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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

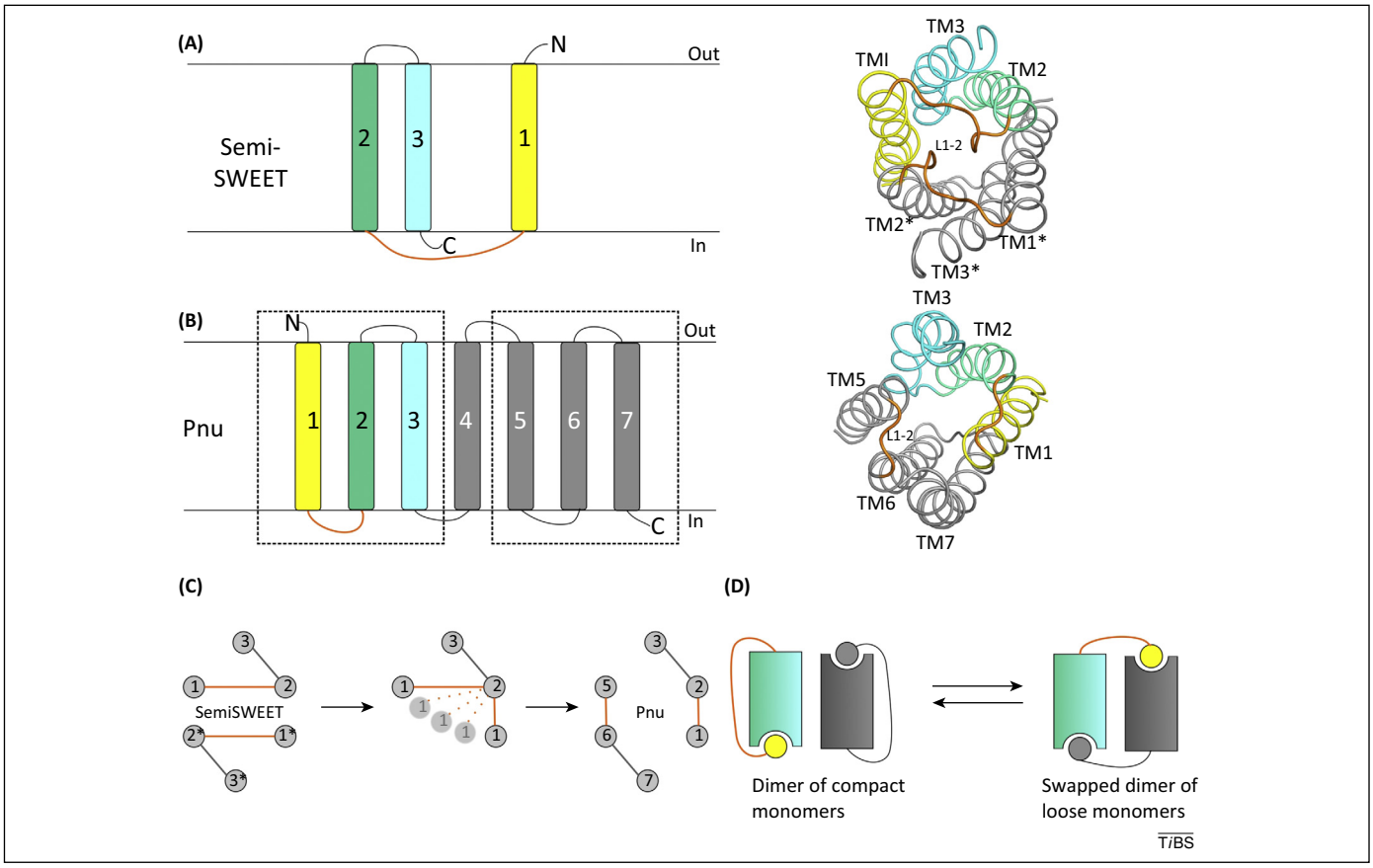

Figure 1. Comparison of the structures of SemiSWEET and PnuC. **(A)** Left: topology of SemiSWEET three-helix bundle (TM1 in yellow, TM2 in green, and TM3 in cyan, loop L1-2 in orange). Right: the structure of the SemiSWEET homodimer in secondary structure ribbon representation (PDB entry 4QNC). The 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).

