interests cover function and regulation of the proteins that mediate the transport of small solutes (with emphasis on oligopeptides) across the plasma membranes of epithelial cells.

This review was presented at the symposium *Fishing with flies, worms and bacteria. Emerging models for mammalian membrane transport and trafficking*, which took place at Experimental Biology 2013, Boston, MA, USA on 21 April 2013.



DOI: 10.1113/jphysiol.2013.259622

physiology, ecology and behaviour as well as many other facets of their biology (Nelson, 2006). It is widely acknowledged that the extraordinary diversity of teleost fish has been generated by a number of peculiar features in their genomes (Volff, 2005; Ravi & Venkatesh, 2008). For instance, a round of whole-genome duplication (tetraploidization/rediploidization) has taken place during the early evolution of the ray-finned fish (Actinopterygian) lineage – to which teleost fish belong – and hundreds of duplicate gene pairs generated by this event have been maintained in teleost fish genomes over 300–450 million years evolution, thus providing an original evolutionary framework for the diversification of gene functions, the generation of diversity and the speciation process. In addition, teleost fish genomes have gone through frequent gene-linkage disruptions (due to the high rate of chromosomal rearrangements) compared to other vertebrates. Also, protein-coding sequences in teleost fish seem to have evolved faster than in mammals, and irrespective of their singleton or duplicated status. Finally, a significant number of conserved non-coding elements that are shared between, for example, cartilaginous fishes (sharks, rays) and tetrapods (amphibians, reptiles, birds and mammals) have diverged beyond recognition in teleost fish, a process that seems initiated in basal ray-finned fishes before the whole-genome duplication event. Because of their enormous genomic variability, teleost fish are extremely attractive models for studying a variety of biological questions, included those related to the evolution of gene functions and/or of their regulatory control (Kassahn *et al.* 2009; Sato *et al.* 2009).

## The SLC family series in teleost fish

Human genes for passive transporters (facilitated or facilitative transporters or uniporters), ion-coupled transporters (symporters) and exchangers (antiporters) are included in the so-called solute carrier (SLC) gene series (Hediger *et al.* 2004; see also http://www.genenames.org/), to date consisting of 52 families and 398 genes (http://www.bioparadigms.org). Teleost fish genes for SLC proteins have also been described in the last two decades, and preliminarily classified in SLC-like form in 50 families and at least 338 genes after mining in the publicly available GenBank database between December 2010 and March 2011 (Verri *et al.* 2012). Despite the incompleteness in the annotation process and/or the limited amount of submitted sequence information in this public database, in this survey transporters of the SLC series were retrieved from 49 teleost fish species. *Danio rerio* (zebrafish) was the most relevant species with 304 genes found, followed by *Salmo salar* (Atlantic salmon) with 53 genes, *Oncorhynchus mykiss* (rainbow trout) with 22, *Takifugu rubripes* (torafugu) with 16, *Takifugu obscurus* (mefugu) with 11, and *Anguilla japonica* (Japanese eel) with 10. SLC genes from various teleost fish models popular in genetics, developmental biology studies, and/or genome analysis, including *Oryzias latipes* (Japanese medaka), *Gasterosteus aculeatus* (three-spined stickleback), and *Tetraodon nigroviridis* (spotted green pufferfish), as well as from various teleost fish species economically important in aquaculture, including *Dicentrarchus labrax* (European sea bass) and *Gadus morhua* (Atlantic cod), were also found, although less represented. As expected, several SLC genes were found in duplicate. A comprehensive description of this kind of information is given in Verri *et al.* (2012), together with a number of other facts. For instance, among the teleost fish SLC families and their members as resulting from the above study, only 41 gene products had functionally been characterized, i.e. the cloned transporters had been tested for transport function by expression in suitable heterologous expression systems (such as *Xenopus laevis* oocytes; Romero *et al.* 1998; Bossi *et al.* 2007; Markovich, 2008). According to He *et al.* (2009) – which included the SLC families in 12 groups based on the nature of their substrates – 15 of these were genes encoding proteins involved in inorganic cation–anion transport, two in amino acid–oligopeptide transport, six in transport of glucose and other sugars, six in metal ion transport, two in transport of bile salts and organic anions, 10 in transport of urea, neurotransmitters and biogenic amines, ammonium, and choline. Most of the functional studies on the SLC proteins were performed to define the role(s) of the transporters in the fundamental processes that allow teleost fish to live in aquatic environments at, for example, different salinities (e.g. cation, anion, urea, ammonium transporters) and oxygen levels (e.g. glucose and other sugar transporters). Studies were also conducted to establish how the uptake of biologically relevant (and/or toxic) metal ions (including iron, copper and cadmium) affects fish development. Information, although more limited, has also been made available on the molecular transport processes that mediate the uptake of amino acids and oligopeptides, due to the physiological relevance of such nutrients' intestinal absorption in teleost fish growth (mostly with regard to the cultured species). Many transporters were cloned from more than one species to compare the function in different teleost fish backgrounds. Again, zebrafish was the most popular teleost fish model used for functional studies. In particular, due to the good annotation of its genes and genome, zebrafish has often been used to extract from the database good full-length sequences that are useful both to generate/recruit adequate molecular tools for functional analysis in zebrafish itself and/or to generate/design molecular tools to jump to (an)other teleost fish species. In this respect, zebrafish can reasonably be considered a sort of reference teleost fish model in the solute carriers field to date. In a survey

performed *ad hoc* for this review (July 2013), at least 275 'official' genes for zebrafish SLC proteins (belonging to 51 families) have been found in the GenBank database. Of these more than 50 have formally been designated as duplicate genes (for details see Table 1).

## The teleost fish PEPT1(SLC15A1) $H^+$–oligopeptide transporter: a case study

During the digestive process, hydrolysis of dietary proteins leads to high levels of di- and tripeptides (di/tripeptides) in the intestinal lumen. The di/tripeptides released are either further hydrolysed to their constituent amino acids or directly taken up in intact form into intestinal epithelial cells. Following the apical influx, di/tripeptides are hydrolysed in the cytosol and the resulting amino acids cross the basolateral membrane using amino acid-transporting systems. Peptides not undergoing hydrolysis can exit the cell by (a) not-yet-molecularly-identified basolateral peptide transport system(s) (possibly responsible for both cell-to-blood efflux and blood-to-cell uptake; among others see: Dyer *et al.* 1990; Thwaites *et al.* 1993*a*,*b*; Thamotharan *et al.* 1996; Terada *et al.* 1999; Irie *et al.* 2004; Pieri *et al.* 2010; Berthelsen *et al.* 2013) and/or by other basolateral solute transporters that have been shown to allow transport of selected peptides (Daniel, 2004). Di/tripeptide transport is mediated at the apical membrane of enterocytes by a single transporter, named PEPT1 (peptide transporter 1) or SLC15A1 (solute carrier 15 family member A1) (Daniel & Kottra, 2004; Smith *et al.* 2013). PEPT1 operates as a $Na^+$-independent, $H^+$-dependent transporter for an enormous number of di/tripeptides. Di/tripeptide transport is electrogenic and responds to both an inwardly directed transmembrane $H^+$ gradient and a transmembrane internal negative electrical potential (Daniel, 2004). Transport is enantio-selective and apparently involves a variable $H^+$–substrate stoichiometry for uptake of neutral and (positively or negatively) charged peptides. PEPT1 is also responsible for the transport of orally active drugs, including $\beta$-lactam antibiotics, aminopeptidase and angiotensin-converting enzyme inhibitors, $\delta$-aminolevulinic acid and many selected pro-drugs (Rubio-Aliaga & Daniel, 2002, 2008; Smith *et al.* 2013), although the option that some molecules can simply interact without being transported cannot be excluded (Knütter *et al.* 2008; Brandsch, 2009).

In teleost fish, the initial observation of intestinal di/tripeptide absorption dates back to more than 30 years ago, when in rainbow trout the rate of intestinal absorption of Gly-Gly was compared *in vivo* to that of Gly (Bogé *et al.* 1981). The first description of the underlying mechanisms that allow di/tripeptide transport across the plasma membranes in teleost fish was provided ten years later, when the evidence of peptide transport was reported in brush-border membrane vesicles (BBMV) of *Oreochromis mossambicus* (Mozambique tilapia) intestinal epithelial cells by monitoring uptake of Gly-L-Phe *vs.* L-Phe (Reshkin & Ahearn, 1991). One year later the first evidence that dipeptides are cotransported with $H^+$ was provided, showing Gly-Gly-dependent intravesicular acidification in eel intestinal BBMV (Verri *et al.* 1992). Since then, the existence of a carrier-mediated, $H^+$-dependent transport of di/tripeptides in the brush-border membrane of fish enterocytes has been confirmed in many teleost species by using various biochemical/physiological approaches, thus providing basic kinetics and substrate specificities of the transport activity (Verri *et al.* 2010). In 2003, the first peptide transporter from a teleost fish, i.e. the zebrafish PEPT1, was cloned and functionally characterized in *X. laevis* oocytes as a low-affinity/high-capacity system (Verri *et al.* 2003). The transporter had affinities for the peptide substrates in the 0.1–10 mM range, depending on the structure and physicochemical nature of the tested substrate. After the molecular and functional characterization of this transporter, the interest for PEPT1 in teleost fish increased progressively, and cloning and functional characterization of PEPT1 orthologs from other species, some of them of the highest commercial value, were carried out in the last few years (Table 2). For three species, namely European sea bass, Atlantic salmon and *Chionodraco hamatus* (Antarctic icefish), detailed kinetic characterizations using *X. laevis* oocytes have been carried out, providing the basis for functional comparison among teleost fish and among teleost fish and mammalian expressed clones. Notably, with increasing the number of sequences available it became evident that teleost fish PEPT1 is duplicated, as initially observed by Gonçalves *et al.* (2007), and thus PEPT1A(SLC15A1A) and PEPT1B(SLC15A1B) genes have to be considered as existing in teleost fish genomes (Table 2, Supplemental Figs S1 and S2). However, at the moment functional transport data are available from PEPT1B transporters only (Table 2), which for the sake of clarity will be called PEPT1 when referring to functional studies, since they are named this way in all the literature published so far. When the functional properties of the expressed proteins (in terms of kinetic parameters, substrate specificities and inhibition patterns, sensitivity to extracellular factors, etc.) were studied in detail, teleost fish PEPT1 transporters revealed a surprising 'molecular diversity' with respect to the well-known mammalian counterparts, which possibly reflects peculiar adaptation to the physiology of the organ(ism) and/or to the environment where the teleost fish lives. In the following paragraphs of this review we will focus on some forms of anatomical (next paragraph) and functional (following three paragraphs) adaptation of the teleost fish PEPT1, placing them ideally alongside the well-established human (Daniel & Kottra, 2004; Smith *et al.* 2013), mammalian (Daniel, 2004) and avian (Gilbert

