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Rempel, S; Stanek, W K; Slotboom, D J

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Annual Review of Biochemistry

Energy-Coupling Factor–Type ATP-Binding Cassette Transporters

S. Rempel,¹ W.K. Stanek,¹ and D.J. Slotboom^{1,2}

¹Groningen Biomolecular and Biotechnology Institute, University of Groningen, 9474AG Groningen, The Netherlands; email: s.r.rempel@rug.nl, w.k.stanek@rug.nl, d.j.slotboom@rug.nl

²Zernike Institute for Advanced Materials, University of Groningen, 9747AG Groningen, The Netherlands

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Keywords

ATP-binding cassette transporter, ABC transporter, energy-coupling factor type, ECF-type, prokaryotic vitamin uptake, membrane transport, mechanism of transport, ATP-driven transport

Abstract

Energy-coupling factor (ECF)–type ATP-binding cassette (ABC) transporters catalyze membrane transport of micronutrients in prokaryotes. Crystal structures and biochemical characterization have revealed that ECF transporters are mechanistically distinct from other ABC transport systems. Notably, ECF transporters make use of small integral membrane subunits (S-components) that are predicted to topple over in the membrane when carrying the bound substrate from the extracellular side of the bilayer to the cytosol. Here, we review the phylogenetic diversity of ECF transporters as well as recent structural and biochemical advancements that have led to the postulation of conceptually different mechanistic models. These models can be described as power stroke and thermal ratchet. Structural data indicate that the lipid composition and bilayer structure are likely to have great impact on the transport function. We argue that study of ECF transporters could lead to generic insight of membrane protein structure, dynamics, and interaction.

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DISCOVERY OF ECF TRANSPORTERS

The name energy-coupling factor (ECF) was first used in the late 1970s by Henderson et al. (1–10) in a series of studies on uptake of folate, biotin, and thiamine in the Gram-positive bacterium *Lactobacillus casei*. Transport of each of these substrates depended on a specific, membrane-bound binding protein (now termed the S-component) and a shared component, for which different S-components compete, termed the energy-coupling factor (now the ECF module). In these studies, it was shown that transport was most likely dependent on ATP hydrolysis, and it was speculated that the energy-coupling factor might resemble the HisP component of the histidine transport system from *Salmonella typhimurium* (7, 11). We now know that both the ECF module and the histidine transport system contain ATPases belonging to the ATP-binding cassette (ABC) superfamily (**Figure 1**; also see the sidebar titled Overview of the ABC Transporter Superfamily) but that the ECF-type ABC transporters form a structurally and mechanistically distinct group within this superfamily (12, 13). Although the molecular identity of ECF-type ABC transporters remained elusive at the time, the results cumulated in the remarkably accurate description of the function of ECF transporters by Henderson et al. (7, p. 1308), who postulated the “hypothesis that the folate, thiamine, and biotin transport systems of *L. casei* each function via a specific binding protein, and that they require, in addition, a common component..., [which] may be a protein required for the coupling of energy to these transport processes.”

In the 2000s, new ABC transporters were identified for the import of biotin (BioMN) in *Sinorhizobium meliloti* and the import of Co^{2+} and Ni^{2+} (CbiMNQO and NikMNQO, respectively) in *Salmonella enterica* and *Rhodobacter capsulatus* (14–16). These ABC transporters did not appear to make use of identifiable periplasmic substrate-binding proteins (SBPs) (see the sidebar titled Overview of the ABC Transporter Superfamily), which were invariably associated with the bacterial ABC importers known at the time, but a connection with the earlier work on ECF transporters was not made (14, 15, 17). Additionally, the genes encoding S-components of ECF transporters were picked up, without initially being linked to Henderson’s work (18–24). The protein RibU (initially named YpaA) was found to be involved in riboflavin transport in *Bacillus subtilis* and *Lactococcus lactis* and was assumed to be a new transport system (22, 24). This assumption was found to be incorrect later, when it was recognized that RibU is an S-component of an ECF transporter; nonetheless, the incorrect assumption was propagated in the interpretation of the first crystal structure of RibU (25). The thiamine binding protein ThiT and folate