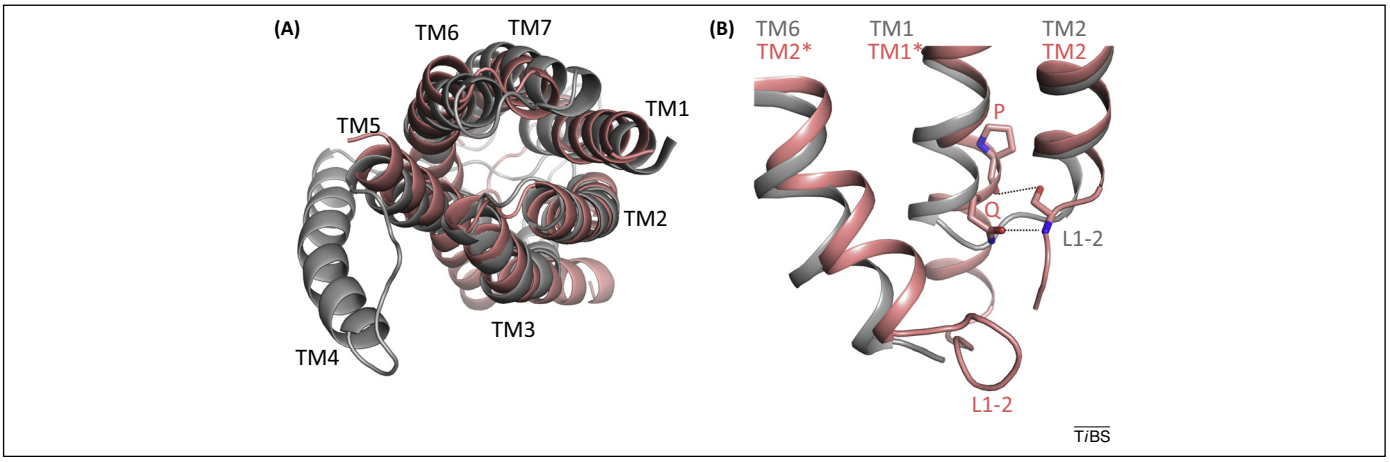

Figure 2. Superposition of *Neisseria mucosa* PnuC (gray) and *Leptospira biflexa* SemiSWEET (light red) crystal structures. **(A)** The two three-helix bundles in PnuC are linked via an auxiliary helix 4 (TM4). **(B)** In SemiSWEETs, the PQ motif in TM1 forms hydrogen bonds with TM2* of the adjacent protomer, thus stabilizing the close proximity of the homodimer. By contrast, in PnuC a short loop between the helices provides an alternative way to achieve close proximity, without the need for the PQ motif. Note that in PnuC, TM1 ends exactly at the position of this motif.

similar in structure (Table 1 and Figure 2A) with their N termini located on the periplasmic side of the membrane and the C termini inside. However, they show a prominent difference in their protein topology. In case of the SemiSWEETs, TM3 is located between TM1 and TM2 on the corners of the hexagon whereas in the structure of PnuC the related TMs have a sequential spatial arrangement (Figure 1).

To facilitate the structural comparison of the protein families we named the TMs of the two protomers of SemiSWEET differently: TM1, 2, and 3 for one protomer, and TM 1*, 2*, and 3* for the other. When TMs 2 and 3 of SemiSWEET and PnuC are structurally aligned, TMs 1 do not superimpose at all. Instead, TM1 of SemiSWEET is located at the position of TM5 in PnuC (Figures 1 and 2). Similarly, TM1 of PnuC is located at the position of TM1* from SemiSWEET. It is noteworthy that the differences in position of the secondary structure elements cannot be the result of circular permutation [27] for two reasons: first, the odd number of helices in the three-helix bundle causes the termini to be located on different sides of the membrane, thus precluding circular permutation [28]. Second, circular permutation would lead to a different connectivity of structurally related helices (TM1–TM3–TM2), which is not the case.

