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# Proton-coupled oligopeptide transporter family SLC15: Physiological, pharmacological and pathological implications

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## Abstract

Mammalian members of the proton-coupled oligopeptide transporter family (SLC15) are integral membrane proteins that mediate the cellular uptake of di/tripeptides and peptide-like drugs. The driving force for uphill electrogenic symport is the chemical gradient and membrane potential which favors proton uptake into the cell along with the peptide/mimetic substrate. The peptide transporters are responsible for the absorption and conservation of dietary protein digestion products in the intestine and kidney, respectively, and in maintaining homeostasis of neuropeptides in the brain. They are also responsible for the absorption and disposition of a number of pharmacologically important compounds including some aminocephalosporins, angiotensin-converting enzyme inhibitors, antiviral prodrugs, and others. In this review, we provide updated information on the structure-function of PepT1 (SLC15A1), PepT2 (SLC15A2), PhT1 (SLC15A4) and PhT2 (SLC15A3), and their expression and localization in key tissues. Moreover, mammalian peptide transporters are discussed in regard to pharmacogenomic and regulatory implications on host pharmacology and disease, and as potential targets for drug delivery. Significant emphasis is placed on the evolving role of these peptide transporters as elucidated by studies using genetically modified animals. Whenever possible, the relevance of drug-drug interactions and regulatory mechanisms are evaluated using *in vivo* studies.

### Keywords

Mimetic; Peptide; Peptide transporters; SLC15

## 1. Introduction

The acceptance of mammalian peptide transporters, as distinct from amino acid transporters, for the intestinal absorption and renal reabsorption of nutritional nitrogen was pushed to the



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forefront by studies in brush border membranes vesicles (BBMV) prepared from intestine and kidney (Ganapathy and Leibach, 1985; Ganapathy and Leibach, 1986). The BBMV studies clearly demonstrated that dipeptides, tripeptides and peptide-like drugs were actively transported into vesicles by a process that was coupled to the movement of protons down an electrochemical proton gradient (Figure 1). With the advent of expression cloning techniques, the molecular basis for peptide transporters was first identified in mammals by Hediger and co-workers (Fei et al., 1994) and Daniel and coworkers (Boll et al., 1994) in which the cloning of *Slc15a1* from a rabbit intestinal cDNA library and functional characterization of the encoded PepT1 was evaluated. Orthologs of PepT1 were soon to follow in other mammalian species as were PepT1 paralogs such as PepT2 (encoded by *SLC15A2*), PhT1 (encoded by *SLC15A4*) and PhT2 (encoded by *SLC15A3*). These proton-coupled oligopeptide transporters of the SLC15 family were shown to be phylogenetically conserved integral membrane proteins (Wang et al., 2010) and, as demonstrated in cell cultures, *Xenopus* oocytes and other heterologous expression systems (Brandsch et al., 2008; Rubio-Aliaga and Daniel, 2008), were responsible for the symport of protons and peptides/mimetics across biological membranes. Many studies have addressed the expression, localization and structure-function of the high-capacity, low-affinity “professed” intestinal peptide transporter PepT1 and the low-capacity, high-affinity “professed” renal peptide transporter PepT2. In contrast, much less is known about the peptide/histidine transporters PhT1 and PhT2. The proton-coupled oligopeptide transporter family (SLC15) belongs to the major facilitator superfamily (MFS) as detailed in the “transporter classification system” operated by the Saier Lab Bioinformatics Group (http://www.tcdb.org).

## 2. Structure-function

The oligopeptide transporters PepT1 and PepT2 mediate proton-coupled active transport of a broad range of dipeptides and tripeptides, including zwitterionic, anionic and cationic peptides as well as a variety of peptide-like drugs (e.g., cefadroxil, enalapril, valacyclovir). Numerous studies aimed at investigating the influence of charge of the dipeptide on PepT1 function. Thus, the parameters voltage, ion-dependence and proton-coupling were investigated. Kinetics of transport were extensively measured by the two-electrode voltage clamp (TEVC) technique and radiolabeled oligopeptide uptake by PepT1 expressed in cRNA-injected *Xenopus laevis* oocytes. Experiments with human PepT1 showed that the intensively used model dipeptide glycylsarcosine (GlySar) as well as the dipeptides Ala-Val, Ala-Asp and Ala-Lys all induce, at physiological pH 6.0 of the unstirred layer near the intestinal brush border membrane, characteristic steady-state current-voltage relationships (Steel et al., 1997). Moreover, the transport efficiency depends on the net charge of the peptides (neutral, negative and positive, respectively). The apparent Michaelis-Menten affinity constant (Km) observed were the same for all these transported dipeptides (Mackenzie et al., 1996). Different results were observed for rabbit PepT1 byAmasheh et al. (1997) for zwitterionic (Gly-Gln), anionic (Gly-Asp) and cationic (Gly-Lys) dipeptide. Noteworthy, it was also shown that all these dipeptides were transported electrogenically regardless of their net charge. However, the measured Km and maximal inward current (Imax) values seemed to be differently affected as a function of membrane potential (Vm) and external pH for neutral and charged substrates. The authors showed that a complex inter-relationship between membrane potential and external pH determines substrate affinity and consequently transport rates. Indeed, it was found that a decrease in external pH induces an increase in the rate of transport of negatively charged dipeptide, and to a lesser extent of neutral substrates, while an increasing pH is concomitant with transport of positively charged dipeptides. *In vitro* observations byLister et al. (1997) confirmed these results. They investigated the effect of net charge on the transmembrane transport of hydrolysis-resistant dipeptides (Phe-Ala, Phe-Gln and Phe-Glu) in isolated jejunal loops of rat small intestine

and the influence of luminal pH. Indeed, transport of neutral and negatively charged dipeptides was stimulated by lowering the luminal pH to 6.8, whereas the contrary was described for positively charged dipeptides (Lister et al., 1997). Tripeptide-like β-lactam antibiotics such as anionic cefixime and zwitterionic cefadroxil were also tested for transport by rabbit PepT1, associated with an additional technique of intracellular pH recording by fluorometric measurements. These experiments showed that only the neutral species of the driven substrate was transported (Wenzel et al., 1996). The proton stoichiometry of peptide transport for neutral and charged dipeptides was investigated for the first time byTemple et al. (1995) using renal brush-border membrane vesicles (BBMV). The proton to substrate ratio was 1:1, 2:1 and 0:1 for neutral, acidic and basic dipeptides, respectively. As renal BBMV contain two isoforms of PepT,Steel et al. (1997) calculated the coupling ratio of flux mediated specifically by rabbit PepT1 during expression in oocytes. The ratio was found to be 1:1, 1:1 and 2:1 for neutral (GlySar,), anionic (Gly-Glu) and cationic (Gly-Lys) dipeptides, respectively (Steel et al., 1997). In 2002, Kottra et al. used the TEVC technique combined with intracellular pH recordings and giant patch clamp (GPC) experiments to demonstrate the electrogenic bidirectional character of dipeptide transport by rabbit PepT1 expressed in *Xenopus laevis* oocytes. Interestingly, it was also shown that anionic dipeptides such as Gly-Asp and Asp-Gly were transported in their neutral and negatively charged forms, with high and low affinities, respectively. On the other hand, cationic dipeptides seemed to be transported in neutral and positively charged forms, resulting in an excess of transport current as compared with neutral substrates. These results also confirmed the 2:1 proton to anionic dipeptide (Gly-Asp) stoichiometry. A study of hPepT1 expressed in CHO cells showed that valacyclovir (prodrug of the anthiherpetic agent acyclovir) in conditions of lower pH exists as a cationic species and has lower uptake, whereas, at higher pH, it exists predominantly as neutral species exhibiting higher uptake values (Balimane and Sinko, 2000). According to the literature, charged substrates were reported to have different binding affinities under various pH conditions depending on their ionization state. The exact characterization of the pH-dependent substrate transport mechanism by PepT1 still needs further investigation to be more completely understood. Unlike PepT1, PepT2 has been little studied and appears to have distinct electrogenic transport properties. Indeed, it has been reported that rat PepT2 expression in Xenopus oocytes reflected an $H^+$: substrate stoichiometry of 2:1 and 3:1 for neutral and anionic dipeptides, respectively (Chen et al., 1999)