a Importers

b Exporters

Figure 1

Comparison of the seven different structural classes of ABC transporters as well as solitary S-components. The color scheme is as follows: ATPase domains (*magenta* and *gray*), C-terminal domains (*medium blue* and *light green*), integral membrane domains (*turquoise blue* and *dark blue*), SBPs and S-components (*yellow*), and additional domains (*orange* and *red*). All structures were aligned on their NBDs in PyMOL, which reveals different distances of the NBDs from the membrane in various systems. (a) ABC importers are known only for prokaryotes and are classified as type I, represented by the alginate transporter AlgM1M2SSQ2 (PDB code 4TQU), type II, represented by the cobalamin importer BtuCDF (PDB code 2QI9), and ECF-type. Types I and II depend on an extracellular SBP, whereas ECF-type ABC transporters use integral membrane S-components. ECF-type ABC transporters are represented by the group II folate transporter ECF-FoIT2 and the group I Co^{2+} transporter CbiMNQO (CbiN subunit missing in the structure; PDB codes 5JSZ and 5X3X, respectively). Solitary S-components are represented by BtuM (PDB code 6FFV). (b) ABC exporters are classified as type I that can be heterodimeric, sometimes with a degenerate ATPase site, or homodimeric; they are represented by the prokaryotic multidrug exporter Tm287/288 (PDB code 4Q4H) and Sav1866 (PDB code 2HYD), respectively. The human sterol exporter ABCG5/G8 (PDB code 5DO7) is a type II ABC exporter. Type III and type IV ABC exporters are also known as ABC mechanotransducers and export lipopolysaccharide, in the case of LptBFG (PDB code 5X5Y), or antibiotics and enterotoxin, in the case of MacB (PDB code 5LIL) (30, 37, 38, 55, 117–123). Abbreviations: ABC, ATP-binding cassette; ECF, energy-coupling factor; NBDs, nucleotide-binding domains; PDB, Protein Data Bank; SBPs, substrate-binding proteins.

OVERVIEW OF THE ABC TRANSPORTER SUPERFAMILY

ATP-binding cassette (ABC) transporters contain two conserved cytosolic nucleotide-binding subunits or domains (NBDs) that bind and hydrolyze ATP and are associated with a pair of integral membrane subunits (12, 13, 17, 54, 114). Seven different structural classes of membrane subunits have been discovered to date (Figure 1). Type I importers, type II importers, ECF-type transporters, and solitary S-components are dedicated to nutrient import in prokaryotes. Type I and II importers make use of water-soluble substrate-binding proteins (SBPs) or domains that provide substrate specificity.

In ABC transporters, the binding and hydrolysis of ATP lead to conformational changes in the ATPase subunits, which are then transmitted to the transmembrane domains or subunits via α -helical structures on the cytoplasmic side of the membrane subunits (coupling helices). The transmembrane subunits cycle through different conformations that allow access of the transported substrate alternately to the extracellular side of the membrane and to the cytosol.

binding protein FolT were the first to be associated with Henderson's work (26). Then, in 2009, a comprehensive study combined bioinformatic and experimental data and made the link with the energy-coupling factor described in the 1970s by Henderson et al. (7, 13).

ECF transporters are strict importers and widespread among prokaryotes (13). They are not present in eukaryotes, with a single possible exception in plants (27). ECF transporters are specific for substrates that are needed in small quantities, including enzymatic cofactors or their precursors (such as B-type vitamins B₁, B₂, B₃, B₆, B₇, B₉, and B₁₂), the divalent cations nickel and cobalt, and a few other compounds such as tryptophan (Table 1). ECF transporters are genuine ABC transporters (Figure 1) consisting of two ATP-hydrolyzing, nucleotide-binding domains (NBDs), termed the ECF-A, A-component, or EcfA; two transmembrane proteins, termed the ECF-T, T-component, or EcfT (see the sidebar titled Nomenclature Issues of ECF-Type ABC Transporters); and the S-component (13, 17, 27). The NBDs and ECF-T together form the tripartite ECF module (13). Although the ECF-A subunits are similar to the NBDs from all other ABC transporters, the integral membrane subunits of ECF transporters are not related to those of other ABC transporters. ECF transporters do not make use of periplasmic or extracellular binding proteins (SBPs), which are essential components of prokaryotic type I and type II ABC importers (13, 17, 28). Instead, the membrane-embedded S-component solely confers substrate specificity to the uptake system.

