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## Digesting New Elements in Peptide Transport

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In this issue of *Structure*, [Beale et al. \(2015\)](#) define structurally and functionally a large extracellular domain unique to mammalian peptide transporters and its implications for the transport of basic di- and tri-peptides ([Beale et al., 2015](#)).

In mammals, the uptake of diet-derived di- and tri-peptides, as well as pharmaceutically important drug molecules such as antibiotics and anti-viral medications, is mediated by PepT1 and PepT2, members of the conserved proton-dependent oligopeptide transporter (POT) family. The POT family belongs to the major facilitator superfamily (MFS), members of which contain 12 transmembrane (TM) helices that form two domains each containing six TM helices related by a pseudo two-fold symmetry.

Early crystal structures of bacterial POT members revealed the architecture of the transporter in a number of distinct transport states ([Newstead et al., 2011](#); [Solcan et al., 2012](#)). Significant attention has lately been focused on investigating the substrate binding site promiscuity for both PepT1 and PepT2 and the tailoring of pro-drugs in an effort to improve the uptake of poorly absorbed or retained medications via these transporters (see [Brandsch, 2013](#), for review). These efforts have been complemented by recent structures of bacterial POT homo-

logs in complex with natural and unnatural di- and tri-peptides, revealing at least two binding modes for di- and tri-peptides depending on their amino acid composition ([Doki et al., 2013](#); [Guettou et al., 2014](#); [Lyons et al., 2014](#)). Subsequent thermodynamic measurements on PepT from *Streptococcus thermophilus* supported a two transport mechanism model as underscored by the different measured proton:peptide transport stoichiometries for di- and tri-peptides ([Parker et al., 2014](#)). Together, these results provide a platform from which to also guide pro-drug development.

Comparison of the various peptide-transporter complexes highlight an asymmetry to the domain movements where the transition from the occluded to the inward open state is via bulk movements of the C-terminal domain. This asymmetrical movement of the TM helices of the C-terminal bundle is largely incompatible with the classic rigid-body rocker-switch model of transport as proposed from structural studies on GlpT and LacY

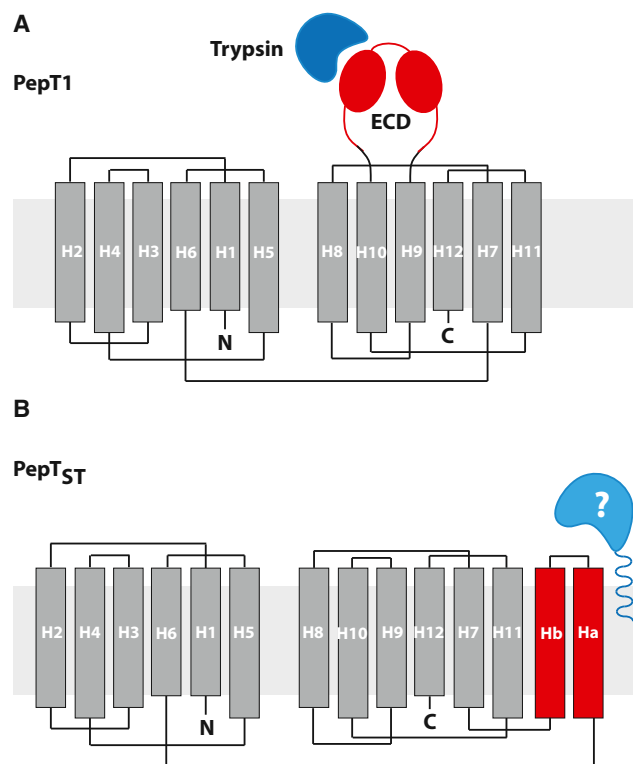
([Abramson et al., 2003](#); [Huang et al., 2003](#)). As an alternative mechanism, it has been postulated that a dynamic movement of helices within the two six-helix bundles is required for the substrate binding site to be alternately accessible to both sides of the membrane ([Fowler et al., 2015](#)). Similar observations were first highlighted for a plant phosphate transporter, where contrary to the peptide transporter, the N-terminal bundle undergoes the analogous movements ([Pedersen et al., 2013](#)).

A significant difference between bacterial, fungal, plant and mammalian peptide transporters has long been known though from topology and sequence analyses that identified the presence of a sizeable extracellular “loop” exclusive to the mammalian transporters. This additional sequence is located between TM helices 9 and 10 of the C-terminal TM bundle and is not assigned with any function. Here, the authors shed light on the structure and function of this extracellular loop, revealing that it is comprised of two consecutive immunoglobulin-like domains

(Figure 1). Of particular note, however, is that the removal of these extracellular domains (ECD) from PepT2 had no significant effect on the core transport activity for peptides or the antibiotic cefaclor. Using surface plasmon resonance and microscale thermophoresis studies to analyze putative interaction partners, the authors found transient binding of the intestinal protease trypsin to the ECD with a micromolar binding constant, high enough to propose a relevant interaction at the significant concentrations of trypsin in the small intestinal mucosa. This binding was attributed to the presence of two highly conserved acidic residues in the ECD, mutations of which abolished trypsin binding.

Based on the transient complex formation between the ECD and trypsin as well as prior reports of trypsin localizing to and binding the mucosa of the small intestine, the authors present an enticing, yet speculative, physiological role for this complex (Beale et al., 2015). They propose that the adaptation to include an ECD to sequester trypsin results in an increase in the local concentration of di- and tri-peptides containing basic residues in the vicinity of the transporter. As the transport of basic peptides by PepT1 is less efficient, this serves also to overcome this hurdle, thus promoting their transport. While the trypsin-ECD interaction has been verified in vitro, in vivo confirmation and analysis of such an interaction will be an important next step in verifying its physiological role. Furthermore, the proposed increase of transport of positively charged peptides due to trypsin will also need to be shown in vitro.

Such a modular adaptation to the classical MFS scaffold supports an intriguing avenue by which evolution adds sophistication to a target protein without adverse



**Figure 1. Schematic Diagrams of Human PepT1 and the *Streptococcus thermophilus* Peptide Transporter PepT<sub>St</sub>**

(A) The mammalian PepT1 contains a large extracellular loop between helices 9 and 10 that Beale et al. (2015) show to consist of two immunoglobulin-fold domains that appear to interact with the intestinal protease trypsin. (B) Bacterial peptide transporters have two additional TM helices inserted between the two 6 TM repeat units that are placed at the periphery of the transporter core. This insert might serve to associate with membrane-bound proteases.

consequences to its core function. Interestingly, the bacterial PepT structures to date contain two TM helices that are inserted between the N- and C-terminal domains, i.e., between TM 6 and 7, and are designated Ha and Hb (Figure 1). These helices, which are unique to bacterial members, form a hairpin in the membrane and are located in the periphery of the MFS fold; their function to-date remains unclear. It is tempting to speculate that these additional helices also serve to associate appropriate membrane-associated or membrane-inserted proteases to the transporter.

In summary, the new structures of the PepT ECDs offer an important extension on the knowledge derived from structural and functional studies on bacterial peptide transporters, thus increasing our

current understanding of peptide and drug uptake in mammals and, in general, how the MFS has adapted to integrate additional structural elements to improve function in eukaryotic systems. Moreover, future studies that aim to establish the physiological role of the trypsin ECD interactions will be of great interest.

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