Since the mid-1990s, many approaches were investigated to define the membrane topology of the human PepT and its functional domains. Several hydropathy plots were predicted using different calculation methods and all of them describe a protein composed of 12 transmembrane helices (TMH or H) with segments 9 and 10 being connected by a long extracellular loop. This consensus model was partly confirmed by "epitope-tagging," which also indicated that the C-terminus was oriented towards the cytoplasm (Covitz et al., 1998). The amino acid sequences of the loop regions of PepT1 and PepT2 are less conserved than that of the TMH and PepT1 and PepT2 exhibit different kinetic properties, namely substrate affinity for the dipeptide GlySar, commonly used for assaying peptidomimetic transport by PepT. Several experiments were performed with chimeras combining different regions of the human PepT1 and rat PepT2. Variable results were observed from these studies. Indeed, it was shown that the functional characteristics of two peptide transporters seem to be inherent either in H1 to 9 (Doring et al., 1996), or in H7, 8 and 9 (Fei et al., 1998) or H1 to 6 (Terada et al., 2000). Other experiments based on model predictions and molecular modeling suggested for PepT1 that the channel for substrate translocation was formed by H1, 3, 5, 7 and 10 (Bolger et al., 1998). Site-directed mutagenesis experiments, namely $Y^{12}A$ (H1), $Y^{167}A/F/H/S$ (H3), $R^{282}A$, $W^{294}A$ (H7) and $Y^{588}F$, $E^{595}A$ (H10), located in conserved H1, 5, 7, and 10, supported this hypothesis and the authors described these amino acids as key residues for transport through the supposed transmembrane channel (Figure 2B,

Bolger et al., 1998; Yeung et al., 1998; Lee et al., 1999). Yeung and co-workers (1998) proposed a different organization: H7, 8, 9 and 10 form one half of the channel and H1, 3 and 5 the other half; this could also explain the different results obtained with the chimeric transporters. Tyrosyl residues at positions 56, 64 and 167, the latter of which belongs to the "PTR motif" also appears to be involved in the binding of the proton (Chen et al., 2000; Yeung et al., 1998) and $Y^{91}$ is the counterpart in the rabbit PepT (Pieri et al., 2009). However, the role of the large extracellular loop between H9 and 10 is still unknown. It seems to have several putative glycosylation sites. Several studies have shown that substrate affinity for PepT1 and PepT2 and pH-dependent transport are linked to the N-terminus of the protein, and more specifically to the conserved histidinyl residues $H^{57}$, $H^{121}$ and $H^{260}$ (Chen et al., 2000; Doring et al., 2002; Fei et al., 1997; Terada et al., 1996; Uchiyama et al., 2003). Mutation of $His^{57}$ and $F^{28}Y$ of PepT1 altered substrate affinity and pH-dependent transport and in the case of human PepT2, $R^{57}H$ led to a complete loss of function (Anderle et al., 2006; Terada et al., 2004). Cysteine scanning experiments complicated the understanding of the model involving $N^{171}$, $S^{174}$, $F^{293}$, $L^{296}$ and $F^{297}$ in the transport of GlySar by human PepT1 (Kulkarni et al., 2003a; Kulkarni et al., 2003b). In addition, the very recent high-resolution 3D structure of a prokaryotic homologue of the mammalian PepT provides new insights in the elucidation of the molecular mechanism of transport. This structure is that of $PepT_{So}$ from *Shewanella oneidensis* and it was obtained by X-ray crystallography at a 3.6-Å resolution (Newstead et al., 2011). This transporter consists of 14 TMH, the first N-terminal half of the protein (H1 to 6) being connected to the second C-terminal half (H7 to 12) by two additional membrane helices, HA and HB (Figure 2A). The resulting V-shaped structure whose base is oriented toward the extracellular side defines two hydrophilic cavities, each one having a functional role (Figure 2C). It is proposed that the central cavity is the peptide-binding site connected to a small cavity corresponding to the extracellular binding site of $H^+$. Furthermore, the authors propose that the substrate binding site involves residues $R^{25}/Y^{29}/R^{32}$ (H1), $Y^{68}$ (H2), $K^{127}$ (H4), $Y^{154}/I^{157}$ (H5) in the N-terminal half, providing a positively charged side of the chamber and residues with $W^{312}/F^{315}$ (H7), $E^{419}/S^{423}$ (H10), $W^{446}$ (H11) in the C-terminal half thus providing a patch of negatively charged residues (Figure 2C). This peptide binding site differs from that described recently byFoley et al. (2010) thanks to computer modelling. The only feature common to both structures is the involvement of residues $E^{595}$ (H10), $Y^{167}$ (H5) and $W^{294}$ (H7). However, many questions remain unsolved regarding the exact residues involved in the molecular mechanism of transport. Solving the protein structures of human PepT1 and PepT2 that are not yet known would help shed light on these still open questions.

## 3. Localization-expression

The physiological, pharmacological and pathological functions of mammalian SLC15 family members are determined, to a large extent, by their expression levels and cellular localization, especially in regard to intestine, kidney and brain. In the intestine, PepT1 protein is abundantly expressed at the apical membrane of enterocytes in mouse and human duodenum, jejunum and ileum, with little or no expression in normal colon (Groneberg et al., 2001; Jappar et al., 2010; Walker et al., 1998). Although PepT2 has been reported in glial cells and tissue-resident macrophages of the enteric nervous system (Ruhl et al., 2005), it is unlikely that PepT2-mediated absorption is involved in these deep neuromuscular layers of the gastrointestinal tract. Transcripts of *SLC15A4* and *SLC15A3* encoding PhT1 and PhT2 have also been found in human and rat intestinal tissue segments (Herrera-Ruiz et al., 2001), and immunohistochemical analyses have indicated that PhT1 is expressed in the villous epithelium of small intestine (Bhardwaj et al., 2006). However, their relevance in peptide/mimetic absorption has not been established. Thus, it appears that the high-capacity, low-affinity intestinal transporter PepT1 is solely responsible for the absorption of di/tripeptides arising from dietary proteins and gastrointestinal secretions.