**Table 1. List of the solute carrier (SLC) families in zebrafish (*Danio rerio*) compared to human**

| | | | Zebrafish | | | | |
|---|---|---|---|---|---|---|---|
| Family | Name | Human Total genes | Total genes | Non-duplicated genes | Duplicated genes designated as 'a' and 'b' | Duplicated genes designated as 'tandem duplicate' | Possibly duplicated genes and/or genes not obviously designated |
| SLC1 | High affinity glutamate and neutral amino acid transporter family | 7 | 9 | 5 | 4 | 0 | 0 |
| SLC2 | Facilitative GLUT transporter family | 14 | 12 | 6 | 2 | 0 | 4 |
| SLC3 | Heavy subunits of the heteromeric amino acid transporters | 2 | 2 | 1 | 1 | 0 | 0 |
| SLC4 | Bicarbonate transporter family | 10 | 6 | 3 | 3 | 0 | 0 |
| SLC5 | Sodium glucose cotransporter family | 12 | 8 | 7 | 0 | 0 | 1 |
| SLC6 | Sodium- and chloride-dependent neurotransmitter transporter family | 20 | 13 | 8 | 4 | 0 | 1 |
| SLC7 | Cationic amino acid transporter/ glycoprotein-associated family | 14 | 10 | 6 | 0 | 0 | 4 |
| SLC8 | $Na^+$–$Ca^{2+}$ exchanger family | 4 | 4 | 1 | 3 | 0 | 0 |
| SLC9 | $Na^+$–$H^+$ exchanger family | 13 | 7 | 5 | 1 | 1 | 0 |
| SLC10 | Sodium bile salt cotransport family | 7 | 4 | 4 | 0 | 0 | 0 |
| SLC11 | Proton-coupled metal ion transporter family | 2 | 1 | 1 | 0 | 0 | 0 |
| SLC12 | Electroneutral cation-coupled Cl cotransporter family | 9 | 8 | 5 | 1 | 1 | 1 |
| SLC13 | Human $Na^+$-sulfate–carboxylate cotransporter family | 5 | 4 | 3 | 0 | 0 | 1 |
| SLC14 | Urea transporter family | 8 | 1 | 1 | 0 | 0 | 0 |
| SLC15 | Proton oligopeptide cotransporter family | 4 | 3 | 2 | 1 | 0 | 0 |
| SLC16 | Monocarboxylate transporter family | 14 | 10 | 7 | 2 | 0 | 1 |
| SLC17 | Vesicular glutamate transporter family | 9 | 5 | 3 | 2 | 0 | 0 |
| SLC18 | Vesicular amine transporter family | 4 | 3 | 2 | 1 | 0 | 0 |
| SLC19 | Folate/thiamine transporter family | 3 | 3 | 2 | 0 | 0 | 1 |
| SLC20 | Type III $Na^+$-phosphate cotransporter family | 2 | 2 | 1 | 1 | 0 | 0 |
| SLC21 | Organic anion transporting family | 11 | 11 | 11 | 0 | 0 | 0 |
| SLC22 | Organic cation/anion/zwitterion transporter family | 23 | 5 | 4 | 1 | 0 | 0 |
| SLC23 | $Na^+$-dependent ascorbic acid transporter family | 4 | 2 | 2 | 0 | 0 | 0 |
| SLC24 | $Na^+$/($Ca^{2+}$–$K^+$) exchanger family | 5 | 4 | 3 | 1 | 0 | 0 |
| SLC25 | Mitochondrial carrier family | 53 | 35 | 26 | 8 | 0 | 1 |
| SLC26 | Multifunctional anion exchanger family | 11 | 8 | 7 | 0 | 0 | 1 |
| SLC27 | Fatty acid transport protein family | 6 | 4 | 2 | 2 | 0 | 0 |
| SLC28 | $Na^+$-coupled nucleoside transport family | 3 | 0 | 0 | 0 | 0 | 0 |
| SLC29 | Facilitative nucleoside transporter family | 4 | 3 | 2 | 0 | 0 | 1 |
| SLC30 | Zinc efflux family | 10 | 9 | 8 | 1 | 0 | 0 |
| SLC31 | Copper transporter family | 2 | 2 | 2 | 0 | 0 | 0 |
| SLC32 | Vesicular inhibitory amino acid transporter family | 1 | 1 | 1 | 0 | 0 | 0 |
| SLC33 | Acetyl-CoA transporter family | 1 | 1 | 1 | 0 | 0 | 0 |

**Table 1. Continued**

| | | | Zebrafish | | | | |
|---|---|---|---|---|---|---|---|
| Family | Name | Human Total genes | Total genes | Non-duplicated genes | Duplicated genes designated as 'a' and 'b' | Duplicated genes designated as 'tandem duplicate' | Possibly duplicated genes and/or genes not obviously designated |
| SLC34 | Type II $Na^+$-phosphate cotransporter family | 3 | 2 | 0 | 1 | 0 | 1 |
| SLC35 | Nucleoside-sugar transporter family | 30 | 19 | 16 | 3 | 0 | 0 |
| SLC36 | Proton-coupled amino acid transporter family | 4 | 1 | 1 | 0 | 0 | 0 |
| SLC37 | Sugar-phosphate/phosphate exchanger family | 4 | 3 | 2 | 1 | 0 | 0 |
| SLC38 | System A & N sodium-coupled neutral amino acid transporter family | 11 | 8 | 6 | 1 | 0 | 1 |
| SLC39 | Metal ion transporter family | 14 | 10 | 10 | 0 | 0 | 0 |
| SLC40 | Basolateral iron transporter family | 1 | 1 | 1 | 0 | 0 | 0 |
| SLC41 | MgtE-like magnesium transporter family | 3 | 1 | 1 | 0 | 0 | 0 |
| SLC42 | Rh ammonium transporter family | 3 | 6 | 5 | 0 | 0 | 1 |
| SLC43 | $Na^+$-independent, system-L like amino acid transporter family | 3 | 3 | 0 | 3 | 0 | 0 |
| SLC44 | Choline-like transporter family | 5 | 4 | 2 | 2 | 0 | 0 |
| SLC45 | Putative sugar transporter family | 4 | 4 | 4 | 0 | 0 | 0 |
| SLC46 | Folate transporter family | 3 | 2 | 2 | 0 | 0 | 0 |
| SLC47 | Multidrug and toxin extrusion (MATE) family | 2 | 2 | 2 | 0 | 0 | 0 |
| SLC48 | Heme transporter family | 1 | 1 | 0 | 1 | 0 | 0 |
| SLC49 | FLVCR-related transporter family | 4 | 4 | 3 | 0 | 0 | 1 |
| SLC50 | Sugar efflux transporters | 1 | 1 | 1 | 0 | 0 | 0 |
| SLC51 | Transporters of steroid-derived molecules | 2 | 1 | 1 | 0 | 0 | 0 |
| SLC52 | Riboflavin transporter family RFVT/SLC52 | 3 | 2 | 2 | 0 | 0 | 0 |
| Total | | 398 | 275 | 201 | 51 | 2 | 21 |

The total number of 'official members' (i.e. the members for which an official symbol has been assigned) in each family is shown for human and zebrafish. The number of zebrafish non-duplicated, duplicated and possibly duplicated/not obviously designated genes is also shown. Data have been obtained by GenBank database search on the Reference Zv9 Primary Assembly (July 2013).

*et al.* 2008) morphological/structural/functional backgrounds.

## Organ/tissue distribution of PEPT1 in teleost fish

In mammals, PEPT1 is abundantly expressed in the epithelial cells from small intestine (duodenum, jejunum and ileum, with no or little expression in normal colon) and kidney (proximal tubule S1 segments), and to a lesser extent in pancreas, bile duct and liver (Daniel, 2004; Daniel & Kottra, 2004; Gilbert *et al.* 2008; Smith *et al.* 2013). In contrast to most species, ruminants (sheep and cattle) express PEPT1 in the stomach (omasum and rumen; Chen *et al.* 1999; Pan *et al.* 2001; Gilbert *et al.* 2008). In birds, there is considerable expression of PEPT1 in the ceca in addition to the small intestine and the kidney (Chen *et al.* 2002; Gilbert *et al.* 2008).

In teleost fish, PEPT1 is primarily expressed in the intestine and found to a much lower extent in a variety of other organs/tissues, including kidney, liver, spleen and others (Table 3). The analysis of mRNA expression along the alimentary canal indicates that PEPT1 expression is invariably restricted to the

**Table 2. PEPT1-related nucleotide sequences in teleost fish available in GenBank (annotated) or derived by computational analysis (predicted)**

| Description | Species | GenBank Acc. No. | Functional expression | Reference (listed by year of publication) |
|---|---|---|---|---|
| mRNA, complete cds (annotated) | | | | |
| PEPT1B | Zebrafish (*Danio rerio*) | NM_198064.1 | Yes | Verri *et al.* 2003 |
| PEPT1B | Atlantic cod (*Gadus morhua*) | AY921634.1 | No | Rønnestad *et al.* 2007 |
| PEPT1A | China rockfish (*Sebastes nebulosus*) | EU160494.1 | No | GenBank submission[a] |
| PEPT1B | European sea bass (*Dicentrarchus labrax*) | FJ237043.2 | Yes | Terova *et al.* 2009; Sangaletti *et al.* 2009 |
| PEPT1B | Atlantic salmon (*Salmo salar*) | NM_001146682.1 | Yes | Rønnestad *et al.* 2010 |
| PEPT1A | Zebrafish (*Danio rerio*) | XM_001919879.3 | No | GenBank submission[b] |
| PEPT1B | Common carp (*Cyprinus carpio*) | JN896885.1 | No | GenBank submission[c] |
| PEPT1A | Mummichog (*Fundulus heteroclitus macrolepidotus*) | JN615008.1 | No | Bucking & Schulte 2012 |
| PEPT1B | Mummichog (*Fundulus heteroclitus macrolepidotus*) | JN615007.1 | No | Bucking & Schulte 2012 |
| PEPT1B | Yellow perch (*Perca flavescens*) | GQ906471.2 | No | Kwasek *et al.* 2012 |
| PEPT1B | Red crucian carp (*Carassius auratus red var.*) | JQ411137.1 | No | GenBank submission[d] |
| PEPT1B | Tetraploid hybrids of red crucian carp and common carp (*Carassius auratus x Cyprinus carpio*) | JQ411139.1 | No | GenBank submission[d] |
| PEPT1B | Triploid hybrids of tetraploid hybrids of red crucian carp and common carp and Japanese crucian carp (*Carassius auratus x Cyprinus carpio x Carassius cuvieri*) | JQ411138.1 | No | GenBank submission[d] |
| PEPT1B | White grouper (*Epinephelus aeneus*) | JX122768.1 | No | GenBank submission[e] |
| PEPT1A | Japanese eel (*Anguilla japonica*) | AB762417.1 | No | GenBank submission[f] |
| PEPT1B | Grass carp (*Ctenopharyngodon idella*) | JN088166.1 | No | Liu *et al.* 2013 |
| PEPT1B | Antarctic icefish (*Chionodraco hamatus*) | AY170828.2 | Yes | Rizzello *et al.* 2013 |
| mRNA, complete cds (predicted) | | | | |
| PEPT1B | Torafugu (*Takifugu rubripes*) | – | – | Tiziano Verri, unpublished data[g] |
| PEPT1B | Japanese medaka (*Oryzias latipes*) | – | – | Tiziano Verri, unpublished data[g] |
| PEPT1B | Southern platyfish (*Xiphophorus maculatus*) | – | – | Tiziano Verri, unpublished data[g] |
| PEPT1B | Spotted green pufferfish (*Tetraodon nigroviridis*) | – | – | Tiziano Verri, unpublished data[g] |
| PEPT1B | Three-spined stickleback (*Gasteosteus aculeatus*) | – | – | Tiziano Verri, unpublished data[g] |
| PEPT1A | Atlantic cod (*Gadus morhua*) | – | – | Tiziano Verri, unpublished data[g] |
| PEPT1A | Torafugu (*Takifugu rubripes*) | – | – | Tiziano Verri, unpublished data[g] |
| PEPT1A | Japanese medaka (*Oryzias latipes*) | – | – | Tiziano Verri, unpublished data[g] |
| PEPT1A | Nile tilapia (*Oreochromis niloticus*) | – | – | Tiziano Verri, unpublished data[g] |
| PEPT1A | Southern platyfish (*Xiphophorus maculatus*) | – | – | Tiziano Verri, unpublished data[g] |
| PEPT1A | Three-spined stickleback (*Gasteosteus aculeatus*) | – | – | Tiziano Verri, unpublished data[g] |

