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The twisted relation between Pnu and SWEET transporters

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The evolutionary relation between sugar and vitamin transporters from the SWEET and Pnu families is unclear. They have similar 3D structures, but differ in the topology of their secondary structure elements, and lack significant sequence similarity. Here we analyze the structures and sequences of different members of the SWEET and Pnu transporter families and propose an evolutionary pathway by which they may have diverged from a common ancestor. A 3D domain swapping event can explain the topological differences between the families, as well as the puzzling observation that a highly conserved and essential sequence motif of the SWEET family (the PQ loop) is absent from the Pnu transporters.

Structural similarity between membrane transporter families

With the increasing number of available crystal structures, it has become apparent that many different families of membrane transporters share structural similarities even though they are not related in sequence. This observation raises a fundamental question about their evolution: did the folds arise from divergent or convergent evolution? (see Glossary) [1]. A prominent example is the LeuT fold, which is adopted by many different protein families [2–9] that lack significant sequence similarity. It is possible that the structural similarity is the result of convergent evolution of unrelated proteins to a fold that is particularly suited for membrane transport. However, in this case divergent evolution is usually assumed because the presence of both structural and functional similarity is a considered strong indication for homology even in the absence of sequence similarity [10–12].

Bacterial SemiSWEET and Pnu transporters also lack obvious sequence similarity but recent crystal structures have revealed that they share a novel fold [13–16]. In addition, they are both facilitators and catalyze similar translocation processes, arguing for homology. Yet, they contain unique topological differences, which must be accounted for in any putative divergent evolutionary pathway. Here we provide an analysis of the structures of the Pnu and SWEET transporter families and suggest an evolutionary pathway that could relate them by divergence.

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We stress that an alternative scenario, which would relate Pnu and SWEET transporters by convergent evolution cannot be excluded. Hypotheses on the evolutionary relation between proteins lacking significant levels of sequence similarity are always somewhat speculative because there is no widely accepted approach that satisfactorily addresses the likelihood of analogy between protein families sharing the same fold [10,11]. The analysis presented here is not intended to rule out a relation by convergence but rather aims to demonstrate the possibility of an evolutionary relation between Pnu and SWEET transporters based on homology.

The SWEET and Pnu folds are similar

SWEET transporters (Sugars Will Eventually be Exported Transporters) are low-affinity sugar transporters classified into the SLC 50 family [17]. They mediate the facilitated diffusion of substrates down their concentration gradients across cell membranes [18]. Plants encode numerous homologous SWEET transporters [19] involved in processes such as phloem loading [18], nectar secretion [20], and nutrient sequestration by plant pathogens [19]. Humans contain only one SWEET gene, which might code for a glucose transporter in the basolateral membrane of enterocytes in the intestine [17]. SWEET transporters consist of seven α-helical transmembrane segments (TMs), organized in a 3+1+3 membrane topology, where the two

Glossary

Analogy: if two similar traits are derived from convergent evolution, they are analogous.
Circular permutation: circular permutation is the rearrangement of an amino acid sequence, when old N- and C termini become linked, and new N- and C termini formed.
Convergent evolution: leads to the manifestation of similar design and function in two traits that have different, unrelated ancestors.
Divergent evolution: leads to the establishment of similar traits by independent evolution from a common identical ancestor. For example, the mutations in related protein sequences in two different organisms lead to divergence, while retaining similarity.
Domain swap: 3D domain swapping is a mechanism for two or more protein molecules to form a dimer or higher oligomer by exchanging an identical structural element ('domain').
Homology: two traits that share a common ancestor are homologous.
Membrane topology: is more narrowly defined than protein topology as the number of transmembrane segments in membrane embedded proteins and the location of their N- and C termini.
Parallel evolution: is the development of new traits of similar design and function by natural selection from different ancestors, which themselves are similar and related by homology.
Protein topology: the topology of a protein describes the spatial arrangement of structural units (for instance beta strands and alpha helices) and the chain connectivity among them.