In the kidney, reverse-transcription PCR of microdissected tubular segments and *in situ* hybridization studies have shown that PepT1 and PepT2 are differentially expressed in rat proximal tubule (Smith et al., 1998). Whereas *Slc15a1* transcripts encoding PepT1 are found in early parts of the proximal tubule (pars convoluta), *Slc15a2* transcripts encoding PepT2 are expressed preferentially (but not exclusively) in latter parts of the proximal tubule (pars recta). Neither transporter is expressed anywhere else in the nephron. Definitive confirmation was provided by immunolocalization studies in rats (Shen et al., 1999) where PepT1 protein was detected in brush border membranes of proximal tubule S1 segments, with progressively weaker expression in deeper cortical regions. In contrast, PepT2 protein was localized primarily to brush border membranes of proximal tubule S3 segments with strong immunostaining of the outer, but not inner, stripe of outer medulla. PepT1 and PepT2 proteins are also found in brush border membranes of mouse kidney (Hu et al., 2008). Indirect localization studies of β-galactosidase expression and fluorophore-conjugated dipeptide accumulation in kidney have shown a similar sequential expression of PepT1 and PepT2, respectively, in mouse proximal tubule (Rubio-Aliaga et al., 2003) to that of rat (Shen et al., 1999). Thus, it appears that PepT1 (a high-capacity, low affinity transporter) and PepT2 (a low-capacity, high-affinity transporter) work in concert to efficiently reabsorb peptide-bound amino acids from the tubular fluid.

In the brain, *Slc15a2* mRNA is expressed in rat astrocytes, subependymal cells, ependymal cells, and epithelial cells of choroid plexus (Berger and Hediger, 1999). Subsequent immunoblot and immunohistochemical studies in rat found that PepT2 has its strongest expression in cerebral cortex, with strong expression also being observed in the olfactory bulb, basal ganglia, cerebellum, hindbrain, epithelial cells of the choroid plexus, and ependymal cells (Shen et al., 2004). In addition, PepT2 is expressed exclusively on apical membranes of choroid plexus epithelia (i.e., cerebrospinal fluid- or CSF-facing) in both adult and neonatal animals. PepT2 protein is found in neurons (adult and neonate) and astrocytes (neonate but not adult), and exhibits an age-related decline in cerebral cortex expression levels as a function of age (fetal and neonatal tissue > adult). On the other hand, there is no molecular or functional evidence for PepT2 in endothelial cells of the blood-brain-barrier, or for PepT1 expression in brain. Although the peptide-histidine transporters PhT1 (Yamashita et al., 1997) and PhT2 (Sakata et al., 2001) are present in brain, their functional importance is unknown. Thus, it appears that PepT2 is involved in the removal of neuropeptides, peptide fragments, and peptide-like drugs from CSF, and may play a role in regulating neuropeptide homeostasis in the extracellular fluid.

Studies on the absorption of PepT1 substrates in intestine, the reabsorption of PepT1/PepT2 substrates in kidney, and the tissue uptake of PepT2 substrates in choroid plexus address the cellular influx of peptides/mimetics from the apical surface of these membranes. Once inside the cell, unstable di/tripeptides can be degraded into constituent amino acids and unstable drugs into transformed metabolites which can be active or inactive. Whereas many transporters are available on the basolateral surface for the efficient translocation of constituent amino acids from cells (Daniel, 2004; Ganapathy et al., 2006; Redzic et al., 2005), little is known about how stable peptides and peptide-like drugs are transported across these basolateral membranes into the blood. Functional studies have shown that basolateral transport systems for peptides/mimetics in intestine, kidney and choroid plexus are distinct from that of PepT1 and PepT2 (Shu et al., 2001; Shu et al., 2002; Smith et al., 2004; Terada and Inui, 2004). However, because they have not been cloned, the precise nature of the basolateral transporter(s) has yet to be resolved.

In regard to other tissues, PepT1 has been reported in the pancreas, bile duct and liver, and PepT2 in the lung, mammary gland and spleen (Brandsch et al., 2008; Rubio-Aliaga and Daniel, 2008). Likewise, PhT1 has been reported in the eye (Yamashita et al., 1997) and

PhT2 in the lung, spleen and thymus (Sakata et al., 2001). Still, the role and relevance of SLC15 family members remain to be elucidated in these tissues.

## 4. Pharmacogenomics

Many genetic variants have been reported for the SLC15 family members PepT1 and PepT2, encoded by *SLC15A1 and SLC15A2,* in humans (Brandsch et al., 2008; Zair et al., 2008; UCSF Pharmacogenetics of Membrane Transporters Database, http://pharmacogenetics.ucsf.edu). With respect to PepT1, only two variants are worth noting, both rare, in their ability to alter transporter activity. The first one, a P586L variant, demonstrated a significantly reduced transport capacity of GlySar in transfected HeLa cells because of the 10-fold lower value for Vmax (Zhang et al., 2004). The second one, a F28Y variant (expressed exclusively within African-Americans), displayed a significantly reduced uptake of GlySar in transfected HEK293 cells because of the 3-fold greater value for Km (Anderle et al., 2006). Regarding disease, a bidirectional association was found between a functional *SLC15A1* SNP (rs2297322, Ser117Asn) and Crohn's disease susceptibility in two cohorts of Swedish (increased risk) and Finnish patients (increased protection) (Zucchelli et al., 2009). The reason for this "flip-flop" phenomenon between populations is currently unknown as is whether or not this polymorphism truly contributes to disease susceptibility.

With respect to PepT2, a rare R57H variant showed a complete loss of function for GlySar uptake in transfected HEK293 cells and Xenopus oocytes, despite normal expression of protein at the plasma membranes (Terada et al., 2004). In another study (Pinsonneault et al., 2004), haplotype analysis revealed the existence of two main variants (*PepT2*1 and *2*), present in substantial frequencies, in an ethnically diverse group of subjects. Both haplotypes displayed similar Vmax values for GlySar uptake in transfected Chinese hamster ovary cells, but 3-fold higher Km values in PepT2*2 as compared to PepT2*1 genetic variants. These authors also reported differences in their pH sensitivity for GlySar transport. A subsequent clinical study in healthy Asian subjects reported no significant differences between genotypic groups (n=10 for alleles *1/*1 and n=5 for alleles *2/*2) in cephalexin pharmacokinetics after a single 1000 mg oral dose of drug (Liu et al., 2009). Finally, a higher blood lead burden was observed in children with the PepT2*2 polymorphism suggesting that, in addition to mutations in delta-aminolevulinic acid dehydratase, genetic variations in PepT2 may serve as a biomarker for increased vulnerability to the neurotoxicity of lead exposure (Sobin et al., 2009).

Collectively, the limited variability (and frequency) of *SLC15A1* and *SLC15A2* polymorphisms suggest a high evolutionary pressure on their gene products, thereby, preventing the survival of mutations that may be severely detrimental to human health. Nevertheless, the total number of human subjects that have been studied so far is sparse as are *in vivo* pharmacokinetic studies evaluating the functional influence of human SLC15 mutations. Moreover, the possible role of PepT1 and PepT2 (and possible mutations) in pathophysiology is in its infancy.

## 5. Studies in wild-type and genetically-modified mice

Major advances have been made in the structure-function, tissue and cellular localization, and regulatory properties of SLC15 family members using cellular, molecular and biochemical methodologies. However, these experimental approaches are often limited because they lack an intact blood supply, appropriate residence times of substrate at biological membranes, and the ability to study a particular gene product under physiological conditions. The development of genetically-modified mice has provided a unique and powerful tool to evaluate the evolving role and relevance of PepT1 and PepT2 in peptide

physiology along with drug absorption, distribution, excretion, pharmacology and toxicology.