cds, coding sequence. [a]Amberg JJ, Anderson CL, Hill RA, Rust MB & Hardy RW. Submitted (17-SEP-2007) Aquaculture Research Institute, University of Idaho, Hagerman, Idaho, USA; [b]Record predicted by automated computational analysis (this record is derived from a genomic sequence NW_003039818 annotated using the gene prediction method GNOMON). Submitted (23-MAR-2011) NCBI Annotation staff; [c]Nie GX, Yan X, Wang JL, Ming H, Wang B, Zheng JL, Li XJ & Kong XH. Submitted (24-OCT-2011) College of Life Sciences, Henan Normal University, Xinxiang, Henan, China; [d]Liu Z, Feng JC, Liu SJ & Liu Y. Submitted (14-JAN-2012) College Life Science, Hunan Normal University, Changsha, Hunan, China; [e]Terova G, Preziosa E, Gliozheni E & Rimoldi S. Submitted (31-MAY-2012) Biotechnology and Molecular Sciences, University of Insubria, Varese, Italy; [f]Ahn H, Watanabe S, Yamada Y, Tsukamoto K & Kaneko T. Submitted (15-NOV-2012) The University of Tokyo, Graduate School of Agricultural and Life Sciences, Tokyo, Japan; [g]mRNA, complete cds predicted by computational analysis using nucleotide (genomic, partial mRNA, EST, etc.) sequences stored in GenBank and Ensembl (for reference see Bucking & Schulte, 2012; Liu *et al.* 2013) in combination with a variety of prediction and annotation methods (GenScan, GenomeScan, FGenesh, Artemis, direct alignment, etc.).

intestine (Verri *et al.* 2010). However, the pattern of expression may significantly differ from (one group of) fish to (another group of) fish. Thus, while in zebrafish, carps and weatherloaches (order *Cypriniformes*) PEPT1 expression is strictly confined to the proximal portion(s) of the intestine, in cod (order *Gadiformes*) and mummichogs (order *Cyprinodontiformes*) PEPT1 is ubiquitously expressed for the whole length of the intestinal canal. In between these extremes, are salmon and trout (order *Salmoniformes*) that exhibit a steady decrease in PEPT1 expression along proximal-to-distal adjacent segments of the intestinal canal, and sea bass, sea bream and icefish (order *Perciformes*) in which the proximal-to-distal drop in expression seems steeper than in salmonids. Whenever present, the pyloric ceca always express PEPT1 at very high levels, which strongly suggests that this organ specialization largely participates in the absorption of the di/tripeptides arising from

**Table 3. Organ/tissue distribution of PEPT1 mRNA in teleost fish (with emphasis on the localization along the post-gastric intestinal tract)**

| Description | Order | Species | GenBank Acc. No. | Organ/tissue distribution | Distribution along the (post-gastric) intestinal tract | Assay(s) | References |
|---|---|---|---|---|---|---|---|
| PEPT1B | *Cypriniformes* | Zebrafish (*Danio rerio*) | NM_198064.1 | Intestine >> spleen > kidney (adult) | Proximal intestine (intestinal bulb) (larva) | Semi-quantitative RT-PCR and *in situ* hybridization | Verri *et al.* 2003 |
| PEPT1B | *Cypriniformes* | Grass carp (*Ctenopharyngodon. idella*) | JN088166.1 | Intestine >> Muscle > kidney = heart = liver > spleen | Foregut >>> midgut > hindgut (juvenile) | Quantitative real-time PCR | Liu *et al.* 2013 |
| PEPT1B | *Cypriniformes* | Asian weatherloach (*Misgurnus anguillicaudatus*) | DQ668370.1 | Heart > liver | Anterior zone of the foregut > posterior zone of the foregut > midgut (adult) | RT-PCR | Gonçalves *et al.* 2007 |
| PEPT1B | *Salmoniformes* | Atlantic salmon (*Salmo salar*) | NM_001146682.1 | Intestine >>> Brain > belly flap = skin = heart > eye = ovary > others (juvenile) | Pyloric ceca > mid-gut > hindgut (juvenile) | Quantitative real-time PCR | Rønnestad *et al.* 2010 |
| PEPT1B | *Salmoniformes* | Rainbow trout (*Oncorhynchus mykiss*) | | | Pyloric ceca > mid-gut > hindgut (juvenile) | Quantitative real-time PCR | Kamalam *et al.* 2013 |
| PEPT1B | *Gadiformes* | Atlantic cod (*Gadus morhua*) | AY921634.1 | Intestine >> kidney = spleen (adult) | Midgut = hindgut (larva) S2 = S3 = S4 = S5 = S6 = S7 ≥ S8 ≥ S9 > S10 (S2, pyloric area; S3, pyloric ceca (inner segments); S4, pyloric ceca (outer segments); S5-S10, six adjacent segments in the remainder of the intestine, starting after the pyloric ceca (S5) and ending with the anus (S10) – based on divisions of the three loops present in the dissected gut. The last segment also comprises the hindgut) (adult) S1 = S2 = S3 > S4 > S5 (S1, pyloric ceca; S2–S5, equally long parts from the remainder of the intestine) (juvenile) | Quantitative real-time-PCR Semi-quantitative RT-PCR Quantitative real-time-PCR | Amberg *et al.* 2008 Rønnestad *et al.* 2007 Bakke *et al.* 2010 |
| PEPT1A | *Cyprinodontiformes* | Mummichog (*Fundulus heteroclitus macrolepidotus*) | JN615008.1 | | Anterior intestine = posterior intestine (adult) | Quantitative real-time PCR | Koven & Schulte 2012 |
| PEPT1B | *Cyprinodontiformes* | Mummichog (*Fundulus heteroclitus macrolepidotus*) | JN615007.1 | | Anterior intestine = posterior intestine (adult) | Quantitative real-time PCR | Koven & Schulte 2012 |
| PEPT1B | *Perciformes* | European sea bass (*Dicentrarchus labrax*) | FJ237043.2 | Intestine >>> gills >brain > heart > liver > spleen > muscle > ovary (adult) | Pyloric ceca ≤ intestinal segment (IS) 1 < IS2 > IS3 (segments 1–3 approx. correspond to the first 3 cm) > IS4 > IS5 > IS6 > IS7 > IS8 > IS9 > IS10 >> rectum (adult) | Quantitative real-time-PCR | Terova *et al.* 2009 |
| PEPT1B | *Perciformes* | Gilthead seabream (*Sparus aurata*) | GU733710.1 | Intestine >>> brain > muscle > spleen (adult) | Intestinal segment (IS) 1 = IS2 > IS3 (segments 1–3 approx. correspond to the first 3 cm) > IS4 > IS5 > IS6 > IS7 (adult) | Quantitative real-time-PCR | Terova *et al.* 2013 |
| PEPT1B | *Perciformes* | Antarctic icefish (*Chionodraco hamatus*) | AY170828.2 | Intestine >>> kidney >>> brain (adult) | Intestine, no regions specified (adult) | Semi-quantitative RT-PCR and real-time PCR | Rizzello *et al.* 2013 |

dietary proteins. Interestingly, spatio-temporal expression of PEPT1 intestinal mRNA has been reported to vary significantly during ontogeny (Verri *et al.* 2003; Amberg *et al.* 2008; Liu *et al.* 2013) and in response to nutritional states (such as food deprivation/refeeding; Hakim *et al.* 2008; Terova *et al.* 2009; Rønnestad *et al.* 2010; Bucking & Schulte, 2012; Koven & Schulte, 2012; Liu *et al.* 2013; reviewed by Verri *et al.* 2011), dietary challenges (Gonçalves *et al.* 2007; Bakke *et al.* 2010; Ostaszewska *et al.* 2010*a*,*b*; Kwasek *et al.* 2012; Liu *et al.* 2013; Kamalam *et al.* 2013; Ostaszewska *et al.* 2013; Terova *et al.* 2013), and, intriguingly, aquatic environment (freshwater/seawater adaptation; see e.g. Kalujnaia *et al.* 2007; Bucking & Schulte, 2012). To our knowledge, there is only one paper in which both PEPT1A and PEPT1B mRNA expression have contemporarily been analysed (Bucking & Schulte, 2012).

One of the current limits of expression studies in teleost fish is the lack of adequate information at the protein level because of the lack of PEPT1 species-specific antibodies. Protein expression data are, however, available in teleost fish, the first coming from a study performed in *Epinephelus coioides* (orange-spotted grouper), using a commercial polyclonal rabbit anti-PEPT1 antibody that was shown to cross-react with orange-spotted grouper PEPT1 protein epitopes (Yuen *et al.* 2007). PEPT1 protein is found constitutively expressed along the brush-border membrane of the intestinal mucosa in the proximal intestine of the juvenile orange-spotted grouper. PEPT1 protein expression is restricted to absorptive epithelial cells and is more evident in enterocytes migrating towards the tips of the mucosal folds, whereas the mucus-secreting goblet cells are negative for PEPT1. Using the same antibody, PEPT1 protein has been detected in rainbow trout (Ostaszewska *et al.* 2010*b*), *Cyprinus carpio* (common carp; Ostaszewska *et al.* 2010*a*) and *Perca flavescens* (yellow perch; Ostaszewska *et al.* 2013), with similar results to the orange-spotted grouper intestine. Therefore, as in higher vertebrates (Daniel, 2004), the PEPT1 protein strongly associates with differentiated and mature absorptive enterocytes.

To summarize, in any fish species tested, PEPT1 mRNA is primarily expressed at the intestinal level, in the portion of the intestinal tract that is directly involved in digestion and absorption. Where competition for different functions occurs in the gut (e.g. in the Asian weatherloach which uses the hindgut as an accessory air-breathing organ), PEPT1 always marks the portion that is devoted to digestion and absorption. Expression is restricted to the brush-border membrane of mature enterocytes (which makes PEPT1 a marker of terminal enterocyte differentiation; Chen *et al.* 2009; Li *et al.* 2011). Expression in other tissues is always lower than in the gut. The functional role of PEPT1 in such tissues is still unknown in fish.

