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SYMPOSIUM REVIEW

Transcriptional and functional regulation of the intestinal peptide transporter PEPT1

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Abstract Dietary proteins are cleaved within the intestinal lumen to oligopeptides which are further processed to small peptides (di- and tripeptides) and free amino acids. Although the transport of amino acids is mediated by several specific amino acid transporters, the proton-coupled uptake of the more than 8000 different di- and tripeptides is performed by the high-capacity/low-affinity peptide transporter isoform PEPT1 (SLC15A1). Its wide substrate tolerance also allows the transport of a repertoire of structurally closely related compounds and drugs, which explains their high oral bioavailability and brings PEPT1 into focus for medical and pharmaceutical approaches. Although the first evidence for the interplay of nutrient supply and PEPT1 expression and function was described over 20 years ago, many aspects of the molecular processes controlling its transcription and translation and modifying its transporter properties are still awaiting discovery. The present review summarizes the recent knowledge on the factors modulating PEPT1 expression and function in *Caenorhabditis elegans*, *Danio rerio*, *Mus musculus* and *Homo sapiens*, with focus on dietary ingredients, transcription factors and functional modulators, such as the sodium–proton exchanger NHE3 and selected scaffold proteins.

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Abbreviations AARE, amino acid responsive element; NHE3, sodium–proton exchanger 3; NHERF, sodium–proton exchanger regulation factor; PDZ, PSD95-disc large-ZO1; PEPT, di- and tripeptide transporter; PPAR, peroxisome proliferator-activated receptor; RNAi, RNA interference; SLC, solute carrier.

Introduction

The digestion of dietary proteins in the intestinal lumen results in the release of free amino acids and small peptides. Although the transport of amino acids via the enterocyte plasma membrane is mediated by several classes of amino acid transporter (for a review, see Broer, 2008), the transport of di- and tripeptides is

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driven solely by the intestinal peptide transporter isoform PEPT1. PEPT1 (SLC15A1; SLC, solute carrier) is a proton-dependent peptide transporter and belongs to the SLC15 family of membrane transporters, which also includes the renal peptide transporter isoform PEPT2 (SLC15A2) and two PHT proteins that transport histidine and selected peptides (Daniel & Kottra, 2004). The electrochemical proton gradient across the membrane allows the uptake of di- and tripeptides against a concentration gradient, enabling higher intracellular than extracellular peptide concentrations, and is strictly dependent on the function of the sodium–proton exchanger NHE3 in the apical membrane (Kennedy *et al*. 2002; Watanabe *et al*. 2005). With their proton gradient-dependent uptake mechanism, peptide transporters belong to the group of 'archaic' transporters. They are present in all living organisms and developed early in evolution. Various isoforms are present in the cell membranes of prokaryotes and simple eukaryotic cells (Daniel *et al*. 2006). In multicellular eukaryotes with a defined tissue distribution, the separation into the intestinal PEPT1-like form and the renal PEPT2-like form can be found from worms (*Caenorhabditis elegans*) (Fei *et al*. 1998; Meissner *et al*. 2004) to fish (*Danio rerio*, *Gadus morhua*) (Verri *et al*. 2003; Ronnestad *et al*. 2007) and birds (*Gallus gallus domesticus*) (Chen *et al*. 2002) up to mammals (*Oryctolagus cuniculus*, *Mus musculus*, *Homo sapiens*) (Fei *et al*. 1994, 2000*b*; Liang *et al*. 1995). Both PEPT isoforms have a broad substrate pattern that includes mostly all di- and tripeptides formed from L-alpha amino acids, as well as a large variety of derivatives, including drugs such as beta-lactam antibiotics, selected angiotensin-converting enzyme inhibitors, protease inhibitors and antivirals (for a review, see Rubio-Aliaga & Daniel, 2008). As a result of their role in mediating the absorption and bioavailability of drugs, they are of considerable importance for pharmacology. Therefore, detailed knowledge about the mechanisms modulating PEPT1 expression and function will enhance its potential in clinical aspects of nutritional and drug therapy. The present review focuses on the known transcriptional, translational and post-translational regulatory mechanisms for the intestinal peptide transporter PEPT1 and summarizes data from studies in various organisms.