*Slc15a1* null mice were first developed and validated byHu et al. (2008), long after this intestinal transporter was identified as having a critical function in the luminal uptake of dietary di/tripeptides in small intestine. In this regard, PepT1 accounts for the great majority of protein digestion products entering the enterocytes and, after efficient hydrolysis, constituent amino acids are effluxed into the portal vein primarily by basolateral amino acid transporters (Daniel, 2004; Ganapathy et al., 2006). It was, therefore, surprising that no pathological phenotype was observed between gender-matched wild-type and *Slc15a1*-deficient mice (Hu et al., 2008). In fact, the *Slc15a1* null animals were found to be viable, fertile, grew to normal body size, body and organ weight, and had no differences in serum clinical chemistry or histology. Interestingly, the lack of apparent biological effects was not due to adaptive changes in response to *Slc15a1* deletion. Other SLC15 family members (i.e., *Slc15a2*, *Slc15a4* and *Slc15a3*) were not upregulated in the small intestine, colon, or kidney of *Slc15a1* null mice. As reported by Nassl and co-workers (2011a), transcriptome and proteome analyses of intestinal tissue revealed no change in the expression and functional activity of amino acid transporters. Likewise, *Slc15a2* gene expression was unaltered in the gut enteric nervous system as was PepT2 protein expression in the kidney of *Slc15a1*-deficient animals. Although it remains unclear why an obvious phenotype was not observed during *Slc15a1* ablation in mice, it is possible that the reciprocal axial gradient of PepT1 and peptidases/amino acid transporters in progressive segments of the intestines, along with diffusive/paracellular permeabilities and substantial residence times, may account for this "apparent" incongruity (Ganapathy et al., 2006). In other words, protein digestion products are absorbed in early small intestine primarily as di/tripeptides in the presence of intestinal PepT1 whereas protein digestion products are absorbed in late small intestine and colon primarily as amino acids in the absence of PepT1 (i.e., biological redundancy exists).

*In vitro*, *in situ* and *in vivo* studies in mice agree that intestinal PepT1 has a significant influence on the absorption of peptide/mimetic compounds. Thus, studies using everted jejunal rings (Ma et al., 2011) and single-pass intestinal perfusions (Jappar et al., 2010) demonstrated the dominant effect of PepT1 where residual uptake of a stable dipeptide, GlySar, in *Slc15a1* knockout mice was about 20% and 10%, respectively, of that in wild-type animals. Moreover, there was general concordance between protein expression levels and PepT1 functional activity such that GlySar permeability in duodenum jejunum > ileum (Jappar et al., 2010). No PepT1 protein was detected in the colon as reflected by very low, non-significant differences in GlySar permeability being observed between the two genotypes. More modest changes were observed during *in vivo* studies in which the systemic exposure (area under the plasma concentration-time curve or AUC) of orally administered GlySar in *Slc15a1* knockout mice was one-half that of wild-type mice (Jappar et al., 2011; Ma et al., 2012). This finding highlights the fact that, although mechanistically valid, *in vitro/in situ* study designs do not necessarily reflect what happens when physiologic conditions are operative, including effects of differing luminal concentrations, segmental intestinal absorption, and gastrointestinal residence times. Studies are currently underway evaluating the role and relevance of intestinal PepT1 on *in vivo* drug absorption. Preliminary studies indicate that after oral dosing, the systemic exposure of acyclovir when administered as valacyclovir (Yang et al., 2011) and cefadroxil (Posada and Smith, 2011a) in *Slc15a1* knockout mice are one-half and one-fifth that of wild-type animals, respectively. The intestinal permeabilities of representative PepT1 substrates are displayed in Table 1.

*Slc15a2* null mice were developed and validated byShen et al. (2003) and by Rubio-Aliaga et al. (2003) at about the same time. As with *Slc15a1* null mice, Shen and co-workers (2003) reported that no discernible phenotype was evident during *Slc15a2* gene deletion. The

*Slc15a2* null mice were viable, grew to normal size and weight, had normal base-line blood and urine chemistries, and were without obvious kidney or brain abnormalities. However, targeted disruption of the *Slc15a2* gene reduced GlySar uptake in isolated choroid plexus by 90%, demonstrating that PepT2 was the primary SLC15 family member responsible for dipeptide uptake in this tissue. Rubio-Aliaga and co-workers (2003) also reported that *Slc15a2* null mice showed no obvious phenotypic abnormalities including no significant alteration in urinary protein and free amino acid levels after disruption of *Slc15a2*. The importance of PepT2 in reabsorption of filtered di/tripeptides was demonstrated in null mice, however, by the significantly reduced renal accumulation of a fluorophore-labeled and a radiolabeled dipeptide after intravenous and intraperitoneal injections, respectively. These authors concluded that PepT2 was the main SLC15 responsible for tubular reabsorption of peptide-bound amino acids, despite the fact that gene deletion did not lead to major changes in the renal excretion of protein or free amino acids. *Slc15a2* null mice did not exhibit an adaptive up-regulation in the expression levels of related genes in both the kidney and brain (Ocheltree et al., 2005; Rubio-Aliaga et al., 2003). Moreover, mRNA, protein and metabolic profiling of kidney tissue in *Slc15a2* null mice revealed subtle, but relatively minor, changes in differentially expressed gene products involved in amino acid and nitrogen metabolism (Frey et al., 2007). Thus, analogous to the intestine, it seems that biological redundancy may favor the reabsorption of di/tripeptides in the presence of renal tubular PepT2 while amino acid reabsorption is favored, after hydrolysis by renal peptidases, in the absence of PepT2.

*In vivo* studies in wild-type and *Slc15a2* knockout mice have provided definitive evidence that PepT2 is the predominant peptide transporter in kidney. Based on the sequential expression of PepT1 and PepT2 in the proximal tubule, a renal clearance model was developed so that the individual contributions of PepT1 and PepT2 could be determined with respect to peptide/mimetic reabsorption. Thus, PepT2 accounted for 86% of the reabsorption of the synthetic model dipeptide GlySar (Ocheltree et al., 2005), 95% of the reabsorption of the aminocephalosporin antibiotic cefadroxil (Shen et al., 2007), and 83% of the reabsorption of the endogenous dipeptide carnosine (Kamal et al., 2009), which can also be taken exogenously as a dietary supplement. With respect to systemic exposure, PepT2 ablation resulted in 2- to 3-fold reductions in the AUC of GlySar (Ocheltree et al., 2005), cefadroxil (Shen et al., 2007), and carnosine (Kamal et al., 2009) because of the reduced tubular reabsorption and greater clearance of these substrates from the body. However, when adjusted for these differences, the CSF-to-blood (or plasma) concentration ratios in *Slc15a2* knockout mice were 4-fold greater for GlySar (Ocheltree et al., 2005), 6- to 7-fold greater for cefadroxil (Shen et al., 2007), and 8-fold greater for carnosine (Kamal et al., 2009), as compared to wild-type animals. This finding reflects the reduced entry of these substrates into choroid plexus during PepT2 ablation and demonstrates that PepT2 is responsible for effluxing peptide/mimetic substrates from CSF into choroidal tissue. Moreover, it appears that the regional effect of PepT2 in limiting exposure of substrates in the CSF (and presumably extracellular fluid) of brain may be more important for some compounds than its effect in increasing systemic exposure (Kamal et al., 2008).