The enormous plasticity of PEPT1 expression at the intestinal level in teleost fish – already known for several years – renders the previous data of expression observed along the mammalian intestine (e.g. in the colon) in certain disease states (Daniel, 2004; Daniel & Kottra, 2004; Smith *et al.* 2013) less 'atypical'. In this respect, it has just been estimated that PEPT1 protein is unequivocally expressed in healthy distal colonic epithelium in mice, rats and humans, where the protein is functional and contributes to electrolyte and water handling (Wuensch *et al.* 2013).

## Teleost fish PEPT1 and the pH dependence issue

In higher vertebrates, PEPT1 function has been studied in a large number of mammalian and avian species, including human, rat, mouse, rabbit, sheep, chicken and turkey. In these species, PEPT1 transport activity has generally been reported to be pH dependent, with external acidic pH having a boosting effect on the uptake of the transported substrate. In general terms, in mammals this effect appears to correlate to a substantial increase of the apparent affinity for the substrate passing from alkaline to neutral to acidic extracellular pH, with no really consistent (slight-to-no) change in maximal transport rate. In human PEPT1, on the other hand, high acidity increases both substrate affinity and maximal transport rate (Daniel, 2004; Daniel & Kottra, 2004, Gilbert *et al.* 2008; Smith *et al.* 2013).

With the functional characterization of zebrafish PEPT1 (Verri *et al.* 2003), it was noticed that many basic kinetic features of this transporter were essentially similar to those of higher vertebrates, e.g. the increasing apparent affinity with decreasing pH. However, in contrast to higher vertebrates (e.g. rabbit), zebrafish PEPT1 maximal transport rate was unexpectedly found to increase at alkaline extracellular pH. The analysis of European sea bass PEPT1 (Sangaletti *et al.* 2009) led to a second surprising result, i.e. with the typical increase in apparent substrate affinity at acidic pH, no significant effect of pH could be observed on the maximal transport rate of this transporter, that can thus be considered functionally similar to the mammalian (e.g. rabbit) and not to the zebrafish model. Interestingly, when functionally analysed, the Atlantic salmon PEPT1 exhibited only a slight pH dependence of the maximal transport rate (Rønnestad *et al.* 2010) while the Antarctic icefish showed no pH dependence of the maximal transport rate (Rizzello *et al.* 2013), which suggests conformance of both transporters to the rabbit/sea bass paradigm.

Taken together, these results strongly suggest that there is considerable molecular diversity among fish transport proteins. By identifying similarities and differences with respect to the mammalian counterparts, the teleost fish proteins can be ideal for molecular mapping of

functionally important regions. With this in mind, we have built a set of rabbit-zebrafish chimeras that have preliminarily been screened for function, revealing that it is possible to modulate the phenotypic response 'pH dependence of $I_{max}$' and eventually reveal where the allosteric sensor of PEPT1 is located along the sequence (A. Romano, unpublished observations). In the meanwhile, an elegant study has been published that used electrophysiological and biophysical approaches to compare the transport kinetics of rabbit, sea bass and zebrafish PEPT1 (Renna *et al.* 2011). A unified model has been proposed that explains the main electrophysiological properties of the PEPT1 transporters and their pH dependence, describing at the same time the behaviour observed in each of the proteins analysed. This scheme suggests a dual role for $H^+$ in the operational mechanism of PEPT1. On one hand, protons are essential to neutralizing the transporter during the inward substrate translocation and their release in the cytosol uncovers the net negative charge of the empty transporter, which then undergoes an energy-dissipating step to return to the outward-facing conformation (for a schematic description of the model see: Renna *et al.* 2011). On the other hand, protonation of the transporter breaks the transport cycle and counteracts the boosting effects of external acidity on the turnover rate. A different balance between the two roles played by $H^+$ may generate opposite effects on the maximal transport rate, as observed experimentally in human *vs.* rabbit *vs.* fish PEPT1 proteins (Mackenzie *et al.* 1996; Amasheh *et al.* 1997; Nussberger *et al.* 1997; Steel *et al.* 1997; Kottra & Daniel, 2001; Kottra *et al.* 2002; Irie *et al.* 2005; Fujisawa *et al.* 2006; Sala-Rabanal *et al.* 2006; Sangaletti *et al.* 2009; Renna *et al.* 2011). The existence of two apparently contrasting actions of external $H^+$ does not negatively impact the overall efficiency of the substrate uptake, and the model can explain how PEPT1 sustains its function across species and at the expected physiological pH conditions.

## Teleost fish PEPT1 and the temperature dependence issue

Adaptation of organisms to a given temperature requires their proteins to work at optimal thermodynamic conditions. This is particularly true for transport proteins – such as the intestinal PEPT1 – which are responsible for maintenance of nutrient homeostasis at both cellular and organism level. Like enzymes, the catalytic activity of membrane transport proteins largely depends on temperature (Hazama *et al.* 1997; Beckman & Quick 2001; Binda *et al.* 2002; Karakossian *et al.* 2005; Takanaga *et al.* 2005; Bacconi *et al.* 2007; Mackenzie *et al.* 2007). Theoretically, in a given protein higher temperatures should accelerate all reactions of the transport mechanism – which occurs as a cycle of serial reactions – and with increasing temperature an increase in transport rate, and thus in substrate uptake, is expected. This is observed in various transporters (Wadiche & Kavanaugh, 1998; Hilgemann & Lu, 1999; Beckman & Quick, 2001). On the other hand, differences in kinetics are expected to occur in orthologous transport proteins of species living at different temperatures. This is also observed for various transporters, such differences appearing strictly adaptive (Maffia *et al.* 1996, 2003; Xue *et al.* 1999; Elias *et al.* 2001; Dode *et al.* 2001; Marshall *et al.* 2002; Galarza-Muñoz *et al.* 2011).

In PEPT1, the effects of temperature on the functional properties of the transporter have been investigated in detail using *X. laevis* oocytes in combination with electrophysiological methods and the dipeptide Gly-L-Gln as a substrate under different experimental conditions. When the function of the rabbit (homeotherm living at 38–39°C; www.fao.org) PEPT1 was studied in comparison to the zebrafish (tropical poikilotherm living at 18–24°C; www.fishbase.org) and the European sea bass (subtropical poikilotherm living at 8–24°C; www.fishbase.org) at the routine operational temperature of $22 \pm 2$°C marked differences were found in the kinetic behaviour of the proteins (Renna *et al.* 2011). In particular, at the same temperature the two teleost fish proteins showed similar kinetics, while the rabbit exhibited significantly slower kinetics with respect to the teleost fish proteins. On the other hand, at higher (closer to the physiological body) temperatures (30°C), the properties of the rabbit PEPT1 were, both qualitatively and quantitatively, more similar to those of the teleost fish (Bossi *et al.* 2012), suggesting that there may be no differences among species when the transporters operate at their respective physiological conditions, and that the differences in kinetics observed in rabbit and teleost fish may represent adaptive changes.

It is obvious that structural differences have to exist between mammalian and teleost fish proteins that confer enhanced flexibility in the latter to compensate for the lower thermal kinetic energy available at lower temperatures. As far as we know, such increased flexibility is essentially achieved through single-site amino acid substitutions in those regions of the protein that undergo large movements during the catalytic cycle (Fields, 2001; Somero, 2004). However, in the Antarctic icefish (polar poikilotherm living at −1.9°C; www.fishbase.org) it has recently been found that a *de novo* domain composed of one to six repeats of seven amino acids (VDMSRSK), placed as an extra stretch in the cytosolic COOH-terminal region of PEPT1 (Supplemental Fig. S1), contributes in part – but *per se* – to cold adaptation (Rizzello *et al.* 2013). VDMSRSK is in a protein region not involved in transport activity and, notably, when transferred to the COOH terminus of the rabbit (warm-adapted) transporter, it confers cold adaptation to this protein. To our knowledge, this strategy based on the insertion of a *de*

*novo* domain is unique among those already known in proteins from psychrophilic species (Feller & Gerday, 2003; Somero, 2003, 2004; Petricorena & Somero, 2007; Pörtner *et al.* 2007). In addition, the VDMSRSK domain has intriguingly been found in the Antarctic icefish (family *Channichthyidae*) as well as in other three species of the same sub-order *Notothenioidei* (notothenioids; all living in the Antarctic waters) that belong to other two families, namely *Trematomus bernacchii* (emerald rockcod) and *T. pennellii* (sharp-spined notothen; family *Nototheniidae*), and *Histiodraco velifer* (a barbeled plunderfish of the family *Artedidraconidae*). This finding strongly indicates that the acquisition of the VDMSRSK domain dates back to at least the most recent common ancestor of the so-called 'Antarctic clade' (Near *et al.* 2012), thus paralleling the most probable evolutionary origin of the antifreeze glycoproteins (AFGPs). In this respect, the VDMSRSK domain may represent a very useful molecular marker of the evolutionary biological and eco-physiological diversification of the Antarctic fishes. Since it is transferable to other proteins, this domain may also represent a valuable tool for future biotechnological applications.

## Species-specific uptake of L-Lys-containing di/tripeptides via teleost fish PEPT1

Teleost fish, as other vertebrates, rely on a dietary supply of a well-balanced profile of indispensable and dispensable amino acids (Wilson, 2002). Among others, L-Lys and L-Met are known to be growth limiting in animals, and several studies have demonstrated that diets deficient in such essential amino acids may result in poor growth performances in vertebrates, teleost fish included. In particular, L-Lys is considered one of the first limiting amino acids during preparation of fish feeds today (Harris, 1980; Forster & Ogata, 1998; Small & Soares, 2000) and this problem increases when plant-based protein sources are used to replace fishmeal (Zhang *et al.* 2008). L-Lys availability limits protein synthesis, protein accretion and growth of fish (Conceição *et al.* 2007; Espe *et al.* 2007), and impairs metabolism (Walton *et al.* 1984). Based on its fundamental role in fish nutrition, the development of new strategies for the supplementation of this amino acid is thus considered worthwhile, in an attempt to deal with the nutritional challenges posed by the need to feed cultured fish with optimized artificial diets. In this respect, being the route for intake of di/tripeptides, PEPT1 has received considerable attention as a target for delivery of essential amino acids in the form of di/tripeptides, and L-Lys-containing di/tripeptides have especially been studied for transport to elucidate the basic properties of their uptake. This with the confidence that information coming from the observed kinetics would have been highly beneficial in the formulation of new diets containing peptide hydrolysates or individual peptides rather than free amino acids (Zambonino Infante *et al.* 1997; Dabrowski *et al.* 2003, 2005; Aragão *et al.* 2004; Zhang *et al.* 2006; Rønnestad *et al.* 2007).