bundles of three TMs are related in sequence. Bacterial members of the SWEET transporter family are invariably homodimers of half-transporter molecules consisting of three TMs termed SemiSWEETs [21]. SWEETs and SemiSWEETs are also referred to as PQ loop transporters, because they belong to a large superfamily of transporters also including the MtN3 (Medicago truncatula nodulin gene 3) and saliva families, bearing a highly conserved proline-glutamine motif [22]. The crystal structures of four different SemiSWEET proteins have recently been determined (from *Leptospira biflexa*, *Vibrio* sp. N418, *Thermodesulfovibrio yellowstonii*, and *Escherichia coli*) [13,14,16]. The proteins form stable homodimers of a protomer that folds into a compact three-helix bundle (Figure 1A). The two three-helix bundles are related to each other by a two-fold rotational axis perpendicular to the plane membrane. The six TMs in the complex are positioned roughly on the corners of a hexagon and create a putative translocation pore at the center.

The family of Pnu (Pyridine nucleotide uptake) proteins consists of bacterial membrane transporters involved in the uptake of different B-type vitamins. Transporters for thiamin (vitamin B_1, PnuT), riboflavin (vitamin B_2, PnuX), and nicotinamide riboside (vitamin B_3, PnuC) have been identified and constitute subfamilies in the Pnu family [23]. Pnu transporters mediate facilitated diffusion of the vitamin substrates, coupled to metabolic trapping in the cytoplasm by phosphorylation [24]. Vitamin-specific intracellular kinases thus indirectly regulate transport activity [25]. Pnu proteins are widely distributed among bacteria and possess seven or eight TMs. Pnu proteins do not share significant sequence similarity with SWEET transporters (less than 15% identity), and do not contain the PQ motif. Nonetheless, the crystal structure of PnuC (from *Neisseria mucosa*) has a core of six TMs that is very similar to the SemiSWEET structures and also consists of two three-helix bundles that are structurally similar [15]. In this case, the three-helix bundles are linked via an extra TM that is located peripherally to the hexagon, and serves to position both three-helix bundles in a parallel orientation (Figure 1B and 2A) [15]. Such a connecting TM is also present in the full-length SWEET transporters giving rise to their 3+1+3 membrane topology. The similarity in membrane topology and domain organization between the Pnu and SWEET transporters had been noticed before, and is also predicted for other transporter families of the PQ loop superfamily [26].

Topological differences between SemiSWEET and PnuC

The building blocks of Pnu and (Semi)SWEET transporters are the symmetry-related three-helix bundles, which are