The modulation of regional brain disposition by PepT2 has been translated directly into significant changes in drug response. Thus, the CSF clearance of intracerebroventricular injected L-kyotorphin (L-KTP), an endogenous neuropeptide and PepT2 substrate, was slower in *Slc15a2* null mice thereby resulting in a 5-fold greater L-KTP-induced analgesia as compared to equivalent doses of this compound in wild-type animals (Jiang et al., 2009). In contrast, the analgesic effect of its synthetic diastereomer D-KTP, a non-PepT2 substrate, was unchanged between genotypes as judged by dose-response analysis. Based on this study, it appears that PepT2 might function in the control of analgesia through regulation of endogenous neuropeptides as well as potential drug candidates. In another study (Hu et al., 2007) the survivability of *Slc15a2* null mice was reduced, as compared to wild-type animals,

after high-dose subcutaneous administration of the heme precursor 5-aminolevulinic acid (5-ALA). In addition, neuromuscular function worsened in mice with PepT2 ablation after chronic dosing of 5-ALA. Although the plasma concentrations of 5-ALA were similar between genotypes, CSF concentrations were 8- to-30 times greater in *Slc15a2* null mice. The authors suggested that PepT2 may limit the exposure of 5-ALA in CSF and have relevance as a secondary genetic modifier of conditions in which 5-ALA metabolism is altered and elevated 5-ALA toxicity occurs (i.e., acute hepatic porphyria and lead poisoning). By finding appropriate challenges for *Slc15a2* knockout mice, these two studies have established for the first time both pharmacologic and neuroprotective phenotypes of PepT2. Subsequent to these studies, biodistribution studies were combined with quantitative autoradiography in order to build a model for the spatial distribution of peptides/mimetics in specific regions of brain tissue (Smith et al., 2011).

## 6. Regulation

Epithelial SLC15s can be regulated by signals emanating from both endogenous and exogenous sources. These signals can be the result of drugs and hormones, diet and nutritional status, disease states, circadian rhythm, and developmental biology. In particular, the underlying mechanisms can be nonspecific, such as changes in membrane surface area, or specific for a certain carrier. Specific regulation can be brought about by alterations in the electrogenic driving force for transport, by changes in transcription and/or stability of mRNA, by translation and post-translational modifications of the protein, and by shifts in the trafficking of protein from preformed cytoplasmic pools to the apical membrane. Many excellent reviews are available describing these regulatory aspects primarily from a cellular-molecular, *in vitro* perspective (Adibi, 2003; Brandsch et al., 2008; Rubio-Aliaga and Daniel, 2008; Terada and Inui, 2007). This manuscript is intended to expand upon the previous reviews by discussing those regulatory mechanisms that are new or have been validated by *in vivo* experiments.

Calcium channel blockers can indirectly stimulate the sodium-proton antiporter in intestine, via reduced intracellular concentrations of calcium, thereby increasing the proton electromotive force and PepT1 activity (Wenzel et al., 2002). This effect was demonstrated *in vivo* by nifedipine enhancing the bioavailability of amoxicillin in humans (Westphal et al., 1990) and that of cephalexin in conscious rats (Berlioz et al., 2000). Likewise, amiloride reduced the intestinal oral absorption of amoxicillin in humans by direct inhibition of the sodium-proton exchanger (Westphal et al., 1995). Insulin appears to increase the membrane population of PepT1 by increasing its translocation from a preformed cytoplasmic pool (Thamotharan et al., 1999). Although (to our knowledge) no *in vivo* studies have been reported to support this belief, *in situ* perfusions of rat jejunum demonstrated that GlySar uptake was substantially increased in diabetic animals treated with insulin for one month, along with an upregulation of brush border PepT1 staining and protein expression (Bikhazi et al., 2004).

Numerous studies have shown that diet can have a significant effect on intestinal PepT1 expression and activity. For example, the diurnal rhythm of PepT1 in small intestine was the result of food intake, rather than the light cycle (Pan et al., 2002; Pan et al., 2004), as demonstrated by greater peak plasma concentrations and systemic exposure of ceftibuten when dosed in fed rats intraduodenally at 8:00 pm as compared to 8:00 am. (Pan et al., 2003). These same authors (Saito et al., 2008) subsequently showed that a clock-controlled gene, DBP, regulated the circadian oscillation of PepT1 expression during normal and restricted feeding conditions as opposed to other transcription factors (i.e., Sp1, Cdx2 and PPAR-which contribute to the basal, intestine-specific, and fasting-induced expression of PepT1, respectively (Terada and Inui, 2007). Moreover, *in vivo* studies (Ma et al., 2012)

revealed that as little as 16 hours of fasting can cause upregulation of PepT1 protein in duodenal, jejunal and ileal segments of wild-type mice, resulting in significant increases in the oral absorption of GlySar in these mice but not in *Slc15a1* knockout animals.Nassl et al. (2011b) further demonstrated a time-dependence of food intake regulation in male mice fed a high protein diet. Specifically, *Slc15a1* null mice had a reduced consumption of food over the first 4 days but gradually increased their food consumption so, that by 18 days, they had the same intake rate as wild-type animals. Regardless, the *Slc15a1* null mice failed to show any significant weight gain over the entire feeding period, indicating a reduced assimilation of intestinal energy.

The two major forms of inflammatory bowel disease (IBD), Crohn's disease (CD) and ulcerative colitis (UC), are chronic disorders characterized by nonspecific inflammation and intestinal tissue damage (Xavier and Podolsky, 2007). Although the precise mechanisms are unknown, IBD pathogenesis involves a complex interaction of host genetics, gut microbiota, innate immunity, and environmental factors. Increasing evidence suggests an important role for PepT1 in linking the intestinal uptake of chemotactic bacterial peptides in colon to an aggravated inflammatory response. Despite little, if any, PepT1 being present in the large intestine, this transporter is aberrantly upregulated in patients with IBD (Merlin et al., 2001; Wojtal et al., 2009). Moreover, cell culture studies have demonstrated that PepT1 can transport small bacterially-produced peptides such as muramyl dipeptide (Vavricka et al., 2004), fMet-Leu-Phe (Merlin et al., 1998), and Tri-DAP (Dalmasso et al., 2011). Thus, a "working model" was proposed in which bacterial di/tripeptides enter colonic enterocytes via apically-expressed colonic PepT1, interact with NOD-like receptors, trigger the NF- B and MAPK pathways, thereby, leading to downstream proinflammatory cytokine/chemokine production and the subsequent migration of neutrophils into regions of inflammation. It is also possible that, because of intestinal epithelial barrier disruption by IBD, bacterial di/tripeptides may reach PepT1-expressing macrophages in lamina propria via the paracellular pathway and, as a result, contribute to the observed immune response (Ingersoll et al., 2012).

Studies in wild-type, human PepT1 transgenic mice (produced by a β-actin promoter for PepT1 expression in all tissues or by a villin promoter for PepT1 expression in intestinal epithelial cells), Nod2 knockout mice, β-actin-human PepT1 transgenic/Nod2 knockout mice and villin-human PepT1 transgenic/Nod2 knockout mice not only supported the "working model" above, but demonstrated that an active PepT1/NOD2 signaling pathway was required to aggravate DSS-induced colitis in these animals (Dalmasso et al., 2011). Moreover, antibiotic pretreatment in β-actin-human PepT1 or villin-human PepT1 transgenic mice abolished the inflammatory response to DSS-induced colitis, indicating that commensal bacteria are required to exacerbate intestinal inflammation. The latter finding suggests that modulation of normal gut microbiota, such as with probiotics, may be an effective strategy in treating IBD. Thus, studies in wild-type and IL-10 knockout mice (as a model of colitis) were treated with and without the probiotic *Lactobacillus plantarum* (LP) for four weeks (Chen et al., 2010). It was observed that LP treatment increased the expression of intercellular junction proteins (IJP) but decreased PepT1 protein and transport activity in the colon of IL-10 knockout mice. In addition, treatment with LP decreased bacterial translocation and proinflammatory cytokine production in the null animals. These findings indicate that both IJP upregulation and PepT1 downregulation are beneficial for reducing the pathways by which bacteria or bacteria-derived antigens breach the intestinal epithelium. In contrast, *Citrobacter rodentium*, a murine attaching and effacing pathogen, was shown to induce PepT1 expression in mouse colon (Nguyen et al., 2009). Moreover, β-actin-hPepT1 transgenic mice infected with *C. rodentium* had reduced bacterial colonization, production of proinflammatory cytokines, and neutrophil infiltration of the colon as compared to wild-type animals. Thus, colonic PepT1 expression in response to pathogens might be a host defense mechanism via its ability to modulate bacterial-epithelial

interactions and intestinal inflammation, presumably by interfering with those lipid rafts used as docking sites for pathogens to attack host cells.