In teleost fish, the first evidences that L-Lys-containing peptides are transported by PEPT1 came from preliminary observations in the zebrafish (two L-Lys-containing dipeptides tested, with L-Lys-Gly being a better substrate than Gly-L-Lys; Verri *et al.* 2010) and from a systematic analysis in the Atlantic salmon (six dipeptides and a tripeptide tested, exhibiting the following relative scale of transport efficiency: L-Lys-L-Pro > L-Lys-L-Val > L-Lys-L-Pro-L-Val > L-Ala-L-Lys > L-Glu-L-Lys > L-Lys-L-Glu > L-Arg-L-Lys; Rønnestad *et al.* 2010). Interestingly, L-Lys-L-Pro-L-Val, a tripeptide that in mammals exerts potent anti-inflammatory effects at the intestinal mucosa by uptake *via* PEPT1 (Dalmasso *et al.* 2008), was found to be a good substrate of the Atlantic salmon transporter, which raises the possibility of associating PEPT1 functioning not only with fish nutrition but also with the treatment of fish enteritis and associated inflammation (Rønnestad *et al.* 2010). Very recently, a study has been reported in which the kinetic properties of European sea bass, zebrafish and rabbit PEPT1 have been investigated systematically and comparatively to establish similarities and differences in the uptake of di/tripeptides containing Gly, L-Lys and L-Met combined in variable forms (namely six dipeptides: Gly-L-Gln, L-Lys-L-Lys, L-Met-L-Lys, L-Lys-Gly, Gly-L-Lys, L-Lys-L-Met, and a tripeptide: L-Lys-L-Lys-L-Lys; Margheritis *et al.* 2013). When tested under the same experimental conditions, substantial species-specific differences could be observed for the PEPT1 clones in response to the various di/tripeptides. In particular, in a set of (transport current) experiments performed using L-Lys- and L-Met-containing dipeptides (reference substrate Gly-L-Gln), L-Lys-L-Met was found to be the best substrate at all tested potentials in European sea bass and rabbit PEPT1 (with L-Lys-L-Met > L-Met-L-Lys = Gly-L-Gln > L-Lys-L-Lys), while in zebrafish PEPT1 all tested dipeptides produced similar currents independently of the charge position or amino acid composition, except for L-Lys-L-Lys, which at the most negative potentials evoked larger currents with respect to European sea bass and rabbit (i.e. L-Lys-L-Lys > L-Lys-L-Met = L-Met-L-Lys = Gly-L-Gln). As also suggested by the analysis of pH dependence presented above, these results indicate that European sea bass acts more like rabbit than zebrafish PEPT1. Interestingly, Atlantic salmon PEPT1 conforms more to the European sea bass/rabbit model than to the zebrafish model, since when tested in Atlantic salmon PEPT1 L-Arg-L-Lys – which is structurally similar to L-Lys-L-Lys – is found at the bottom of the transport efficiency scale (Rønnestad *et al.* 2010). Furthermore,

focusing on L-Lys- and neutral amino acid (Gly and L-Met)-containing dipeptides, it has been observed that the currents produced by Gly-L-Lys and L-Lys-Gly (on one hand) and L-Met-L-Lys and L-Lys-L-Met (on the other) can be ranked differently in sea bass and rabbit PEPT1 (with L-Lys-Gly > Gly-L-Gln > Gly-L-Lys, and L-Lys-L-Met > Gly-L-Gln = L-Met-L-Lys) and in zebrafish PEPT1 (with Gly-L-Gln > L-Lys-Gly > Gly-L-Lys, and Gly-L-Gln = L-Lys-L-Met = L-Met-L-Lys). While the former scale also pertains to the Antarctic icefish PEPT1 (Rizzello *et al.* 2013), the latter is in accordance with the preliminary findings from zebrafish PEPT1 (Verri *et al.* 2010). Finally, in European sea bass and rabbit PEPT1 the currents elicited by Gly-L-Lys, L-Lys-Gly, L-Met-L-Lys and L-Lys-L-Met were always independent of the external pH, while in zebrafish they were invariably pH dependent, with alkalinization exerting an activation role. Interestingly, the definition of the peculiar features of the rabbit/sea bass *vs.* zebrafish transporter and their combination with the more general structural–functional information coming from both crystallographic (Newstead, *et al.* 2011; Newstead, 2011; Solcan *et al.* 2012) and molecular modelling experiments (Meredith & Price 2006; Pedretti *et al.* 2008; Meredith, 2009) has led to the identification of a natural substitution in transmembrane domain VIII of a threonine (in position 327 in rabbit and 330 in European sea bass) with an isoleucine (in position 334 in zebrafish) that seems to be relevant for the fine tuning of the characteristics of transport in the rabbit/sea bass *vs.* zebrafish PEPT1. The threonine is highly conserved in all vertebrates, fish included, with the exception of zebrafish (and other cyprinids) PEPT1, while it is also present in the duplicated PEPT1A protein (Supplemental Fig. S1). As demonstrated by analysing the function of the mutated (T327I) rabbit transporter, such a residue principally changes the mechanism of interaction of the protein with di/tripeptides, showing impairment in substrate selectivity and affinity and consequently in transport efficiency.

Taken together, these results on the species-specific substrate uptake support the concept that providing detailed analysis of teleost fish PEPT1 transporters can be (a) extremely useful in generating a well-integrated general framework of knowledge on the properties of this transporter and (b) of significant support in suggesting new structure–function paradigms for this protein, in order to understand how the transporter molecularly adapts to match the dietary requirements of the species (Karasov *et al.* 2011; Karasov & Douglas, 2013). In terms of application, the kinetic results described above may also be critical for the complete comprehension of the role of peptide-containing diets in teleost fish nutrition (Dabrowski *et al.* 2010). In this respect, it would be worthwhile integrating the conclusions coming from the molecular data with the preliminary findings that have already led to correlating the effect of L-Lys-Gly dietary supplementation with PEPT1 expression, growth and development in a few teleost fish species, such as rainbow trout, common carp and yellow perch (Table 4).

## Conclusions

The discovery of the genome organization in a steadily increasing number of vertebrate and invertebrate species is making available an immense amount of molecular information that can be easily accessed by public database examination. However, while a given gene/mRNA/amino acid sequence from a given species can now be rapidly analysed and compared with others, information on the biological role and function of a given gene product from an alternative non-mammalian species is very often limited, and when available is generally considered irrelevant due to the current prevailing opinion that it is better to use animal models as close to humans as possible. On the other hand, the increasing use of teleost fish models in developmental biology and genetics research, as well as in ecological and toxicological studies, the increasing number of teleost fish species cultured for human consumption instead of being fished, and attempts – in an increasing number of research programmes – to complement the molecular information from human biology with that from lower vertebrates (e.g. developing fish models of human disease and/or performing comparative analysis of genomes across vertebrates from human to fish) are making the study of the physiology of such non-conventional animal models increasingly important and necessary. Studies of this kind are properly classed as comparative and integrative physiology, as well as applied, environmental and ecological physiology, and will soon be part of translational biology.

For PEPT1, the comparative analysis of orthologs has shown that the teleost fish proteins may exhibit unusual properties in terms of pH dependence, temperature dependence and/or substrate specificity, which is providing new contributions to the discussion of the functional plasticity of the PEPT1 transporter. But can any of the information derived from teleost fish models be used to inform us about how the human transporter works and/or what is(are) its essential role(s) in human physiology? The answer is 'yes, of course' in many instances. For example, findings on the uptake of the Lys-containing peptides in the various teleost fish *vs.* rabbit proteins give good support for the notion that human PEPT1 more closely resembles the rabbit/sea bass than the zebrafish transporter model. In fact, among the protein residues of the substrate-binding pocket that are likely to interact with the dipeptide there is no amino acid substitution in rabbit, sea bass and human PEPT1, except for the threonine T327 in rabbit (T327 in human and T330 in sea bass)

**Table 4. Experimental growth trials involving L-Lys-Gly-based diets (only trials in which PEPT1 expression data have been reported in comparison to growth data have been listed)**

| Species | Developmental stage | Dipeptide | Diets used for experimental feeding | Time of experimental feeding | PEPT1 (mRNA or protein) expression | Growth | Reference |
|---|---|---|---|---|---|---|---|
| Rainbow trout (*Oncorhynchus mykiss*) | Juvenile (alevin) | L-Lys-Gly | Wheat-gluten-protein-based diet containing L-Lys-Gly (PP), or free L-Lys and Gly (AA), or no L-Lys (CON) *vs.* commercial starter Aller Futura (AF) | 28 days | CON≥PP≥AA>AF (mRNA, whole intestine) | PP≥AA≥ AF>CON | Ostaszewska *et al.* 2010*b* |
| Common carp (*Cyprinus carpio*) | Juvenile | L-Lys-Gly | Wheat-gluten-protein-based diet containing L-Lys-Gly (PP), or free L-Lys and Gly (AA), or no L-Lys (CON) or frozen zooplankton (Z) (restricted diet) *vs.* commercial starter Anglo Norse (AN) | 28 days | PP>AN=AA> Z>CON (mRNA, whole intestine) | AN>PP= AA= CON>Z | Ostaszewska *et al.* 2010*a* |
| Yellow perch (*Perca flavescens*) | Juvenile | L-Lys-Gly | Wheat-gluten-protein-based diet containing L-Lys-Gly (LG), or free L-Lys and Gly (FL), or no L-Lys (NL) *vs.* commercial starter Bio Oregon (BO) | 55 days | LG>FL>NL (mRNA, whole digestive tract) | LG≥FL= BO>NL | Kwasek *et al.* 2012 |
| Yellow perch (*Perca flavescens*) | Juvenile | L-Lys-Gly | Wheat-gluten-protein-based diet containing L-Lys-Gly (LG) or free L-Lys and Gly (FL) or no L-Lys (C) *vs.* commercial starter Bio Oregon (BO) | 48 days | LG≥FL=BO>C (protein, anterior intestine) | LG≥FL= BO>C | Ostaszewska *et al.* 2013 |

which is substituted by an isoleucine (I334) in zebrafish PEPT1 (Margheritis *et al.* 2013). As discussed, this change correlates with a different substrate specificity pattern for Lys-containing peptides in zebrafish with respect to rabbit/sea bass PEPT1. In addition, there is substantial coherence in the substrate specificity of rabbit/sea bass (Margheritis *et al.* 2013) and human PEPT1 (among others see e.g. Vig *et al.* 2006). If we consider that the data for pH dependence also support the notion that human PEPT1 is functionally closer to rabbit/sea bass than to zebrafish PEPT1, a rather simple sequence–function scheme can be conceived, in which the human/rabbit/sea bass protein is the paradigm of reference and the zebrafish protein is the paradigm of outward comparison. This scheme may help delineate the relevance of single residues in the human transporter and their role in the definition of the molecular phenotype. For instance, this scheme could be used to test the substrate specificity of a human PEPT1 mutated in a selected residue (such as T327) and observe how the mutated protein responds in terms of substrate specificity for the Lys-containing peptides with respect to the wild type. This would be valuable while waiting for (a) natural mutation(s) (i.e. SNPs) in the human PEPT1 that involve(s) the candidate residue(s) to be found. In the longer term, this molecular tool could be useful to support the development of new strategies for the preparation of original peptide-containing diets for human nutrition. In fact, L-Lys is an essential amino acid not only for animals but also for humans and the supplementation of L-Lys for forms of those Lys-containing peptides that are optimally absorbed *via* PEPT1 can be beneficial for producing improved foods for human consumption and/or for designing novel functional foods. Such food types may also have clinical applications, such as enteral nutrition (see e.g. Braunschweig *et al.* 2001; Gramlich *et al.* 2004; Kudsk, 2007; de Aguilar-Nascimento *et al.* 2010). More generally, the studies conducted on PEPT1 in teleost fish (as well as in other less conventional animal models) may be of inspiration for studies in humans. For example, a large number of papers have explored whether or not any correlations can be established between PEPT1 and growth in farmed animals – teleost fish included as discussed above – during their ontogeny and/or under a variety of dietary challenges. Some of these studies indicate that correlations may exist. Interestingly, many of these studies are typically conducted on