PEPT1 regulation by nutrient supply

Historical background. The daily absorption of amino acids, carbohydrates and fatty acids from the diet provides the nutritional requirements for the survival of an organism. During evolution, often parallel uptake routes for a macronutrient were developed to enable compensation for the loss of one transporter by another. Fatty acid uptake via the intestinal plasma membrane, for example, is mediated by various fatty acid transporters and, in the case of a high dietary fatty acid concentration, is performed by a proton gradient-dependent 'flip–flop' mechanism (Hamilton, 1998). For a long time, it was believed that proteins were completely hydrolysed to free amino acids in the intestinal lumen and that their absorption was mediated by various amino acid transporters. However, in the 1970s, studies revealed that small peptides of two and three amino acids in length (di- and tripeptides) were the main product of intestinal protein digestion (Adibi & Mercer, 1973), which initiated the exploration of the corresponding transport system. About 20 years later, the successful cloning of the intestinal di- and tripeptide transporter of rabbit (Boll *et al*. 1994; Fei *et al*. 1994), human (Liang *et al*. 1995), rat (Saito *et al*. 1995), worm (Fei *et al*. 1998) and mouse (Fei *et al*. 2000*b*) opened up a new field in macronutrient physiology.

It is well known that intestinal anatomy and the overall expression and function of nutrient transporters in enterocytes are triggered by the dietary composition and nutrient supply. As amino acids are building blocks for cellular peptides and proteins, the efficient uptake of amino acids in the form of di- and tripeptides via PEPT1 is tightly linked to the luminal peptide/amino acid concentration. In 1998, Walker and colleagues reported a peptide-mediated increased Pept1 mRNA transcription in human colon carcinoma Caco-2 cells (Walker *et al*. 1998). In this context, protein-rich diets (>20% casein) and supplementation with the dipeptide glycyl-phenylalanine or the amino acid phenylalanine more than doubled the mRNA/protein expression and transport function of PEPT1 in rats (Shiraga *et al*. 1999). In addition, a low supply of PEPT1 substrates in a short-term fasting state also enhances the expression and membrane abundance of their intestinal transporter, as observed in rats (Ogihara *et al*. 1999; Thamotharan *et al*. 1999; Ihara *et al*. 2000), mice (Ma *et al*. 2012), chicken (Madsen & Wong, 2011) and human volunteers (Vazquez *et al*. 1985). These controversial regulations can be explained by the fact that, during short-term fasting, the intestine is prepared for efficient di- and tripeptide transport in the refeeding phase. The increased intestinal PEPT1 expression in a fasting state is dependent on the peroxisome proliferator-activated receptor alpha (PPARalpha). In fasted PPARalpha-deficient mice, the PEPT1 protein expression was unchanged when compared with *ad libitum*-fed animals (Shimakura *et al*. 2006*a*). These studies have identified the products of protein digestion as metabolic signals for modulation of Pept1 mRNA transcription/stability and of the intracellular trafficking of the PEPT1 protein.