Data on the regulation of PepT2, as compared to PepT1, are sparse especially in regard to *in vivo* study conditions. As described earlier, PepT1 exhibited a diurnal rhythm in rat small intestine; however, this phenomenon was not observed for renal PepT1 and PepT2 (Pan et al., 2002). With respect to chronic renal failure (i.e., 5/6 nephrectomy of male rats), PepT2 but not PepT1 protein was upregulated in kidney membranes two weeks after surgery along with enhanced PepT2 transport activity of GlySar in renal brush border membrane vesicles (BBMV) (Takahashi et al., 2001). In contrast, 16 weeks after 5/6 nephrectomy in male rats, both PepT1 and PepT2 transcripts were reduced in kidney as was GlySar transport in renal BBMV (Nakamura et al., 2004). Finally, hypothyroidism induced PepT2 protein expression in the kidney of male rats, however, functional activity was not tested (Doring et al., 2005).

More recently, studies have implicated scaffolding proteins and protein kinases in the regulation of PepT1 and PepT2. Thus, the PDZ domain protein PDZK1 interacted with human PepT2 and enhanced the transport activity of GlySar in co-transfected HEK293 cells (Kato et al., 2004; Noshiro et al., 2006; Sugiura et al., 2006). Moreover, PDZK1 was found to regulate PepT1 activity *in vivo* as demonstrated by the enhanced oral absorption of cephalexin (sampled over 30 min) in wild-type as compared to *Pdzk1* null mice (Sugiura et al., 2008). A study byBoehmer et al. (2008) showed that the protein kinase SGK1 and scaffold protein NHERF2 served as post-translational modulators of PepT2 in which Gly-Gly uptake and protein abundance were enhanced in *Xenopus* oocytes. The glucocorticoid stimulation of PepT1 transport was also dependent upon SGK1 as determined by Gly-Gly jejunal uptake in wild-type and *Sgk1* null mice (Rexhepaj et al., 2009). Finally, pharmacological knockout of PI3K and partial genetic knockout of *Pdzk1* revealed that both protein kinases participated in the regulation of basal PepT1 transport as shown for Gly-Gly in mouse jejunal segments (Rexhepaj et al., 2010). Nevertheless, these findings are limited in scope and the relevance of scaffolding proteins and protein kinases in regulating the *in vivo* physiological, pharmacological and pathological functions of PepT1 (in intestine and kidney) and PepT2 (in kidney and brain) remains to be elucidated.

Finally, the *in vivo* relevance and regulation of peptide/histidine transporters is largely unknown. Still, the expression of PhT1 transcripts was reported to be significantly upregulated in inflamed areas of the colon, but not terminal ileum, of patients with Crohn's disease and ulcerative colitis (Lee et al., 2009). Moreover, using null mice, PhT1 was responsible for the *in vivo* production of cytokines, a process most likely occurring by lysosomal release of tri-DAP, a NOD1-dependent ligand, into the cytosol via PhT1 (Sasawatari et al., 2011). Thus, PhT1, in addition to PepT1, may have a role in IBD. The link to disease and other properties of SLC15 transporters can be found in Table 2.

## 7. human PEPT1-RF: An alternative splice variant?

Saito and co-workers (Saito et al. 1997) isolated cDNA encoding a pH-sensing regulatory factor of oligopeptide transporter (human PEPT1-RF) from the human duodenum library. They suggested this sequence to be a possible alternative splice variant of *SLC15A1* gene, a finding supported byUrtti et al. (2001). However, in the NCBI database the "Gene" human PEPT1-RF (Accession AB001328) is not listed as splice variant of *SLC15A1*, which calls into question this designation. Indeed, this transcript contains several mismatches like insertion/deletions, especially within a region which corresponds in the reference genomic sequence of *SLC15A1* to a large exon. In addition, it includes non-consensus splice sites and lacks multiple consecutive internal exons compared to the majority of publicly available sequence data. The sequence of AB001328 also contains an interspersed repeat (Alu

element) beginning around position 1070, which is not existent in the *SLC15A1* genomic sequence. Furthermore, the coding region contains a stop codon significantly upstream of the last splice junction, a hallmark of candidates for "nonsense-mediated mRNA decay" (NMD). Given the absence of supportive evidence for this transcript (and its predicted protein), no reference sequence (RefSeq) has been created for it by NCBI and it is, at the present time, not officially considered as a splice variant of *SLC15A1*.

## 8. Conclusions and perspective

Substantial progress has been made in understanding the role and relevance of SLC15 mammalian transporters since the previous review by Daniel and Kottra (2004), as part of the mini-review series covering all SLC families (Hediger et al., 2004). In particular, *in vivo* studies have validated many of the prior *in vitro* and *in situ* results demonstrating the importance of PepT1 and PepT2 in nutrition, pharmacologic response, and disease progression. Moreover, our knowledge of PepT1 and PepT2 regulation, from a molecular perspective, has been greatly enhanced, albeit, incomplete. Notwithstanding notable accomplishments, especially in PepT pharmacogenomics and the development of mouse knockout models, major gaps still exist. For example, the basolateral transporter(s) responsible for cellular efflux of peptides/mimetics into blood have not been cloned and, unfortunately, we are no further along in understanding this process. Although a significant discovery was made by determining the crystal structure of a prokaryotic peptide transporter, $PepT_{So}$, the development of a 3D crystal structure of mammalian PepT1 (and PepT2) will be critical to advance pharmaceutical drug design of new chemical entities with enhanced delivery and targeting properties. Another significant gap includes the complex interrelationship of gut microflora, host genetics, and the aberrant expression and polymorphism of PepT1 (and perhaps PhT1) in IBD progression. In addition to disease progression, the aberrant expression of colonic PepT1 offers a strategy for targeting antiinflammatory peptides/mimetics as well as blocking the uptake of harmful chemotactic bacterial products. It can also be argued that inflammation of the gut may have similarities to that in lung and brain, thereby, making PepT2 a viable target for therapy in these organs. Finally, a potential role of PhT1 in IBD and the availability of PhT1 null mice should stimulate a renewed interest in peptide/histidine transporters in general. These "less appreciated" transporters might have an important role in processing lysosomal peptides produced from gut bacteria along with currently unknown roles in other tissues.