(large groups of) individuals at the early stages of their lifespan. In addition, in these animals basic biometric analyses (weight, length, BMI, condition factor, etc.) are carefully conducted, in conjunction with biochemical, physiological and molecular analyses aimed at establishing if and how the variation of certain molecular markers correlate to one or more general growth indexes. All this is important, since even a limited growth loss in a farmed animal during early development may result in substantial monetary loss for farmers (among others see e.g. Gilbert *et al.* 2008; Verri *et al.* 2011; and literature cited therein). If critically analysed, the studies on farmed animals integrate well with results coming from the more conventional basic research models (such as *C. elegans*, mice, etc.) that are typically employed to investigate the role(s) of PEPT1 in growth by detailing the molecular mechanisms that integrate PEPT1 function/regulation into the general physiological scheme of the animal growth response (see e.g. Meissner *et al.* 2004; Spanier *et al.* 2009; Benner *et al.* 2011; Kolodziejczak *et al.* 2013). On the other hand – at least to our knowledge – there has been no paper published to date directly dealing with PEPT1 and growth in humans, and currently this research field can be considered neglected. In summary, studies of PEPT1 function in teleost fish provide a worthwhile area of focus, mostly with regard to aspects relating to the elucidation of the relationship between PEPT1 and growth in certain (early) phases of human ontogeny. In this context it has to be emphasized that subtle and complex interactions may exist between factors that positively (welfare, healthy environment, optimal nutrition, good health state, etc.) or negatively (environmental pollution, malnutrition, potential disease states, life in low/middle income countries, etc.) affect human growth, and the question is even more relevant if we consider that such factors strongly act on human growth in the first 2 years of life. The interplay between these factors may dramatically determine how the rest of an individual's life will evolve in terms of survival, growth responses (height, weight, lean body mass, BMI, etc.), presence/absence of disease states (obesity, insulin resistance, cardio-metabolic diseases, etc.), cognitive development, attained schooling, human capital outcome, etc. (for insights into the long-term importance of the first 1000 days – widely recognized as a particularly sensitive period for child health and development – see e.g. Adair *et al.* 2013).

As this description of PEPT1 function shows, detailed studies of teleost fish transporters can be extremely useful in elucidating membrane transport physiology since they offer an extraordinary assortment of molecular, structural and functional adaptations which could lead to novel breakthroughs in physiological research. Revealing these adaptations may provide new hints for answering relevant questions about both basic and applied transporter research.

## References


Adair LS, Fall CH, Osmond C, Stein AD, Martorell R, Ramirez-Zea M, Sachdev HS, Dahly DL, Bas I, Norris SA, Micklesfield L, Hallal P, Victora CG; COHORTS group (2013). Associations of linear growth and relative weight gain during early life with adult health and human capital in countries of low and middle income: findings from five birth cohort studies. *Lancet* **382**, 525–534.

Amasheh S, Wenzel U, Boll M, Dorn D, Weber WM, Clauss W & Daniel H (1997). Transport of charged dipeptides by the intestinal $H^+$/peptide symporter PepT1 expressed in *Xenopus laevis* oocytes. *J Membr Biol* **155**, 247–256.

Amberg JJ, Myr C, Kamisaka Y, Jordal AE, Rust MB, Hardy RW, Koedijk R & Rønnestad I (2008). Expression of the oligopeptide transporter, PepT1, in larval Atlantic cod (*Gadus morhua*). *Comp Biochem Physiol B Biochem Mol Biol* **150**, 177–182.

Aragão C, Conceição LEC, Martins D, Rønnestad I, Gomes E & Dinis MT (2004). A balanced dietary amino acid profile improves amino acid retention in post-larval Senegalese sole (*Solea senegalensis*). *Aquaculture* **233**, 293–304.

Bacconi A, Ravera S, Virkki LV, Murer H & Forster IC (2007). Temperature dependence of steady-state and presteady-state kinetics of a type IIb $Na^+$/Pi cotransporter. *J Membr Biol* **215**, 81–92.

Bakke S, Jordal AE, Gómez-Requeni P, Verri T, Kousoulaki K, Aksnes A & Rønnestad I (2010). Dietary protein hydrolysates and free amino acids affect the spatial expression of peptide transporter PepT1 in the digestive tract of Atlantic cod (*Gadus morhua*). *Comp Biochem Physiol B Biochem Mol Biol* **156**, 48–55.

Beckman ML & Quick MW (2001). Substrates and temperature differentiate ion flux from serotonin flux in a serotonin transporter. *Neuropharmacology* **40**, 526–535.

Benner J, Daniel H & Spanier B (2011). A glutathione peroxidase, intracellular peptidases and the TOR complexes regulate peptide transporter PEPT-1 in *C. elegans*. *PLoS One* **6**, e25624.

Berthelsen R, Nielsen CU & Brodin B (2013). Basolateral glycylsarcosine (Gly-Sar) transport in Caco-2 cell monolayers is pH dependent. *J Pharm Pharmacol* **65**, 970–979.

Binda F, Bossi E, Giovannardi S, Forlani G & Peres A (2002). Temperature effects on the presteady-state and transport-associated currents of GABA cotransporter rGAT1. *FEBS Lett* **512**, 303–307.

Bogé G, Rigal A & Peres G (1981). Rates of *in vivo* intestinal absorption of glycine and glycylglycine by rainbow trout (*Salmo gairdneri* R.). *Comp Biochem Physiol A Physiol* **69**, 455–459.

Bossi E, Cherubino F, Margheritis E, Oyadeyi AS, Vollero A & Peres A (2012). Temperature effects on the kinetic properties of the rabbit intestinal oligopeptide cotransporter PepT1. *Pflugers Arch* **464**, 183–191.

Bossi E, Fabbrini MS & Ceriotti A (2007). Exogenous protein expression in *Xenopus* oocytes: basic procedures. *Methods Mol Biol* **375**, 107–131.

Brandsch M (2009). Transport of drugs by proton-coupled peptide transporters: pearls and pitfalls. *Expert Opin Drug Metab Toxicol* **5**, 887–905.

Braunschweig CL, Levy P, Sheean PM & Wang X (2001). Enteral compared to parenteral nutrition: a meta-analysis. *Am J Clin Nutr* **74**, 534–542.

Bucking C & Schulte PM (2012). Environmental and nutritional regulation of expression and function of two peptide transporter (PepT1) isoforms in a euryhaline teleost. *Comp Biochem Physiol A Mol Integr Physiol* **161**, 379–387.

Chen H, Pan YX, Wong EA, Bloomquist JR & Webb Jr KE (2002). Molecular cloning and functional characterization of a chicken intestinal peptide transporter (cPepT1) in *Xenopus* oocytes and Chinese hamster ovary cells. *J Nutr* **132**, 387–393.

Chen H, Wong EA & Webb Jr KE (1999). Tissue distribution of a peptide transporter mRNA in sheep, dairy cows, pigs, and chickens. *J Anim Sci* **77**, 1277–1283.

Chen YH, Lu YF, Ko TY, Tsai MY, Lin CY, Lin CC & Hwang SP (2009). Zebrafish cdx1b regulates differentiation of various intestinal cell lineages. *Dev Dyn* **238**, 1021–1032.

Conceição LEC, Ribeiro L, Engrola S, Aragão C, Morais S, Lacuisse M, Soares F & Dinis MT (2007). Nutritional physiology during development of Senegalese sole (*Solea senegalensis*). *Aquaculture* **268**, 64–81.

Dabrowski K, Lee KJ & Rinchard J (2003). The smallest vertebrate, teleost fish, can utilize synthetic dipeptide-based diets. *J Nutr* **133**, 4225–4229.

Dabrowski K, Terjesen BF, Zhang Y, Phang JM & Lee KJ (2005). A concept of dietary dipeptides: a step to resolve the problem of amino acid availability in the early life of vertebrates. *J Exp Biol* **208**, 2885–2894.

Dabrowski K, Zhang YF, Kwasek K, Hliwa P & Ostaszewska T (2010). Effects of protein-, peptide- and free amino acid-based diets in fish nutrition. *Aquacult Res* **41**, 668–683.

Dalmasso G, Charrier-Hisamuddin L, Nguyen HT, Yan Y, Sitaraman S & Merlin D (2008). PepT1-mediated tripeptide KPV uptake reduces intestinal inflammation. *Gastroenterology* **134**, 166–178.

Daniel H (2004). Molecular and integrative physiology of intestinal peptide transport. *Annu Rev Physiol* **66**, 361–384.

Daniel H & Kottra G (2004). The proton oligopeptide transporter family SLC15 in physiology and pharmacology. *Pflugers Arch* **447**, 610–618.

de Aguilar-Nascimento JE, Dock-Nascimento DB & Bragagnolo R (2010). Role of enteral nutrition and pharmaconutrients in conditions of splanchnic hypoperfusion. *Nutrition* **26**, 354–358.

Dode L, Van Baelen K, Wuytack F & Dean WL (2001). Low temperature molecular adaptation of the skeletal muscle sarco(endo)plasmic reticulum $Ca^{2+}$-ATPase 1 (SERCA 1) in the wood frog (*Rana sylvatica*). *J Biol Chem* **276**, 3911–3919.

Dyer J, Beechey RB, Gorvel JP, Smith RT, Wootton R & Shirazi-Beechey SP (1990). Glycyl-L-proline transport in rabbit enterocyte basolateral-membrane vesicles. *Biochem J* **269**, 565–571.

Elias CL, Xue XH, Marshall CR, Omelchenko A, Hryshko LV & Tibbits GF (2001). Temperature dependence of cloned mammalian and salmonid cardiac $Na^+/Ca^{2+}$ exchanger isoforms. *Am J Physiol Cell Physiol* **281**, C993–C1000.

Espe M, Lemme A, Petri A & El-Mowafi A (2007). Assessment of lysine requirement for maximal protein accretion in Atlantic salmon using plant protein diets. *Aquaculture* **263**, 168–178.

Feller G & Gerday C (2003). Psychrophilic enzymes: hot topics in cold adaptation. *Nat Rev Microbiol* **1**, 200–208.

Fields PA (2001). Review: Protein function at thermal extremes: balancing stability and flexibility. *Comp Biochem Physiol A Mol Integr Physiol* **129**, 417–431.

Forster I & Ogata HY (1998). Lysine requirement of juvenile Japanese flounder *Paralichthys olivaceus* and juvenile red sea bream *Pagrus major*. *Aquaculture* **161**, 131–142.

Fujisawa Y, Tateoka R, Nara T, Kamo N, Taira T & Miyauchi S (2006). The extracellular pH dependency of transport activity by human oligopeptide transporter 1 (hPEPT1) expressed stably in Chinese hamster ovary (CHO) cells: A reason for the bell-shaped activity versus pH. *Biol Pharm Bull* **29**, 997–1005.

Galarza-Muñoz G, Soto-Morales SI, Holmgren M & Rosenthal JJ (2011). Physiological adaptation of an Antarctic $Na^+/K^+$-ATPase to the cold. *J Exp Biol* **214**, 2164–2174.