A 3D domain swap can explain the topological differences

At first glance, the lack of sequence similarity and topological differences between the SemiSWEETS and PnuC may suggest that they have converged to the same overall structures. However, we argue that a plausible divergent pathway can also account for the similarities and dissimilarities. The differences between SemiSWEET and PnuC are reminiscent of 3D domain swapping [29,30]. In the SemiSWEET structure, the three-helix bundles adopt a 'compact' conformation, whereas they are in a 'loose' conformation in the PnuC structure. To interconvert between the two, TM1 has to be swapped from one of the three-helix bundles to the other (Figure 1C,D). 3D domain swapping of TM1 in the SemiSWEETs would result in a conformation of the three-helix bundle as observed in PnuC. Swapping is tolerated in homodimeric proteins because the overall structure and the helical interactions of the six-TM core do not change, since identical helices swap places (Figure 1C). 3D domain swapping is not unprecedented

Table 1. Structural comparison of the three-helix bundles (root-mean-square deviation, rmsd)[a]

	PnuC N-terminal bundle	PnuC C-terminal bundle	SemiSWEET 4QNC	SemiSWEET 4QND	SemiSWEET 4NRG	SemiSWEET 4X5M	SemiSWEET 4X5N
PnuC N-terminal bundle	–	1.3 Å	2.2 Å	2.3 Å	1.6 Å	2.3 Å	2.2 Å
PnuC C-terminal bundle		–	2.1 Å	2.6 Å	1.7 Å	3.3 Å	3.3 Å
SSWEET 4QNC			–	1.8 Å	0.9 Å	1.7 Å	1.6 Å
SSWEET 4QND				–	2.4 Å	1.9 Å	2.6 Å
SSWEET 4NRG					–	2.0 Å	1.9 Å
SSWEET 4X5M						–	0.6 Å
SSWEET 4X5N							–

[a]The structural alignments were executed using the Dali Server (DaliLite V3) [35] using pdb files 4QNC (*L. biflexa* serovar Patoc SemiSWEET), 4QND (*Vibrio* sp. N418 SemiSWEET), 4RNG (*T. yellowstonii* DSM 11347 SemiSWEET), 4X5M and 4X5N (*E. coli* SemiSWEET), and 4QTN (*N. mucosa* PnuC).

in the divergence of membrane proteins from a common ancestor: a comparable domain swap was observed when the structure of the human TRAAK [TWIK (Tandem of pore domains in a Weak Inward rectifying K+ channel)-related arachidonic acid-stimulated K(+) channels] potassium channel was compared with other, homologous potassium channels [31].

The different arrangements of the TMs in the three-helix bundles of SemiSWEETs and PnuC correlate with differences in the length of the loop connecting TM1 and 2 (L1-2). In the SemiSWEETs, the loop has to span a longer distance compared to L1-2 and the related L5-6 in the Pnu proteins (Figure 1). A shortening of the SemiSWEET L1-2 would make it impossible for TM3 to be located between TM1 and TM2, but swapping of the position of TM1 would still allow for the integrity of the six-TM core. The difference in the length of L1-2 between SemiSWEETs and Pnus is a general property of the protein families, rather than a peculiarity of the homologs that were crystallized. Multiple sequence alignments show that L1-2 consists of seven residues in the SemiSWEETs and SWEETs (Figure S1 in the supplementary material online, TM boundaries according to [13]) and only of two residues in the Pnus (TM boundaries according to [15]).

The function of the PQ loop motif supports a domain swap hypothesis

The length difference of loop L1-2 also correlates with the presence of the PQ motif. The PQ motif located in TM1/TM1* is the most conserved motif in the SemiSWEETs and other PQ-loop transporters [32,33], but not present in any of the Pnu proteins. A comparison of the structures of SemiSWEETs and PnuC provides a possible explanation for the absence of this motif in PnuC. The glutamine side chain of the PQ motif in TM1 of the SemiSWEETS can form hydrogen bonds with backbone carbonyl and amine groups of TM2* in the other protomer, allowing TM1 and TM2* to be in close proximity (Figure 2B, light red structure). In all SemiSWEETs, TM1 continues for two turns beyond the PQ motif, and is then followed by the relatively long loop to TM2 (Figures 2B and S1 in the supplementary material online). By contrast, TM1 of PnuC ends exactly where the PQ motif is located in the SemiSWEET (Figure 2B), and is followed by the short loop to TM2 (Figure 2B). The short L1-2 in PnuC brings the two TMs in close proximity without the need for non-covalent interactions via the PQ motif as seen in the SemiSWEETs. The differences in the length of TM1 and loop L1-2 between (Semi)SWEET and Pnu transporters are well conserved (Figure 3).