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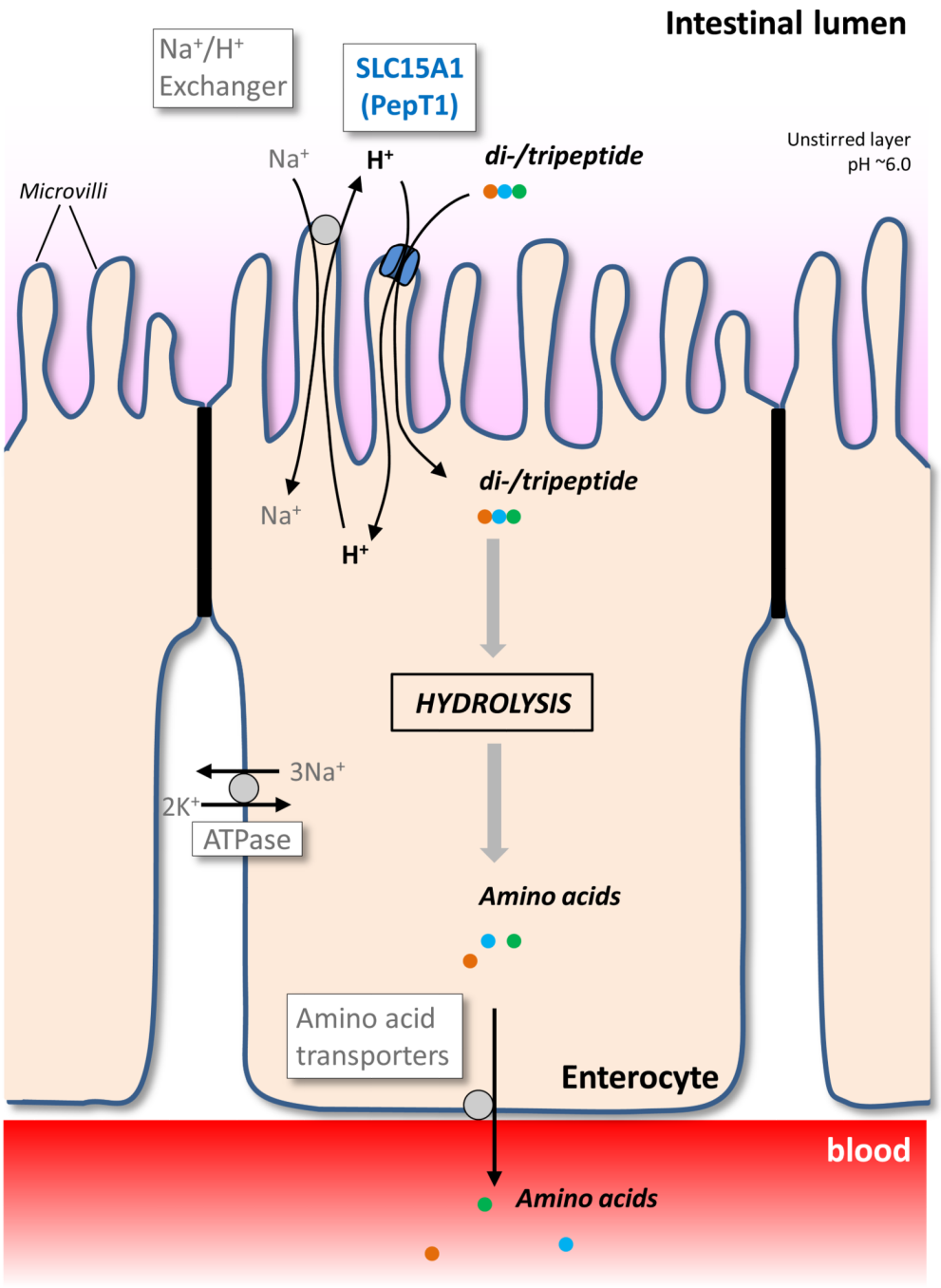
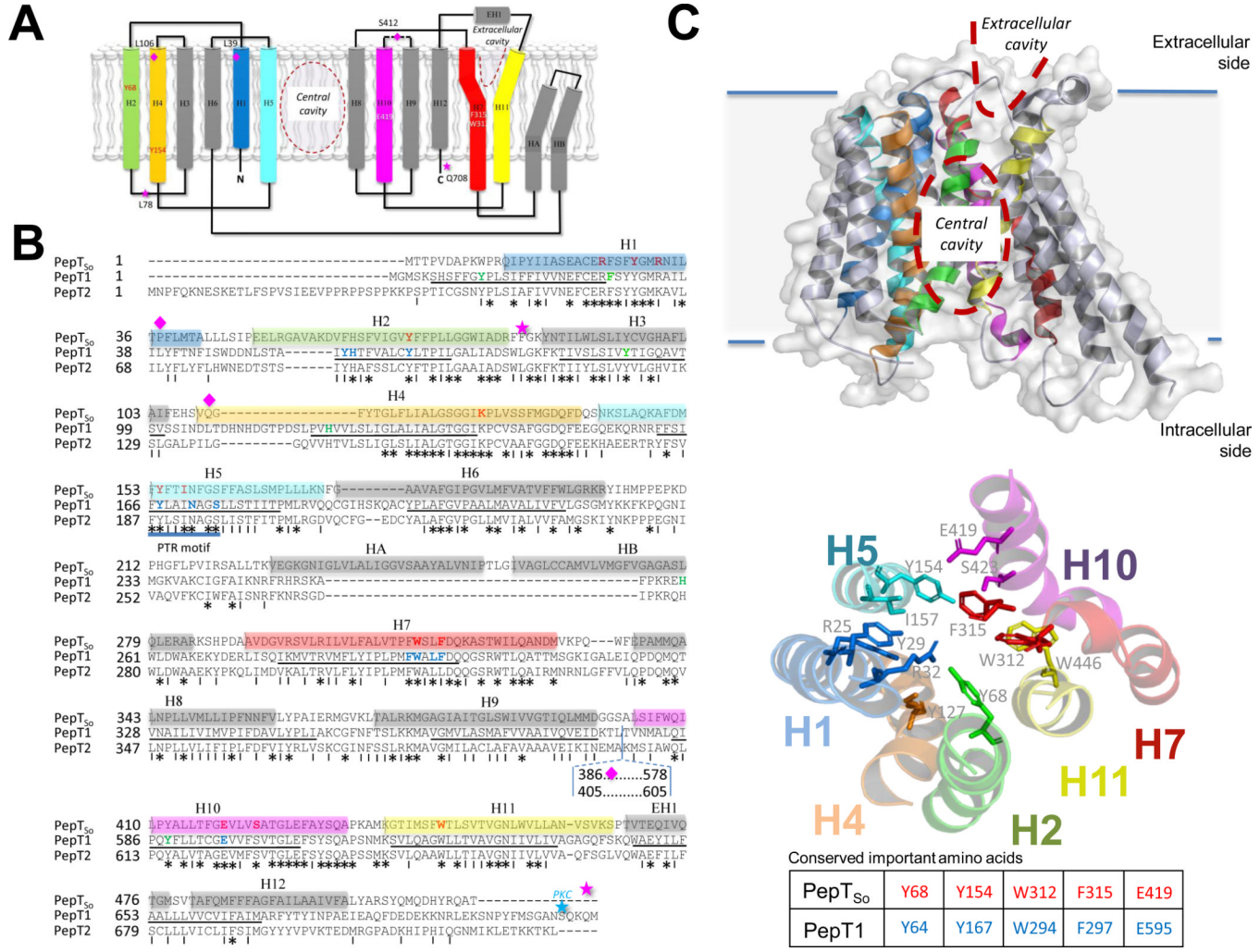