Gilbert ER, Wong EA & Webb KE Jr (2008). Board-invited review: Peptide absorption and utilization: Implications for animal nutrition and health. *J Anim Sci* **86**, 2135–2155.

Gonçalves AF, Castro LF, Pereira-Wilson C, Coimbra J & Wilson JM (2007). Is there a compromise between nutrient uptake and gas exchange in the gut of *Misgurnus anguillicaudatus*, an intestinal air-breathing fish? *Comp Biochem Physiol Part D Genomics Proteomics* **2**, 345–355.

Gramlich L, Kichian K, Pinilla J, Rodych NJ, Dhaliwal R & Heyland DK (2004). Does enteral nutrition compared to parenteral nutrition result in better outcomes in critically ill adult patients? A systematic review of the literature. *Nutrition* **20**, 843–848.

Hakim Y, Harpaz S & Uni Z (2008). Expression of brush border enzymes and transporters in the intestine of European sea bass (*Dicentrarchus labrax*) following food deprivation. *Aquaculture* **290**, 110–115.

Harris LE (1980). Diet stuffs. In *Fish Diet Technology*, ed. Pillay TVR, pp. 111–168. UNDP/FAO, Rome.

Hazama A, Loo DD & Wright EM (1997). Presteady-state currents of the rabbit $Na^+$/glucose cotransporter (SGLT1). *J Membr Biol* **155**, 175–186.

He L, Vasiliou K & Nebert DW (2009). Analysis and update of the human solute carrier (SLC) gene superfamily. *Hum Genomics* **3**, 195–206.

Hediger MA, Romero MF, Peng JB, Rolfs A, Takanaga H & Bruford EA (2004). The ABCs of solute carriers: physiological, pathological and therapeutic implications of human membrane transport proteins. *Pflugers Arch* **447**, 465–468.

Hilgemann DW & Lu CC (1999). GAT1 ($GABA:Na^+:Cl^-$) cotransport function. Database reconstruction with an alternating access model. *J Gen Physiol* **114**, 459–475.

Irie M, Terada T, Katsura T, Matsuoka S & Inui K (2005). Computational modeling of $H^+$-coupled peptide transport via human PEPT1. *J Physiol* **565**, 429–439.

Irie M, Terada T, Okuda M & Inui K (2004). Efflux properties of basolateral peptide transporter in human intestinal cell line Caco-2. *Pflugers Arch* **449**, 186–194.

Kalujnaia S, McWilliam IS, Zaguinaiko VA, Feilen AL, Nicholson J, Hazon N, Cutler CP & Cramb G (2007). Transcriptomic approach to the study of osmoregulation in the European eel *Anguilla anguilla*. *Physiol Genomics* 2007 **31**, 385–401.

Kamalam BS, Panserat S, Aguirre P, Geurden I, Fontagné-Dicharry S & Médale F (2013). Selection for high muscle fat in rainbow trout induces potentially higher chylomicron synthesis and PUFA biosynthesis in the intestine. *Comp Biochem Physiol A Mol Integr Physiol* **164**, 417–427.

Karakossian MH, Spencer SR, Gomez AQ, Padilla OR, Sacher A, Loo DD, Nelson N & Eskandari S (2005). Novel properties of a mouse gamma-aminobutyric acid transporter (GAT4). *J Membr Biol* **203**, 65–82.

Karasov WH & Douglas AE (2013). Comparative digestive physiology. *Compr Physiol* **3**, 741–783.

Karasov WH, Martínez del Rio C & Caviedes-Vidal E (2011). Ecological physiology of diet and digestive systems. *Annu Rev Physiol* **73**, 69–93.

Kassahn KS, Dang VT, Wilkins SJ, Perkins AC & Ragan MA (2009). Evolution of gene function and regulatory control after whole-genome duplication: comparative analyses in vertebrates. *Genome Res* **19**, 1404–1418.

Knütter I, Wollesky C, Kottra G, Hahn MG, Fischer W, Zebisch K, Neubert RH, Daniel H & Brandsch M (2008). Transport of angiotensin-converting enzyme inhibitors by $H^+$/peptide transporters revisited. *J Pharmacol Exp Ther* **327**, 432–441.

Kolodziejczak D, Spanier B, Pais R, Kraiczy J, Stelzl T, Gedrich K, Scherling C, Zietek T & Daniel H (2013). Mice lacking the intestinal peptide transporter display reduced energy intake and a subtle maldigestion/malabsorption that protects them from diet-induced obesity. *Am J Physiol Gastrointest Liver Physiol* **304**, G897–G907.

Kottra G & Daniel H (2001). Bidirectional electrogenic transport of peptides by the proton-coupled carrier PEPT1 in *Xenopus laevis* oocytes: its asymmetry and symmetry. *J Physiol* **536**, 495–503.

Kottra G, Stamfort A & Daniel H (2002). PEPT1 as a paradigm for membrane carriers that mediate electrogenic bidirectional transport of anionic, cationic, and neutral substrates. *J Biol Chem* **277**, 32683–32691.

Koven W & Schulte P (2012). The effect of fasting and refeeding on mRNA expression of PepT1 and gastrointestinal hormones regulating digestion and food intake in zebrafish (*Danio rerio*). *Fish Physiol Biochem* **38**, 1565–1575.

Kudsk KA (2007). Beneficial effect of enteral feeding. *Gastrointest Endoscopy Clin North Am* **17**, 647–662.

Kwasek K, Terova G, Wojno M, Dabrowski K & Wick M (2012). The effect of dietary dipeptide lysine-glycine on growth, muscle proteins, and intestine PepT1 gene expression in juvenile yellow perch. *Rev Fish Biol Fisheries* **22**, 797–812.

Li IC, Chan CT, Lu YF, Wu YT, Chen YC, Li GB, Lin CY & Hwang SP (2011). Zebrafish krüppel-like factor 4a represses intestinal cell proliferation and promotes differentiation of intestinal cell lineages. *PLoS One* **6**, e20974.

Liu Z, Zhou Y, Feng J, Lu S, Zhao Q & Zhang J (2013). Characterization of oligopeptide transporter (PepT1) in grass carp (*Ctenopharyngodon idella*). *Comp Biochem Physiol B Biochem Mol Biol* **164**, 194–200.

Mackenzie B, Loo DDF, Fei YJ, Liu W, Ganapathy V, Leibach FH & Wright EM (1996). Mechanisms of the human intestinal $H^+$-coupled oligopeptide transporter hPEPT1. *J Biol Chem* **271**, 5430–5437.

Mackenzie B, Takanaga H, Hubert N, Rolfs A & Hediger MA (2007). Functional properties of multiple isoforms of human divalent metal-ion transporter 1 (DMT1). *Biochem J* **403**, 59–69.

Maffia M, Acierno R, Cillo E & Storelli C (1996). $Na^+$-D-glucose cotransport by intestinal BBMVs of the Antarctic fish *Trematomus bernacchii*. *Am J Physiol Regul Integr Comp Physiol* **271**, R1576–R1583.

Maffia M, Rizzello A, Acierno R, Verri T, Rollo M, Danieli A, Döring F, Daniel H & Storelli C (2003). Characterisation of intestinal peptide transporter of the Antarctic haemoglobinless teleost *Chionodraco hamatus*. *J Exp Biol* **206**, 705–714.

Margheritis E, Terova G, Oyadeyi AS, Renna MD, Cinquetti R, Peres A & Bossi E (2013). Characterization of the transport of lysine-containing dipeptides by PepT1 orthologs expressed in *Xenopus laevis* oocytes. *Comp Biochem Physiol A Mol Integr Physiol* **164**, 520–528.

Markovich D (2008). Expression cloning and radiotracer uptakes in *Xenopus laevis* oocytes. *Nat Protoc* **3**, 1975–1980.

Marshall C, Elias C, Xue XH, Le HD, Omelchenko A, Hryshko LV & Tibbits GF (2002). Determinants of cardiac $Na^+/Ca^{2+}$ exchanger temperature dependence: $NH_2$-terminal transmembrane segments. *Am J Physiol Cell Physiol* **283**, C512–C520.

Meissner B, Boll M, Daniel H & Baumeister R (2004). Deletion of the intestinal peptide transporter affects insulin and TOR signaling in *Caenorhabditis elegans*. *J Biol Chem* **279**, 36739–36745.

Meredith D (2009). Review. The mammalian proton-coupled peptide cotransporter PepT1: sitting on the transporter-channel fence? *Philos Trans R Soc Lond B Biol Sci* **364**, 203–207.

Meredith D & Price RA (2006). Molecular modeling of PepT1-towards a structure. *J Membr Biol* **213**, 79–88.

Near TJ, Dornburg A, Kuhn KL, Eastman JT, Pennington JN, Patarnello T, Zane L, Fernández DA & Jones CD (2012). Ancient climate change, antifreeze, and the evolutionary diversification of Antarctic fishes. *Proc Natl Acad Sci U S A* **109**, 3434–3439.

Nelson JS (2006). *Fishes of the World*. John Wiley & Sons, New York.

Newstead S (2011). Towards a structural understanding of drug and peptide transport within the proton-dependent oligopeptide transporter (POT) family. *Biochem Soc Trans* **39**, 1353–1358.

Newstead S, Drew D, Cameron AD, Postis VL, Xia X, Fowler PW, Ingram, JC, Carpenter, EP, Sansom MS, McPherson MJ, Baldwin SA & Iwata S (2011). Crystal structure of a prokaryotic homologue of the mammalian oligopeptide-proton symporters, PepT1 and PepT2. *EMBO J* **30**, 417–426.

Nussberger S, Steel A, Trotti D, Romero MF, Boron WF & Hediger MA (1997). Symmetry of $H^+$ binding to the intra- and extracellular side of the $H^+$-coupled oligopeptide cotransporter PepT1. *J Biol Chem* **272**, 7777–7785.

Ostaszewska T, Dabrowski K, Kamaszewski M, Grochowski P, Verri T, Rzepkowska M & Wolnicki J (2010*a*). The effect of plant protein-based diet supplemented with dipeptide or free amino acids on digestive tract morphology and PepT1 and PepT2 expressions in common carp (*Cyprinus carpio* L.). *Comp Biochem Physiol A Mol Integr Physiol* **157**, 158–169.

Ostaszewska T, Dabrowski K, Kamaszewski M, Kwasek K, Grodzik M & Bierla J (2013). The effect of dipeptide, Lys-Gly, supplemented diets on digestive tract histology in juvenile yellow perch (*Perca flavescens*). *Aquacult Nutr* **19**, 100–109.

Ostaszewska T, Kamaszewski M, Grochowski P, Dabrowski K, Verri T, Aksakal E, Szatkowska I, Nowak Z & Dobosz S (2010*b*). The effect of peptide absorption on PepT1 gene expression and digestive system hormones in rainbow trout (*Oncorhynchus mykiss*). *Comp Biochem Physiol A Mol Integr Physiol* **155**, 107–114.

Pan YX, Wong EA, Bloomquist JR & Webb Jr KE (2001). Expression of a cloned ovine gastrointestinal peptide transporter (oPepT1) in *Xenopus* oocytes induces uptake of oligopeptides *in vitro*. *J Nutr* **131**, 1264–1270.