Current results in *C. elegans* and human colon cells. Based on these findings and on the knowledge that the intracellular amino acid pool is also dependent on the peptidase-driven breakdown rate of di- and tripeptides, further research was performed. In 2001, Wenzel and colleagues demonstrated in Caco-2 cells that PEPT1-driven peptide uptake loads the cells with small peptides which are hydrolysed to amino acids, which, in turn, trans-stimulate the uptake of essential amino acids (L-arginine, L-lysine) via the transport system $b^{0,+}$ (Wenzel *et al*. 2001). These mechanisms were dependent on intracellular peptide hydrolysis, as no trans-stimulation was detectable in the presence of the aminopeptidase inhibitor amastatin. However, the impact of amastatin on PEPT1 function was not analysed. For *in vivo* studies, we chose the nematode *C. elegans*, which expresses three peptide transporter isoforms: the intestinal high-capacity/low-affinity PEPT-1 (K04E7.2, formerly OPT-2, PEP-2), the broader expressed (excretory cells, muscles) PEPT-2 (C06G8.2, formerly OPT-1, PEP-1) (Meissner *et al*. 2004) and the neuronal PEPT-3 (F56F4.5, formerly OPT-3), which mainly acts as a proton channel (Fei *et al*. 2000*a*). In a recent study, we demonstrated that RNA interference (RNAi) gene silencing (Fraser *et al*. 2000) of two predicted cytosolic peptidases, ZC416.6 and R11H6.1/PES-9, was sufficient to reduce intestinal *C. elegans* PEPT-1 expression and function (Benner *et al*. 2011). ZC416.9 is an orthologue of the mammalian bifunctional leukotriene A4 hydrolase/aminopeptidase LTA4H, but, as *C. elegans* lacks leukotrienes (Morgan *et al*. 2005), ZC416.6 may act exclusively as an aminopeptidase in the nematode. R11H6.1/PES-9 is structurally related to the mammalian cytosolic dipeptidase CNDP2. As both mammalian peptidases LTA4H and CNDP2 are sensitive to the aminopeptidase inhibitors bestatin (Davies *et al*. 2009) and amastatin (Daniel & Adibi, 1994), the impact of both compounds on *C. elegans* PEPT-1 function was analysed. By taking into account that bestatin itself is a PEPT1 substrate and to exclude competition at the binding site of the transporter, bestatin concentrations lower than 0.1 mM were used. Both inhibitors reduced *C. elegans* PEPT-1 activity in a concentration-dependent manner without changing its mRNA or protein abundance, indicating that both peptidases modulate the intracellular amino acid pool, which, in turn, affects the transport capacity of *C. elegans* PEPT-1. To test whether the mechanism is also conserved in mammals, LTA4H and CNDP2 gene silencing by siRNA was performed in human colon carcinoma Caco-2 cells and resulted in a significantly reduced PEPT1 protein expression (Benner *et al*. 2011). These results support the finding that the intracellular amino acid pool, modulated either by changes in the extracellular peptide supply or by a reduced intracellular peptide hydrolysis, is an evolutionarily conserved key regulator for the intestinal peptide transporter PEPT1.

Transcription factors regulating pept1 gene expression

Although studies on the regulation of Pept1 gene expression in mammals by dietary factors have been known for several years, studies on the underlying molecular mechanisms are limited. Shiraga *et al*. (1999) found that selected amino acids (including leucine and phenylalanine) can control the promoter activity of rat-Pept1 via the amino acid responsive element (AARE). The AARE-like motif in the rat-PEPT1 promoter is located between −277 and −271 bp (5′-CATGGTG-3′) upstream of the start codon and shows high homology to the AARE motif from asparagine synthetase. Furthermore, the authors described a predicted Cdx2 binding site around 640 bp upstream of the start codon in the antisense direction. The systematic analysis of the mouse-Pept1 promoter revealed the relevant cis- and trans-elements within 1140 bp upstream of the transcription start site, including three GC boxes for the binding of the transcription factor Sp1 (Fei *et al*. 2000*b*). This regulatory mechanism is also conserved in humans, where the basal human-Pept1 gene expression is regulated by Sp1 (Shimakura *et al*. 2005) (summarized in Table 1). The direct interaction of Sp1 with two GC boxes in the first 170 bp upstream of the start codon highlights its role in mammalian Pept1 expression. One year later, the same group reported that the intestine-specific expression of Pept1 is controlled by Cdx2. However, the human-Pept1 promoter lacks typical binding motifs for Cdx2. Although the binding site of Cdx2 is not yet known, further studies have uncovered that Cdx2 binding is dependent on Sp1 (Shimakura *et al*. 2006*b*) and on butyrate (Dalmasso *et al*. 2008). In 2008, Inui and colleagues found a circadian regulation of rat-Pept1 transcriptional activation by the clock gene-controlled protein albumin D site-binding protein, which binds to its corresponding binding motif in the distal promoter (Saito *et al*. 2008). These binding motifs were established late in evolution, as the *C. elegans pept-1* core promoter (600 bp upstream of the transcriptional start site) lacks Sp1- and Cdx2-related binding sites, and, in a recent study, we found that knockdown of the homologous genes had no impact on *C. elegans* PEPT-1 protein expression. However, in *C. elegans*, the FOXO transcription factor DAF-16, the key transcription factor of the insulin/insulin growth factor-like signalling pathway, was predicted to be a repressor of *pept-1* gene expression (Meissner *et al*. 2004).