**Figure 1.**
Schematic model of peptide transport in epithelial cells from intestine. Di- and tripeptides are the natural substrates co-transported with protons by peptide transporter isoform 1 (SLC15A1) across the apical epithelial membrane of enterocytes. The activity of the peptide transporter depends upon the electrochemical proton gradient, partly established by the apical $Na^+$, $H^+$ -exchanger, which depends upon the activity of the basolateral $Na^+$, $K^+$ ATPase. Rapidly, di- and tripeptides are hydrolyzed in the cytosol, and free amino acids are released into the blood stream via different types of amino acid transporters located in the basolateral membrane.




| | | | | | |
|---|---|---|---|---|---|
| $PepT_{So}$ | Y68 | Y154 | W312 | F315 | E419 |
| PepT1 | Y64 | Y167 | W294 | F297 | E595 |

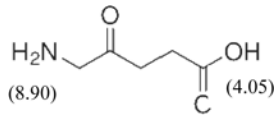
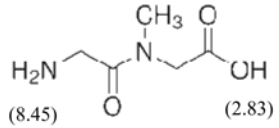
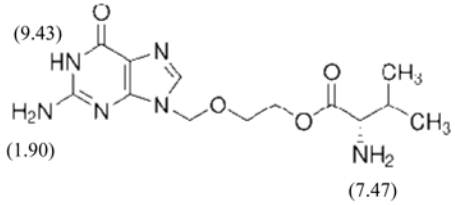
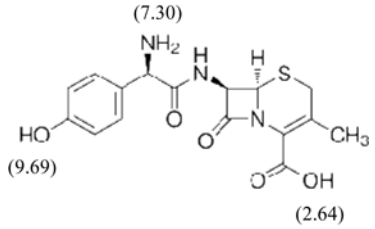
**Figure 2.**

Putative di- and tripeptide binding site of a PepT homologue. **A.** *Schematic topology of the S. oneidensis* $PepT_{So}$ (adapted from Newstead et al., 2011). This representation is based on the 3D structure (PDB ID: 2XUT) as template. There are 14 transmembrane domains (TMD): 12 transmembrane helices (H1 to H12), 2 membrane helices (HA and HB). The peptide binding site domain is colored in blue (H1), green (H2), orange (H4), cyan (H5), red (H7), purple (H10) and yellow (H11), in contrast to the rest of the protein (in grey). The results of labeling experiments using residue accessibility with antibodies are also shown. *Purple diamonds* refer to labeling with antibodies in the absence of permeabilization and *purple stars* refer to labeling after permeabilization. The position of peptide and $H^+$ binding sites are indicated by the central cavity and the extracellular cavity, respectively. **B.** *Amino acid sequence alignment of peptides transporters*. The three sequences were aligned using the Clustal W 2.1 program: bacteria ($PepT_{So}$, Uniprot ID: Q8EKT7), *Homo sapiens* isoform 1 (PepT1, Uniprot ID: B2CQT6) and *Homo sapiens* isoform 2 (PepT2, Uniprot ID: Q16348). Background color code is the same as in A. The putative protein kinase C (PKC) phosphorylation site is denoted by a *blue star*. Identical residues are indicated by asterisks (*) and homologous ones by black sticks (|). Amino acids involved in the binding site are colored in red in $PepT_{So}$ sequence. Amino acids of human PepT1 colored in *blue* are essential to the transport function. The *green* ones play a role in the substrate affinity. Underlined sequences of human PepT1 correspond to the predicted transmembrane

segments according toChen et al. (2000). **C.** *3D structure of* $PepT_{So}$. The predicted structure was visualized by PyMOL v0.99 software in cartoon representation (above) with surface transparency. Helices involved in the binding site are represented using the same color code as in A. The upper cross section (below) allows evidencing the spatial localization of residues involved in the peptide binding site.

**Table 1**

Properties of representative PepT1 substrates in wild-type (WO) and knockout (KO) mice

| Substrate | Classification | MW | pKa (Values)[a] | WT Peff (x $10^{-4}$ cm/s)[b] | KO Peff (x $10^{-4}$ cm/s)[e] |
|---|---|---|---|---|---|
| 5-Amino-levulinic acid | Non-Peptide Mimetic | 131 |  | 2.00 | 0.20 |
| Glycyl-sarcosine | Synthetic Dipeptide | 146 |  | 1.70 | 0.15 |
| Val-acyclovir | Peptide-Like Prodrug | 324 |  | 1.68 (1.66)[c] | 0.05 |
| Cef-adroxil | Peptide-Like Drug | 363 |  | 0.81 (1.56)[d] | 0.09 |

[a] pKa values were reported for 4-aminolevulinic acid (Product Information, Sigma-Aldrich, St. Louis, MO), glycylsarcosine (Irie et al., 2005), valacyclovir (Balimane and Sinko, 2000) and cefadroxil (Tsuji et al., 1981).

[b] Peff is the jejunal permeability of 4-aminolevulinic acid (Xie et al., 2012), glycylsarcosine (Wu et al., 2009), valacyclovir (Yang et al., 2010) and cefadroxil (Posada and Smith, 2011b) in wild-type mice as determined by *in situ* single-pass perfusions.

[c] Peff is the jejunal permeability of valacyclovir in humans as determined by *in vivo* single-pass perfusions (Lennernäs, 2007).

[d] Peff is the jejunal permeability of a similar aminocephalosporin, cephalexin, in humans as determined by *in vivo* single-pass perfusions (Lennernäs, 2007).

[e] Peff is the jejunal permeability of 4-aminolevulinic acid (Xie et al., 2012), glycylsarcosine (Wu et al., 2009), valacyclovir (Yang et al., 2010) and cefadroxil (Posada and Smith, 2011b) in *Slc15a1* knockout mice as determined by *in situ* single-pass perfusions.

**Table 2**

SLC15 - Proton oligopeptide cotransporter family

| Human gene name | Protein name | Aliases | Predominant substrates | Transport type / coupling ions | Tissue distribution and cellular / subcellular expression | Link to disease | human gene locus | Sequence accession ID |
|---|---|---|---|---|---|---|---|---|
| SLC15A1 | PEPT1 | oligopeptide transporter 1, H+-peptide transporter 1 | di- and tri-peptides, protons, beta-lactam antibiotics | C / H+ | apical surface of epithelial cells from small intestine and kidney; pancreas, bile duct and liver | inflammatory bowel disease (Ser117Asn SNP and colonic upregulation) | 13q32.3 | NM_005073 |
| SLC15A2 | PEPT2 | oligopeptide transporter 2, H+-peptide transporter 2 | di- and tri-peptides, protons, beta-lactam antibiotics | C / H+ | apical surface of epithelial cells from kidney and choroid plexus; neurons, astrocytes (neonates), lung, mammary gland, spleen, enteric nervous system | Lead exposure (*2 haplotype associated with higher blood lead burden in male children) | 3q21.1 | NM_021082 |
| SLC15A3 | PHT2 | peptide/histidine transporter 2, PTR3 | di- and tri-peptides, protons, histidine | C / H+ | lung, spleen, thymus, intestine (faintly in brain, liver, adrenal gland, heart) | | 11q12.2 | NM_016582 |
| SLC15A4 | PHT1 | peptide/histidine transporter 1, PTR4 | di- and tri-peptides, protons, histidine | C / H+ | brain, eye, intestine (faintly in lung and spleen) | inflammatory bowel disease (colonic upregulation) | 12q24.32 | NM_145648 |