ECF-type ABC transporters are classified into three groups: group I, group II, and solitary. Group I transporters are dedicated systems, in which only a single S-component forms a complex with the ECF module. Group II transporters are modular, meaning that different S-components for various substrates can interact with a shared ECF module (13, 28). The transport systems described by Henderson et al. (7) in the 1970s belong to this group. Some organisms only encode an S-component and do not contain genes coding for the ECF module (13, 28–30). It is still controversial whether these solitary S-components constitute bona fide transport systems themselves, but recent data support a transport function and suggest that they may be more widespread than previously thought (30).

In the next sections, we provide an overview of the phylogeny and diversity of ECF transporters and review recent structural and biochemical advancements that have led to more detailed mechanistic insights. We discuss different conceptual models for ATP-coupled transport and show that the study of ECF transporters could lead to generic understanding of membrane protein structure, function, and dynamics.

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Table 1 Diversity of ECF-type ABC transporters based on Reference 13

Transporter	For group I: name of ECF-T	For group I: name of ECF-A	S- component	Additional component	Group(s) ^a	Substrate ^b	Notes	Experimental reference(s) ^c
BioMNY	BioN	BioM	BioY	None	Groups I and II	Biotin	NA	14, 16
BioY	Not present	Not present	BioY	None	Solitary	Biotin	NA	29
BtuM	Not present	Not present	BtuM	None	Solitary	Cobalamin	NA	30
ChiMNQO	ChiQ	ChiO	ChiM	ChiN	Group I	Cobalt	NA	15, 38
ChiT	ChiTt	ChiTa	ChiT	None	Groups I and II	Cobalamin precursor	^c	NA
ChrTUV	ChrV	ChrU	ChrT	None	Groups I and II	Cobalamin	NA	32
Cce1529	Cce1531	Cce1530	Cce1529	None	Group I	Unknown	Not in Reference 13; archaeal	NA
Cyano_T	Cyano_T	Not present	Not present	None	Not classified	Unknown	Not in Reference 13	27
FolT	group II only	group II only	FolT	None	Group II	Folate	NA	26
Glr2054	Glr2053	Glr2054	Glr2052	None	Group I	Unknown	Not in Reference 13; cyanobacterial	NA
HtsTUV	HtsU	HtsV	HtsT	None	Groups I and II	Unknown	NA	NA
Ker335	Ker337	Ker336	Ker335	None	Group I	Unknown	Not in Reference 13; archaeal	NA
LipT	Group II only	Group II only	LipT	None	Group II	Lipoate	NA	NA
MtaTUV	MtaV	MtaU	MtaT	None	Group I	Methylthioadenosine	NA	NA
MTH452	MTH453	MTH454	MTH452	None	Group I	Unknown	Not in Reference 13; archaeal	NA
MtsTUV	MtsV	MtsU	MtsT	None	Groups I and II	Methionine precursors	NA	NA
NiaX	Group II only	Group II only	NiaX	None	Group II	Niacin	NA	47
NikMNQO	NikQ	NikO	NikM	NikN	Group I	Nickel	NA	15
PanT	PanTt	PanTa	PanT	None	Groups I and II	Pantothenate	^c	36, 66
PdsU	PdsUt	PdsUa	PdsU	None	Groups I and II	Pyridoxine	^c	NA
PdsU2	Group II only	Group II only	PdsU2	None	Group II	Pyridoxine	Formerly HmpT (27)	35 ^d
QrtTUVVW	QrtU	QrtV, QrtW	QrtT	None	Group I	Queuosine precursor	NA	NA
QueT	QueTt	QueTa	QueT	None	Groups I and II	Queuosine precursor	^c	NA
RibU	RibUt	RibUa	RibU	None	Groups I and II	Riboflavin	Group I only in Bifidobacteria ^e	22, 23
Ssl137	Ssl135	Ssl136	Ssl137	None	Group I	Unknown	Not in Reference 13; archaeal	NA
ThiT	Group II only	Group II only	ThiT	None	Group II	Thiamine	NA	26, 48
ThiW	ThiWt	ThiWa	ThiW	None	Groups I and II	Thiazole	^c	NA
TrpP	TrpPt	TrpPa	TrpP	None	Groups I and II	Tryptophan	^c	NA
YkoEDC	YkoC	YkoD	YkoE	None	Group I	Thiamine	NA	45