PEPT1 regulation by modification of the transmembrane proton flux and the intracellular pH

The function of PEPT1 is strictly dependent on the transmembrane proton gradient in combination with an inside-negative membrane potential. Both factors provide

Table 1. Overview of selected factors modulating the gene and/or protein expression and function of the intestinal peptide transporter PEPT1

Modulator	Effect on PEPT1	Kind of modulation	References
Amino acids leucine and phenylalanine	Control of pept1 promoter activity via the AARE in rats	Transcriptional	Shiraga *et al*. (1999)
Transcription factor SP1	Binds to the PEPT1 promoter and modulates the basal pept1 transcription	Transcriptional	Shimakura *et al*. (2005)
Transcription factor Cdx2	Modulates pept1 transcription in concert with SP1 and with butyrate in mammals	Transcriptional	Shimakura *et al*. (2006*b*) Dalmasso *et al*. (2008)
Transcription factor DAF-16	Represses pept1 transcription in *Caenorhabditis elegans*	Transcriptional	Meissner *et al*. (2004)
Peptidase inhibitors amastatin and bestatin	Decrease intracellular peptide hydrolysis and reduce pept1 function in *C. elegans*	Functional	Benner *et al*. (2011)
RNA interference of intracellular peptidases	Reduced protein expression and function of PEPT1 in Caco-2 cells and *C. elegans*	Translational, functional	Benner *et al*. (2011)
Sodium–proton exchanger NHE3/NHX-2	Proton export is necessary for correct function of PEPT1 in mammalian cells and in *C. elegans*	Functional interaction via proton gradient (protein–protein interaction not yet proven)	Kennedy *et al*. (2002) Watanabe *et al*. (2005) Pieri *et al*. (2010) Benner *et al*. (2011)
Scaffold protein PDZK1	Interacts with mouse PEPT1, trafficking to and anchoring in the plasma membrane	Direct protein–protein interaction	Sugiura *et al*. (2008)

AARE, amino acid responsive element; PDZ, PSD95-disc large-ZO1.

a powerful driving force for the proton-coupled transport of di- and tripeptides into enterocytes. In the presence of the dipeptide glycyl-sarcosine, a decrease in the intracellular pH was detectable in enterocytes of wild-type mice, which was absent in PEPT1-deficient mice, indicating the acid loader function of PEPT1 (Chen *et al*. 2010). To avoid acidification of the cells, Na^+/H^+ exchangers mediate the electroneutral efflux of protons into the gut lumen in exchange with extracellular Na^+, which leaves the cell via the Na^+/K^+-ATPase at the basolateral side. It has been demonstrated in mammalian cell models that NHE3 activity is required for the correct function of PEPT1 in the intestinal epithelium (Kennedy *et al*. 2002; Watanabe *et al*. 2005; Pieri *et al*. 2010), whereas the function of the renal form PEPT2 is dependent on NHE1 and/or NHE2 (Wada *et al*. 2005). These processes are evolutionarily conserved, and the essential action of NHE3 in the mammalian enterocytes has already been established in the *C. elegans* intestine by its orthologue NHX-2 (Nehrke, 2003). We showed *in vivo* that RNAi gene silencing of NHX-2 in *C. elegans*, which results in a moderate but significant decrease in intracellular pH (Nehrke, 2003), leads to reduced protein expression and function of *C. elegans* PEPT-1 (Benner *et al*. 2011) (Fig. 1). Furthermore, both were found to alter other central proton-dependent nutrient transport processes in nematodes. As PEPT1-deficient *C. elegans* accumulates large quantities of body fat (Brooks *et al*. 2009; Spanier *et al*. 2009) and *nhx-2(RNAi) C. elegans* is extremely lean (Nehrke, 2003), a general impact of PEPT1 and NHX-2 function on fatty acid uptake was predicted. Indeed, part of the cellular fatty acid uptake is mediated via a pH-dependent mechanism called the 'fatty acid flip–flop' (Hamilton, 1998). Uncharged fatty acids enter the phospholipid bilayer of the plasma membrane and move to the cytosol by releasing a proton. An increase in intracellular pH, as in nematodes with reduced PEPT1 expression and function, supports the flip–flop mechanism and induces fat accumulation. In contrast, a reduced NHX-2 function slows down the 'fatty acid flip–flop' as a result of a decrease in the intracellular pH, finally leading to lean nematodes (Spanier *et al*. 2009) (Fig. 2).