The crystal structures of *Escherichia coli* SemiSWEET show that the proline of the PQ loop probably acts as a molecular hinge that allows essential conformational changes during the translocation [16]. *E. coli* SemiSWEET was crystallized in two different states, with the substrate-binding site exposed to the cytoplasmic and the periplasmic side of the membrane, respectively. A rigid body movement of the three-helix bundles around the hinge converts one state into the other, and probably is the mechanistic basis for substrate translocation. Strikingly, the three-helix bundles that behave as rigid bodies do not correspond to the three helices that constitute a SemiSWEET protomer, but rather consist of TM 2 and 3 of one protomer and the part of TM 1* that precedes the PQ motif of the other protomer [16]. The bundles that move as the rigid bodies thus correspond to three-helix bundle found in PnuC, which provides further support for our hypothesis that a 3D domain swap relates Pnu and SWEET transporters.

A possible pathway for divergent evolution of the SWEET and Pnu proteins

Based on the topological differences between SemiSWEET and Pnu transporters and the different lengths of the loops connecting TM1 and TM2 we propose a hypothetical evolutionary scheme, which relates the Pnu and SWEET transporters by divergence (Figure 4). The origin may be a primordial membrane protein consisting of a three-helix

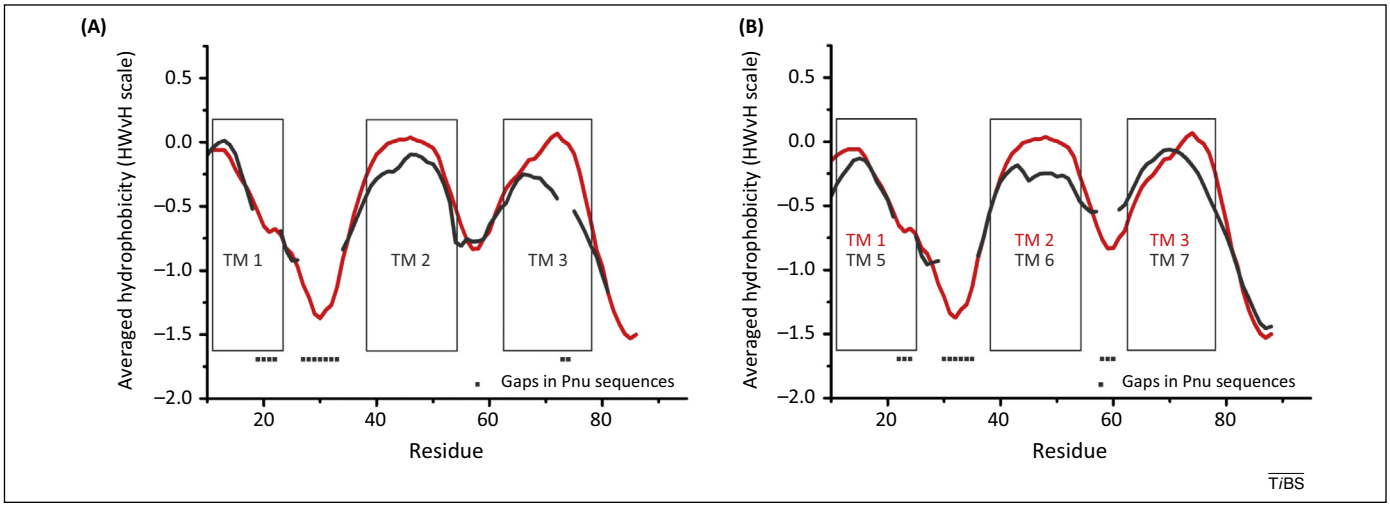

Figure 3. Alignments of hydropathy profiles. The average hydropathy profile of the SemiSWEET three-helix bundle (red) was aligned with the average profile of either the N-terminal **(A)** or the C-terminal three-helix bundle of the Pnu family **(B)** (dark gray). Without exception, loop L1-2 and loop L5-6 in the Pnu family are shorter than the corresponding loop in SemiSWEETs. Similarly, TM1 and TM5 in PnuC are always shorter than TM1 of SemiSWEET. The positions of the gaps that are required to make the alignment are indicated. The corresponding multiple sequence alignments are shown in Figure S1 in the supplementary material online. Hydropathy alignments [36] of multiple sequence alignments were performed using the AlignMe tool [37] with the multiple sequence alignments as input. HWvH is the hydrophobicity scale according to Hessa, White, and von Heijne [38].