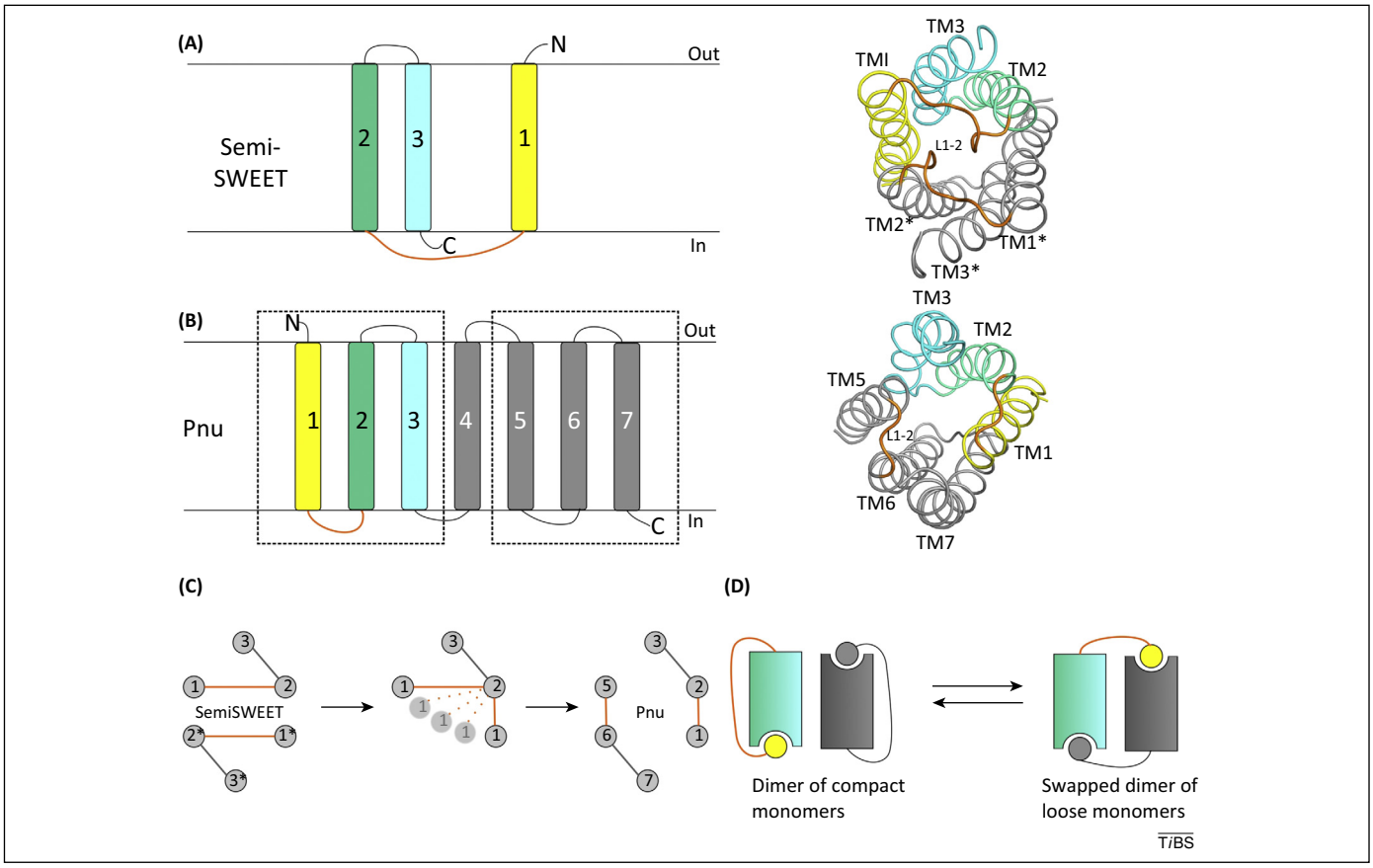

Figure 1. Comparison of the structures of SemiSWEET and PnuC. **(A)** Left: topology of SemiSWEET three-helix bundle (TM1 in yellow, TM2 in green, and TM3 in cyan, loop L1-2 in orange). Right: the structure of the SemiSWEET homodimer in secondary structure ribbon representation (PDB entry 4QNC). The viewpoint is from the cytoplasmic side of the membrane along the two-fold axis. The TMs of the second protomer are denoted with TM1*, 2*, and 3*, respectively (in gray). **(B)** Left: 3+1+3 membrane topology of Pnu transporters. The three-helix bundles are denoted with the broken rectangles, same coloring as in panel (A). Right: the structure of PnuC (PDB entry 4QTN), viewed from the cytoplasmic side of the membrane. TM4 connecting the two halves is located peripherally. **(C)** Relation between the six-TM cores of the Pnu and SemiSWEET transporters. 3D domain swapping of TM1 converts the SemiSWEET connectivity into the PnuC connectivity. The positioning of the helices is based on the crystal structures. Shortening and reorientation of the loop L1-2 would interconvert topologies of the SemiSWEET and Pnu transporters. **(D)** Schematic representation of 3D domain swapping. Left: a dimer of 'compact' protomers (as in the SemiSWEETs). Right: a dimer of 'loose' protomers with swapped yellow TM1 (reminiscent of PnuC).