Diet-dependent phenotypic alterations in PEPT1-deficient mice

Although the loss of the intestinal peptide transporter induces severe phenotypic alterations in *C. elegans*, the phenotype of PEPT1-deficient mice is no different from that of wild-type mice when they are fed on a

carbohydrate-rich standard diet (Hu *et al.* 2008). However, when fed a diet containing 45% of its energy as protein, PEPT1 knockout mice were found to show major changes in the plasma amino acid pattern, whereas the pattern was unchanged when the diet contained less than 21% protein (Nassl *et al.* 2011*a*). Therefore, PEPT1 function is essential under nutritional conditions with high protein content, when the amino acid transporters are saturated and the uptake of small peptides offers additional nutrient supply. As a high-protein diet increases satiety and promotes weight loss, it was tested whether the intestinal peptide transporter is involved in these processes. Interestingly, loss of PEPT1 reduced the food intake in the first days on a high-protein diet more severely than in wild-type mice, an effect that might be driven by increased arginine and corresponding low leptin concentrations in plasma (Nassl *et al.* 2011*b*).

To induce obesity in PEPT1-deficient mice, the knockout animals were challenged with a diet containing 48% of its energy as fat. Interestingly, these mice showed a significantly lower weight gain than their wild-type littermates. This can be explained by a reduced energy intake as a result of nutrient maldigestion/malabsorption in the small intestine and a higher energy loss in faeces. Investigating the origin of the impaired nutrient digestion/absorption, we observed that PEPT1-deficient mice lack the diet-induced ability to modify the architecture of the intestinal mucosa. In contrast with wild-type mice, in which a high-fat diet increases villus length and surface area of the upper small intestine, both factors remain unchanged in PEPT1-deficient animals (Kolodziejczak *et al.* 2013). The gut architecture is modified by various factors in mammals and, recently, interleukin-6 has been reported to be an essential growth promotion chemokine that supports villus elongation (Jin *et al.* 2010). However, PEPT1-deficient animals have a markedly lower systemic interleukin-6 concentration than wild-type mice, which finally might result in reduced intestinal surface area. Furthermore, the loss of PEPT1 as cellular acid loader modifies the proton export of NHE3. As NHE3 also contributes to water absorption, PEPT1-deficient mice show reduced water absorption from the small intestine (Chen *et al.* 2010). Although they do not develop diarrhoea, this impairment could also contribute to the diet-insensitive villus architecture, for example via changes in intestinal trans-mural pressure, which has also been shown to affect interleukin-6 secretion (Kishikawa *et al.* 2002). Most interestingly, mice lacking NHE3 have reduced body weight, increased gut length and increased mass of caecum and colon tissue and content (Schultheis *et al.* 1998), and they display, in part, phenotypic features of PEPT1-deficient mice. Based on these observations, we hypothesize that the different phenotypic outcomes with respect to the body fat content in mice and *C. elegans* might be caused by the ability to modify the surface area of their intestinal epithelium and therefore compensate for food energy absorption. Although the surface of the murine intestinal epithelium is highly flexible depending on the diet, the surface of the 20 enterocytes in *C. elegans* is predicted to be stable. Yet, no studies have been published on the modifications of the intestinal microvilli structure and enterocyte surface area of *C. elegans* based on food source and diet composition.