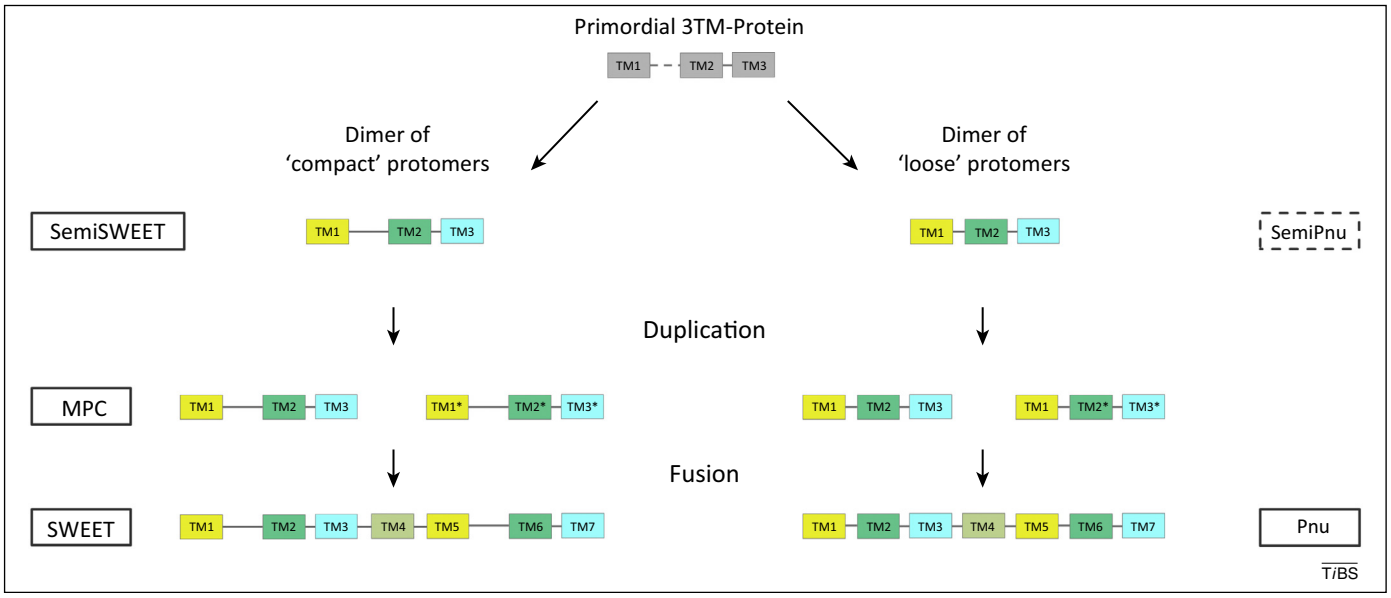

Figure 4. Possible evolutionary history of the SWEET and Pnu families. Three-helix bundles with 'compact' or 'loose' conformations may have arisen from a common ancestor. Homodimers of the 'compact' bundles represent the SemiSWEET transporters and homodimers of the 'loose' bundles may be the putative SemiPnu proteins (Figure S2 in the supplementary material online). The genes then duplicated and fused via insertion of the linker helix TM 4, forming the SWEET and Pnu transporters with 3+1+3 topology. The TMs are colored according to Figure 1. Proteins that are currently found in protein databases are indicated by the black boxes (unbroken lines, experimentally verified; broken lines, predicted). MPC refers to mitochondrial pyruvate carriers, which are heterodimers [39,40].

bundle that formed a homodimer. In the primordial homodimer, a 3D domain swapping event could take place, while maintaining the homodimeric state. This protein diverged into two versions, one with a long L1-2 loop and folded in the 'compact' conformation (SemiSWEET) and the other with a short loop compatible only with the 'loose' conformation. The latter could be tentatively referred to as 'SemiPnu' proteins. We identified putative 'SemiPnus' in the databases (Figure S2 in the supplementary material online), but these proteins have not been experimentally characterized yet. The divergence into the 'compact' and 'loose' forms may thus have been driven by deletion or insertion of amino acids in the loop.