Pedretti A, De Luca L, Marconi C, Negrisoli G, Aldini G & Vistoli G (2008). Modeling of the intestinal peptide transporter hPepT1 and analysis of its transport capacities by docking and pharmacophore mapping. *ChemMedChem* **3**, 1913–1921.

Petricorena ZL & Somero GN (2007). Biochemical adaptations of notothenioid fishes: comparisons between cold temperate South American and New Zealand species and Antarctic species. *Comp Biochem Physiol A Mol Integr Physiol* **147**, 799–807.

Pieri M, Christian HC, Wilkins RJ, Boyd CA & Meredith D (2010). The apical (hPepT1) and basolateral peptide transport systems of Caco-2 cells are regulated by AMP-activated protein kinase. *Am J Physiol Gastrointest Liver Physiol* **299**, G136–G143.

Pörtner HO, Peck L & Somero G (2007). Thermal limits and adaptation in marine Antarctic ectotherms: an integrative view. *Phil Trans R Soc B Biol Sci* **362**, 2233–2258.

Ravi V & Venkatesh B (2008). Rapidly evolving fish genomes and teleost diversity. *Curr Opin Genet Develop* **18**, 544–550.

Renna MD, Sangaletti R, Bossi E, Cherubino F, Kottra G & Peres A (2011). Unified modeling of the mammalian and fish proton-dependent oligopeptide transporter PepT1. *Channels* **5**, 89–99.

Reshkin SJ & Ahearn GA (1991). Intestinal glycyl-L-phenylalanine and L-phenylalanine transport in a euryhaline teleost. *Am J Physio Regul Integr Comp Physioll* **260**, R563–R569.

Rizzello A, Romano A, Kottra G, Acierno R, Storelli C, Verri T, Daniel H & Maffia M (2013). Protein cold adaptation strategy via a unique seven-amino acid domain in the icefish (*Chionodraco hamatus*) PEPT1 transporter. *Proc Natl Acad Sci U S A* **110**, 7068–7073.

Romero M, Kanai Y, Gunshin H & Hediger M (1998). Expression cloning using *Xenopus* laevis oocytes. *Methods Enzymol* **296**, 17–52.

Rønnestad I, Gavaia PJ, Viegas CS, Verri T, Romano A, Nilsen TO, Jordal AE, Kamisaka Y & Cancela ML (2007). Oligopeptide transporter PepT1 in Atlantic cod (*Gadus morhua* L.): cloning, tissue expression and comparative aspects. *J Exp Biol* **210**, 3883–3896.

Rønnestad I, Murashita K, Kottra G, Jordal AE, Narawane S, Jolly C, Daniel H & Verri T (2010). Molecular cloning and functional expression of Atlantic salmon peptide transporter 1 in *Xenopus* oocytes reveals efficient intestinal uptake of lysine-containing and other bioactive di- and tripeptides in teleost fish. *J Nutr* **140**, 893–900.

Rubio-Aliaga I & Daniel H (2002). Mammalian peptide transporters as targets for drug delivery. *Trends Pharmacol Sci* **23**, 434–440.

Rubio-Aliaga I & Daniel H (2008). Peptide transporters and their roles in physiological processes and drug disposition. *Xenobiotica* **38**, 1022–1042.

Sala-Rabanal M, Loo DDF, Hirayama BA, Turk E & Wright EM (2006). Molecular interactions between dipeptides, drugs and the human intestinal $H^+$–oligopeptide cotransporter, hPEPT1. *J Physiol* **574**, 149–166.

Sangaletti R, Terova G, Peres A, Bossi E, Corà S & Saroglia M (2009). Functional expression of the oligopeptide transporter PepT1 from the sea bass (*Dicentrarchus labrax*). *Pflugers Arch* **459**, 47–54.

Sato Y, Hashiguchi Y & Nishida M (2009). Temporal pattern of loss/persistence of duplicate genes involved in signal transduction and metabolic pathways after teleost-specific genome duplication. *BMC Evol Biol* **9**, 127.

Small BC & Soares JH Jr. (2000). Quantitative dietary lysine requirement of juvenile striped bass *Morone saxatilis*. *Aquacult Nutr* **6**, 207–212.

Smith DE, Clémençon B & Hediger MA (2013). Proton-coupled oligopeptide transporter family SLC15: physiological, pharmacological and pathological implications. *Mol Aspects Med* **34**, 323–336.

Solcan N, Kwok J, Fowler PW, Cameron AD, Drew D, Iwata S & Newstead S (2012). Alternating access mechanism in the POT family of oligopeptide transporters. *EMBO J* **31**, 3411–3421.

Somero GN (2003). Protein adaptations to temperature and pressure: complementary roles of adaptive changes in amino acid sequence and internal milieu. *Comp Biochem Physiol B Biochem Mol Biol* **136**, 577–591.

Somero GN (2004). Adaptation of enzymes to temperature: searching for basic "strategies". *Comp Biochem Physiol B Biochem Mol Biol* **139**, 321–333.

Spanier B, Lasch K, Marsch S, Benner J, Liao W, Hu H, Kienberger H, Eisenreich W & Daniel H (2009). How the intestinal peptide transporter PEPT-1 contributes to an obesity phenotype in *Caenorhabditits elegans*. *PLoS One* **4**, e6279.

Steel A, Nussberger S, Romero MF, Boron WF, Boyd CA & Hediger MA (1997). Stoichiometry and pH dependence of the rabbit proton-dependent oligopeptide transporter PepT1. *J Physiol* **498**, 563–569.

Takanaga H, Mackenzie B, Peng JB & Hediger MA (2005). Characterization of a branched-chain amino-acid transporter SBAT1 (SLC6A15) that is expressed in human brain. *Biochem Biophys Res Commun* **337**, 892–900.

Terada T, Sawada K, Saito H, Hashimoto Y & Inui K (1999). Functional characteristics of basolateral peptide transporter in the human intestinal cell line Caco-2. *Am J Physio Gastrointest Liver Physioll* **276**, G1435–G1441.

Terova G, Corà S, Verri T, Rimoldi S, Bernardini G & Saroglia, M (2009). Impact of feed availability on PepT1 mRNA expression levels in sea bass (*Dicentrarchus labrax*). *Aquaculture* **294**, 288–299.

Terova G, Robaina L, Izquierdo M, Cattaneo A, Molinari S, Bernardini G & Saroglia M (2013). PepT1 mRNA expression levels in sea bream (*Sparus aurata*) fed different plant protein sources. *Springerplus* **2**, 17.

Thamotharan M, Zonno V, Storelli C & Ahearn GA (1996). Basolateral dipeptide transport by the intestine of the teleost *Oreochromis mossambicus*. *Am J Physio Regul Integr Comp Physioll* **270**, R948–R954.

Thwaites DT, Brown CD, Hirst BH & Simmons NL (1993*a*). $H^+$-coupled dipeptide (glycylsarcosine) transport across apical and basal borders of human intestinal Caco-2 cell monolayers display distinctive characteristics. *Biochim Biophys Acta* **1151**, 237–245.

Thwaites DT, Brown CD, Hirst BH & Simmons NL (1993*b*). Transepithelial glycylsarcosine transport in intestinal Caco-2 cells mediated by expression of $H^+$-coupled carriers at both apical and basal membranes. *J Biol Chem* **268**, 7640–7642.

Verri T, Kottra G, Romano A, Tiso N, Peric M, Maffia M, Boll M, Argenton F, Daniel H & Storelli C (2003). Molecular and functional characterisation of the zebrafish (*Danio rerio*) PEPT1-type peptide transporter. *FEBS Lett* **549**, 115–122.

Verri T, Maffia M & Storelli C (1992). $H^+$/glycyl-glycine cotransport in eel intestinal brush-border membrane vesicles: studies with the pH-sensitive dye acridine orange. *Biochim Biophys Acta* **1110**, 123–126.

Verri T, Romano A, Barca A, Kottra G, Daniel H & Storelli C (2010). Transport of di- and tripeptides in teleost fish intestine. *Aquacult Res* **41**, 641–653.

Verri T, Terova G, Dabrowski K & Saroglia M (2011). Peptide transport and animal growth: the fish paradigm. *Biology Lett* **7**, 597–600.

Verri T, Terova G, Romano A, Barca A, Pisani P, Storelli C & Saroglia M (2012). The SoLute Carrier (SLC) family series in teleost fish. In *Functional Genomics in Aquaculture*, ed. Saroglia M & Liu Z, pp. 219–320. John Wiley & Sons, Oxford.

Vig BS, Stouch TR, Timoszyk JK, Quan Y, Wall DA, Smith RL & Faria TN (2006). Human PEPT1 pharmacophore distinguishes between dipeptide transport and binding. *J Med Chem* **49**, 3636–3644.

Volff JN (2005). Genome evolution and biodiversity in teleost fish. *Heredity* **94**, 280–294.

Wadiche JI & Kavanaugh MP (1998). Macroscopic and microscopic properties of a cloned glutamate transporter/chloride channel. *J Neurosci* **18**, 7650–7661.

Walton MJ, Cowey C & Adron JW (1984). The effect of dietary lysine levels on growth and metabolic of rainbow trout (*Salmo gairdneri*). *Br J Nutr* **52**, 115–122.

Wilson RP (2002). Amino acid and proteins. In *Fish Nutrition*, ed. Halver JE & Hardy RW, pp. 162–164. Academic Press, London.

Wuensch T, Schulz S, Ullrich S, Lill N, Stelzl T, Rubio-Aliaga I, Loh G, Chamaillard M, Haller D & Daniel H (2013). The peptide transporter PEPT1 is expressed in distal colon in rodents and humans and contributes to water absorption. *Am J Physiol Gastrointest Liver Physiol* **305**, G66–G73.

Xue XH, Hryshko LV, Nicoll DA, Philipson KD & Tibbits GF (1999). Cloning, expression, and characterization of the trout cardiac $Na^+/Ca^{2+}$ exchanger. *Am J Physio Cell Physioll* **277**, C693–C700.

Yuen BB, Wong CK, Woo NY & Au DW (2007). Induction and recovery of morphofunctional changes in the intestine of juvenile carnivorous fish (*Epinephelus coioides*) upon exposure to foodborne benzo[*a*]pyrene. *Aquat Toxicol* **82**, 181–194.

Zambonino Infante JL, Cahu CL & Peres A (1997). Partial substitution of di- and tripeptides for native proteins in sea bass diet improves *Dicentrarchus labrax* larval development. *J Nutr* **127**, 608–614.

Zhang C, Ai Q, Mai K, Tan B, Li H & Zhang L (2008). Dietary lysine requirement of large yellow croaker, *Pseudosciaena crocea* R. *Aquaculture* **283**, 123–127.

Zhang Y, Dabrowski K, Hliwa P & Gomulka P (2006). Indispensable amino acid concentrations decrease in tissues of stomachless fish, common carp in response to free amino acid- or peptide-based diets. *Amino Acids* **31**, 165–172.

## Additional information

### Competing interests

None declared.

### Funding

Work in the authors' laboratory has been supported by grants from the University of Salento (Fondi ex-60%) and in part by grants from the Apulian Region (Codice Identificativo Progetto PS_070).