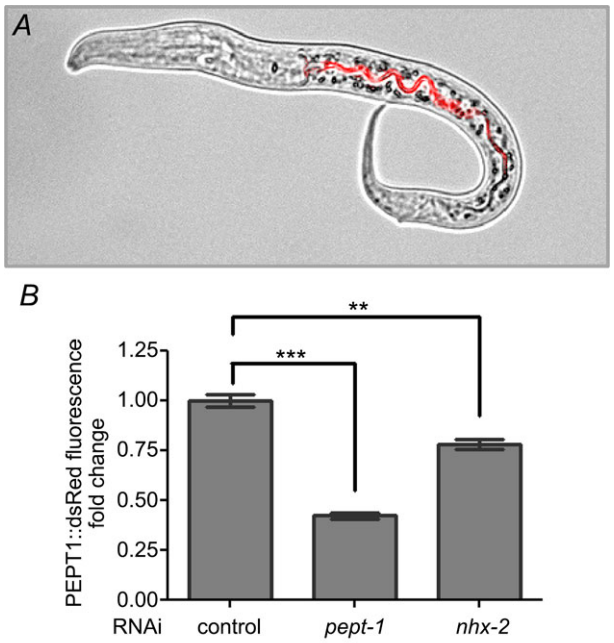

Figure 1
A, representative *Caenorhabditis elegans* MZE91R *(unc-119(ed3) III; cbgIs91[pPept-1:pept-1::DsRed;unc-119(+)]; rrf-3(pk1426) II)* expressing the PEPT1::DsRed fusion protein at the intestinal brush border membrane (red signal). *B*, PEPT1::DsRed expression in MZE91R *C. elegans* after RNA interference (RNAi) gene silencing of *pept-1* and *nhx-2*. The RNAi feeding protocol described in Fraser *et al*. (2000) was used. Controls were fed on bacteria carrying the empty pPD129.36 vector. Values are based on four independent experiments showing mean ± SEM. For statistical analysis, the Kruskal–Wallis test was performed with $^{**}P \leq 0.01$ and $^{***}P \leq 0.001$.

PEPT regulation by PDZ (PSD95-disc large-ZO1) domain proteins

In polarized cells, including all kinds of epithelial cells, the defined localization of membrane proteins is essential for their correct function. Protein–protein interactions with scaffold proteins, which target them to appropriate regions of the plasma membrane and regulate their activity, have been shown for various xenobiotic transporters (Kato *et al.* 2004). However, information on direct protein–protein interactions with peptide transporters is scarce. Both mammalian peptide transporter isoforms

PEPT1 and PEPT2 contain the class I PDZ binding motif S/T-X-Ø (S/T, serine/threonine; X, any residue; Ø, hydrophobic residue) at their C-terminus and are recognized by PDZ domain proteins. The PDZ domain is typical for scaffold proteins and essential for protein–protein contact (Sheng & Sala, 2001). A direct interaction of mouse PEPT1 with the scaffold protein PDZK1 has been reported (Sugiura *et al*. 2008), whereas binding of human-PEPT1 to sodium–proton exchanger regulation factor 1 (NHERF1) and NHERF2 was undetectable (Boehmer *et al*. 2008). However, interaction of human-PEPT2 with PDZK1 (Noshiro *et al*. 2006) and NHERF2 (Boehmer *et al*. 2008) was described. Just recently the homologous gene for the mammalian NHERF family of PDZ domain proteins was found in *C. elegans*. The nematode contains only one NHERF family protein, named NRFL-1 (C01F6.6, formerly TAG-60), which is essential for the anchoring of the amino acid transporter AAT-6 (homologue of the light subunit of heteromeric amino acid transporters) to the apical plasma membrane (Hagiwara *et al*. 2012). Whether *C. elegans* PEPT-1, which contains a predicted class I PDZ binding motif (TFD) at its C-terminus, is