The genes for the Semi transporters then duplicated, diverged in sequence, and fused via insertion of TM4 to yield the full-length SWEET and Pnu proteins found in eukaryotes and bacteria, respectively (Figure 4). Domain swapping must have preceded these events, because only in a homodimeric arrangement the identical TMs 1 can be swapped without compromising structural integrity. It is therefore likely that evolutionary events giving rise to the fused full-length proteins took place in parallel and independently in the Pnu and SWEET transporters [10,34].

Concluding remarks

The analysis presented here suggests that (Semi)SWEET and Pnu transporters are topological pseudo-isomers in which the positions of the individual helices are similar, but they are contributed from different three-helix bundles. We hypothesize that the length of the loop connecting TM1 and 2 is an important determinant for adopting a Pnu- or a SemiSWEET-like conformation within the three-helix bundle. Additionally, the structures provide a molecular explanation for the missing PQ motif in the Pnu transporters and support the structural importance of this motif in SWEET and other PQ-loop transporters. It may be possible to experimentally test the hypothesis of 3D domain swapping by engineering SemiSWEET proteins with shortened loops between TM1 and TM2. Similarly, the putative SemiPnu proteins could be used for the same purpose. The Pnu and SWEET families are particularly suitable for tracing the relatedness of their fold, because both the half- and full-length transporters have been identified and structurally characterized. For no other membrane transporter family is such rich information currently available.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tibs.2015.02.002.

References

1 Theobald, D.L. and Miller, C. (2010) Membrane transport proteins: surprises in structural sameness. *Nat. Struct. Mol. Biol.* 17, 2–3
2 Yamashita, A. *et al.* (2005) Crystal structure of a bacterial homologue of Na^+/Cl^--dependent neurotransmitter transporters. *Nature* 437, 215–223
3 Ressl, S. *et al.* (2009) Molecular basis of transport and regulation in the Na(+)/betaine symporter BetP. *Nature* 458, 47–52
4 Schulze, S. *et al.* (2010) Structural basis of Na(+)-independent and cooperative substrate/product antiport in CaiT. *Nature* 467, 233–236
5 Gao, X. *et al.* (2009) Structure and mechanism of an amino acid antiporter. *Science* 324, 1565–1568
6 Fang, Y. *et al.* (2009) Structure of a prokaryotic virtual proton pump at 3.2 A resolution. *Nature* 460, 1040–1043
7 Faham, S. *et al.* (2008) The crystal structure of a sodium galactose transporter reveals mechanistic insights into Na^+/sugar symport. *Science* 321, 810–814
8 Ehrnstorfer, I.A. *et al.* (2014) Crystal structure of a SLC11 (NRAMP) transporter reveals the basis for transition-metal ion transport. *Nat. Struct. Mol. Biol.* 21, 990–996

