A POTluck of peptide transporters

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The uptake of diet-derived peptides is mediated by the conserved proton-dependent oligopeptide transporter (POT) family. Crystal structures of bacterial transporters in the inward-open conformation and in an occluded conformation published in *The EMBO Journal* provide structural insight into the proton-driven peptide transport cycle.

Lipid bilayers are generally not permeable to amino acids, peptides and their derivatives. Cells therefore rely on active transporters or facilitators for the uptake and redistribution of these valuable compounds. In the late 1970s, many transporter activities were identified and characterized from brain synaptic or kidney/intestinal brush border membranes, including for example transporters for serotonin, glutamate and alanine (Fass *et al.*, 1977; Rudnick, 1977; Kanner and Sharon, 1978). Their activities were shown to be dependent on the Na⁺ gradient maintained by the Na⁺, K⁺-ATPase, and for a while, an attractive model was that active, secondary transporters of the plasma membrane in animals are driven by the Na⁺ gradient whereas those in plants, fungi and bacteria are coupled to the proton gradient.

This distinction changed when it was shown that transport of a glycyl-L-proline dipetide across rabbit kidney brush border membranes was in fact dependent on a proton gradient (Ganapathy and Leibach, 1983). In 1994, the rabbit PEPT1 protein was successfully cloned, expressed and characterized as the proton-dependent peptide transporter (Fei *et al.*, 1994) responsible of this activity, and it became a founding member of the large proton-dependent oligopeptide transporter (POT) family.

Human PEPT1 is recognized for its key function in the uptake of peptide nutrients from the intestinal tract. However, along with the PEPT2 isoform, it exerts also a range of other important peptide transport functions in various tissues such as kidney, brain, glandular and endocrine tissues and placenta. For a general overview of PEPT1 tissue distribution, see for example <http://www.proteinatlas.org/ENSG00000088386>.

POT proteins are found in eukaryotes and eubacteria, while being surprisingly absent in archaea. In 1989, Konings and coworkers identified an H⁺-dependent transporter for di- and tripeptides in *Lactococcus lactis* (Smid *et al.*, 1989), and many bacterial members of the family have since then been

Figure 1 Alternating access mechanism of the proton-dependent oligopeptide transporter (POT) family. To the right, the PEPT_{St} transporter is shown in the inward-open conformation (Solcan *et al.*, 2012, PDB 4APS), in the middle, the PEPT_{So} in the inward-occluded conformation (Newstead *et al.*, 2011, PDB 2XUT). To the left, another protein of the Major Facilitator Family, the Fucose transporter is shown in the outward-facing conformation (Dang *et al.*, 2010, PDB 3O7Q).

characterized and recognized as members of the POT family, such as the *E. coli* yjdL transporter (Jensen *et al.*, 2011).

For many years, the mechanism of POT proteins, which selectively transport di- or tripeptides of any kind, remained puzzling. Quite clearly, recognition of the N- and C-termini of the peptide, rather than interactions with the side chains, would be expected to determine optimal cargo recognition. Furthermore, the question remained of how transport would be coupled to an H⁺ gradient.

Two reports from the Newstead/Iwata group published in *The EMBO Journal* provide critical new insight into the structure and transport mechanism of the POT family (Newstead *et al.*, 2011; Solcan *et al.*, 2012). Based on a widespread approach in membrane protein crystallography of using bacterial homologues of mammalian membrane proteins, two different bacterial species of POT transporters—the PEPT_{So} from *Shewanella oneidensis* and the PEPT_{St} from *Streptococcus thermophilus* were crystallized and their structures determined at medium resolution. Overall, the POT structure is similar to other proteins of the Major Facilitator Superfamily (MFS) such as the sugar permeases (see Figure 1). A simple mechanistic model has been proposed for MFS proteins, the rocking bundle model. In this model, two related ‘halves’ of the transporter (transmembrane spanning segments 1–6 and 7–12, respectively) form a V-shaped structure with the substrate site in the middle that is exposed to either the outside (uptake) or the inside (release) of the cell via an occluded intermediate state. The proton gradient is thought to stimulate the switch between these two conformations. In real life, the picture is not so simple as seen here for the POT transporters: the first structure, PEPT_{So}, revealed an asymmetric, occluded state with an undefined substrate in a buried cavity. Transition to the inward-open conformation, as revealed from the latest structure and

further supported by e.g. molecular dynamics simulations and mutational studies, shows both rigid-body motions and more flexible changes. A markedly polar environment with charged residues along the transport pathway is the basis for the proton-coupled mechanism, which involves the formation and dissociation of salt bridges to open and close gates to the intracellular and extracellular environment. In the occluded state, substrate binding is mediated by conserved tyrosines and a glutamate in a manner homologous to what has been predicted for PEPT1 and yjdL (Meredith *et al.*, 2000; Jensen *et al.*, 2011).

Besides their critical function as peptide transporters, PEPT1 and 2 are also responsible for the uptake of drugs with peptide-like moieties, such as penicillin antibiotics and PEPT1 and 2 are widely recognized as drug uptake vehicles in drug development (Nielsen and Brodin, 2003). Clearly, we need more detailed information of how specific compounds are recognized and the kinetics of active transport is determined. Furthermore, in agricultural livestock production, the efficiency of protein nutrient uptake is extremely important and relies on the optimal match between uptake kinetics and the available resources of feed (D’Inca *et al.*, 2011). There is no doubt that we wish to see the POTluck increasing from many sides.

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Conflict of interest

The author declares that he has no conflict of interest.

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