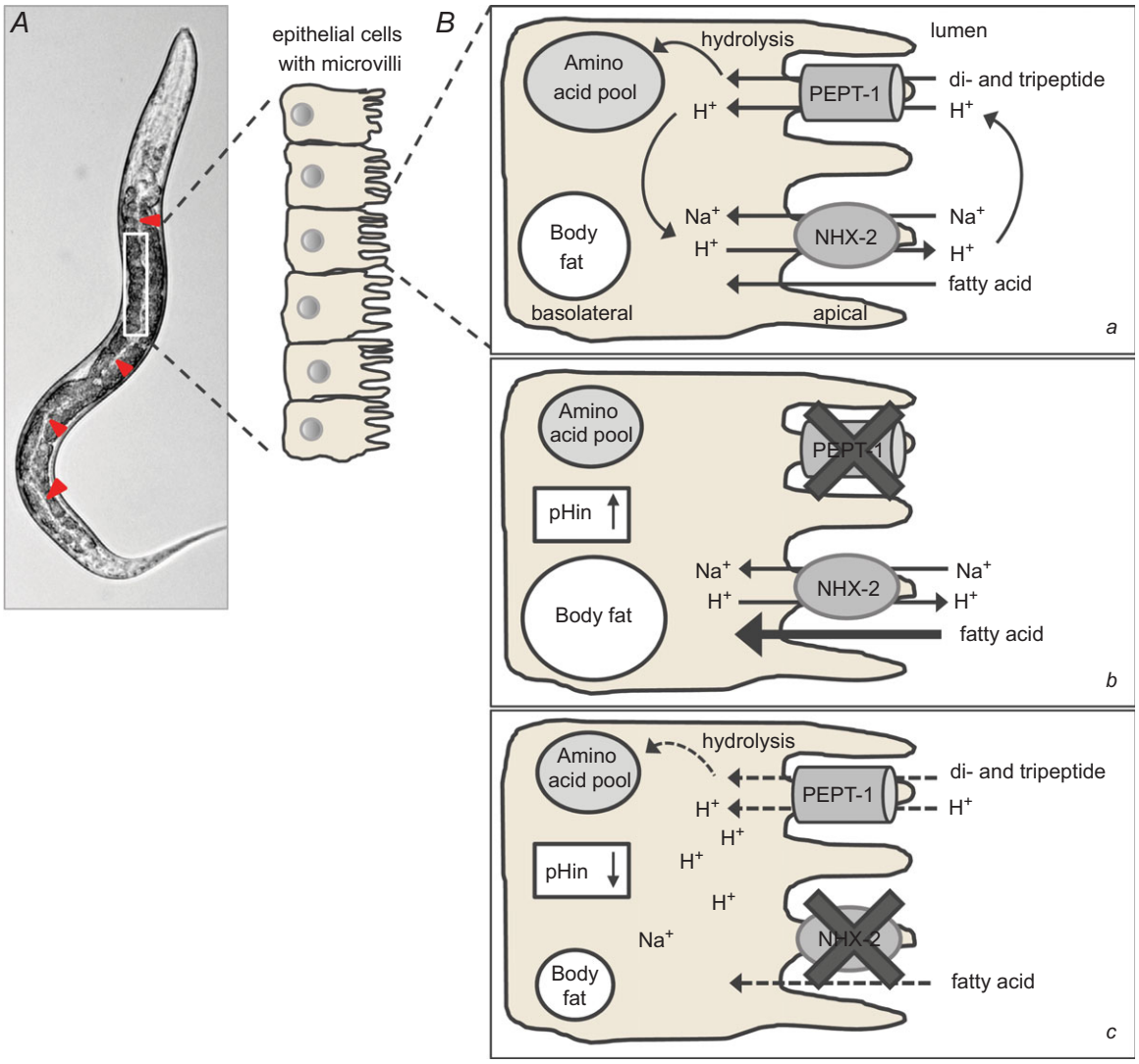

Figure 2
A, image of *Caenorhabditis elegans* and schematic magnification of the intestinal epithelium. The nematode's head faces to the top and arrowheads mark the intestinal lumen. The scheme focuses on epithelial cells with the basolateral side to the left and the apical side to the right side. *B*, interplay of peptide transporter PEPT-1 and sodium–proton exchanger NHX-2 in enterocytes. *Ba*, in the wild-type situation, both proteins are active in the apical membrane and stabilize amino acid homeostasis and intracellular pH (pHin). *Bb*, loss of PEPT-1 protein stops the peptide and proton influx, leading to lower intracellular amino acid concentrations and an increase in pHin. Uptake of fatty acids via the fatty acid flip–flop mechanism is supported and induces obesity. *Bc*, when NHX-2 is missing, proton export stops and pHin decreases. Uptake of fatty acids via the flip–flop mechanism is decreased, leading to nematodes with empty body fat depots.

also an interaction partner of NRFL-1 awaits further analysis.

Concluding remarks

During the last two decades, many factors that directly or indirectly influence the expression and function of the intestinal peptide transporter PEPT1 have been evaluated. Without any doubt, the close cooperation of PEPT1 with the sodium–proton exchanger NHE3 in enterocytes is of high relevance and influences other central nutrient transport processes, such as fatty acid transport via the brush border membrane. However, evidence for direct protein–protein interactions with PEPT1 is scarce. Therefore, future research should focus on the identification of interaction partner proteins to PEPT1 and NHE3 in the apical membrane of enterocytes. Although technically challenging, as the proteins of interest are membrane proteins, this will expand the repertoire of modulators of this nutrient transport system.

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Additional information

Competing interests

No conflicts of interest exist for the author.

Author contributions

BS planned, wrote and revised the manuscript.

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