9 Weyand, S. *et al.* (2008) Structure and molecular mechanism of a nucleobase-cation-symport-1 family transporter. *Science* 322, 709–713
10 Murzin, A.G. (1998) How far divergent evolution goes in proteins. *Curr. Opin. Struct. Biol.* 8, 380–387
11 Theobald, D.L. and Wuttke, D.S. (2005) Divergent evolution within protein superfolds inferred from profile-based phylogenetics. *J. Mol. Biol.* 354, 722–737
12 Dietmann, S. and Holm, L. (2001) Identification of homology in protein structure classification. *Nat. Struct. Biol.* 8, 953–957
13 Xu, Y. *et al.* (2014) Structures of bacterial homologues of SWEET transporters in two distinct conformations. *Nature* 515, 448–452
14 Wang, J. *et al.* (2014) Crystal structure of a bacterial homologue of SWEET transporters. *Cell Res.* 24, 1486–1489
15 Jaehme, M. *et al.* (2014) Crystal structure of the vitamin B_3 transporter PnuC, a full-length SWEET homolog. *Nat. Struct. Mol. Biol.* 21, 1013–1015
16 Lee, Y. *et al.* (2015) Structural basis for the facilitative diffusion mechanism by SemiSWEET transporter. *Nat. Commun.* 6, Published online January 19, 2015, http://dx.doi.org/10.1038/ncomms7112
17 Wright, E.M. (2013) Glucose transport families SLC5 and SLC50. *Mol. Aspects Med.* 34, 183–196
18 Chen, L-Q. *et al.* (2012) Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. *Science* 335, 207–211
19 Chen, L-Q. *et al.* (2010) Sugar transporters for intercellular exchange and nutrition of pathogens. *Nature* 468, 527–532
20 Lin, I.W. *et al.* (2014) Nectar secretion requires sucrose phosphate synthases and the sugar transporter SWEET9. *Nature* 508, 546–549
21 Xuan, Y.H. *et al.* (2013) Functional role of oligomerization for bacterial and plant SWEET sugar transporter family. *Proc. Natl. Acad. Sci. U.S.A.* 110, 3685–3694
22 Jézégou, A. *et al.* (2012) Heptahelical protein PQLC2 is a lysosomal cationic amino acid exporter underlying the action of cysteamine in cystinosis therapy. *Proc. Natl. Acad. Sci. U.S.A.* 109, 3434–3443
23 Jaehme, M. and Slotboom, D.J. (2015) Diversity of membrane transport proteins for vitamins in bacteria and archaea. *Biochim. Biophys. Acta* 1850, 565–576
24 Kurnasov, O.V. *et al.* (2002) Ribosylnicotinamide kinase domain of NadR protein: identification and implications in NAD biosynthesis. *J. Bacteriol.* 184, 6906–6917
25 Merdanovic, M. *et al.* (2005) Coupling of NAD^+ biosynthesis and nicotinamide ribosyl transport: characterization of NadR ribonucleotide kinase mutants of *Haemophilus influenzae*. *J. Bacteriol.* 187, 4410–4420
26 Yee, D.C. *et al.* (2013) The transporter-opsin-G protein-coupled receptor (TOG) superfamily. *FEBS J.* 280, 5780–5800
27 Heinemann, U. and Hahn, M. (1995) Circular permutations of protein sequence: not so rare? *Trends Biochem. Sci.* 20, 349–350
28 Mackin, K.A. *et al.* (2014) An empirical test of convergent evolution in rhodopsins. *Mol. Biol. Evol.* 31, 85–95
29 Liu, Y. and Eisenberg, D. (2002) 3D domain swapping: as domains continue to swap. *Protein Sci.* 11, 1285–1299
30 Bennett, M.J. *et al.* (1994) Domain swapping: entangling alliances between proteins. *Proc. Natl. Acad. Sci. U.S.A.* 91, 3127–3131
31 Brohawn, S.G. *et al.* (2013) Domain-swapped chain connectivity and gated membrane access in a Fab-mediated crystal of the human TRAAK K^+ channel. *Proc. Natl. Acad. Sci. U.S.A.* 110, 2129–2134
32 Ruivo, R. *et al.* (2012) Mechanism of proton/substrate coupling in the heptahelical lysosomal transporter cystinosin. *Proc. Natl. Acad. Sci. U.S.A.* 109, 210–217
33 Ponting, C.P. *et al.* (2001) Novel protein domains and repeats in *Drosophila melanogaster*: insights into structure, function, and evolution. *Genome Res.* 11, 1996–2008
34 Pearce, T. (2011) Convergence and parallelism in evolution: a neo-Gouldian account. *Br. J. Philos. Sci.* 63, 429–448
35 Holm, L. and Rosenström, P. (2010) Dali server: conservation mapping in 3D. *Nucleic Acids Res.* 38, 545–549
36 Lolkema, J.S. and Slotboom, D.J. (1998) Hydropathy profile alignment: a tool to search for structural homologues of membrane proteins. *FEMS Microbiol. Rev.* 22, 305–322
37 Khafizov, K. *et al.* (2010) A study of the evolution of inverted-topology repeats from LeuT-fold transporters using AlignMe. *Biochemistry* 49, 10702–10713
38 Hessa, T. *et al.* (2007) Molecular code for transmembrane-helix recognition by the Sec61 translocon. *Nature* 450, 1026–1030
39 Bricker, D.K. *et al.* (2012) A mitochondrial pyruvate carrier required for pyruvate uptake in yeast, *Drosophila*, and humans. *Science* 337, 96–100
40 Herzig, S. *et al.* (2012) Identification and functional expression of the mitochondrial pyruvate carrier. *Science* 337, 93–96