Abbreviations: ABC, ATP-binding cassette; ECF, energy-coupling factor; NA, not applicable.

^aAll names are given for the group I transporters. Names for the corresponding group II transporters are given as “ECF-” followed by the name of the S-component—for example, the vitamin B₁₂-specific ECF transporter is termed ChrTUV for group I and ECF-ChrT for group II (32). The grouping was derived from entries in the SEED database and may differ from Reference 28; it does not include solitary S-components (same substrate specificity assumed).

^bBold names indicate experimental evidence is available. For the other substrates, the specificity is taken from predictions from Reference 13.

^cIn Reference 13, these S-components were identified as exclusively occurring in group II ECF transporters, but group I examples can now be found in the SEED database. Names are given as the term of the S-component with “c” or “a” added for ECF-T or ECF-A, respectively.

^dOnly the structure of the *apo* complex is reported; no functional data proving substrate specificity are included.

^eThe relevant experimental studies that show the substrate specificity of the respective transport systems are referenced. If no reference is given, the substrate specificity is taken from predictions from Reference 13.

NOMENCLATURE ISSUES OF ECF-TYPE ABC TRANSPORTERS

Many databases wrongly annotate the ECF-T and ECF-A subunits of ECF transporters as CbiQ or CbiO, respectively, although these terms are specific for the cobalt ECF transporter CbiMNQO.

The name of the S-component HmpT has changed to PdxU2, because genome context analysis predicted that the protein is specific for pyridoxine (13, 27). The most prominent example of the use of the obsolete name HmpT is in the structure of the ECF-HmpT full complex (35).

No uniform naming rules for individual components have been adapted because many gene and protein names were assigned before the discovery of ECF transporters, leading to situations in which CbiM or BioM are S-component or ATPase, respectively. We advise using the unique names of all subunits for group I transporters (e.g., CbrTUV for the vitamin B₁₂-specific transporter). For group II transporters, we propose using the name ECF for the ECF module, followed by the name of the S-component (e.g., the name ECF-CbrT for the vitamin B₁₂-specific group II transporter).

DIVERSITY AND PHYLOGENY OF ECF TRANSPORTERS

The highly curated SEED database (<http://pseed.theseed.org/?page=Home>) currently contains 1,876 prokaryotic genomes encoding ECF transporters with a variety of different substrate specificities (31) (Table 1). This number reduces to 1,445 representative organisms upon removal of substrains of the same species. Of these, 717 are Firmicutes, 378 are Proteobacteria, 175 are Actinobacteria, 51 are Tenericutes, and 124 belong to other phyla or Archaea.

The dedicated group I transporters are defined as ECF transporters that have all subunits encoded in a single operon (13, 28). There are 1,927 individual group I ECF transporters (Figure 2a), but the number of organisms containing these transporters is smaller because they can carry more than one group I ECF transporter. The occurrence of more than one group I ECF transporter is most pronounced in Archaea and Actinobacteria (e.g., six group I transporters are present in *Thermofilum pendens*) (13, 27, 28). Although most prevalent in Firmicutes, group I systems are present in a wider range of organisms than are group II ECF transporters (Figure 2a).

Group II transporters are defined as ECF transporters that have the ECF module subunits encoded in one operon and have one or multiple S-component genes scattered over the genome (13, 27, 28). Collectively, 787 group II ECF modules are predicted to interact with 4,387 S-components to allow for transport of different substrates (Figure 2b). Almost all group II transporters are found in Firmicutes (Figure 2b). The number of S-components and group II ECF modules per organism is highly variable. For example, *Enterococcus faecium* DO carries as many as 21 S-components and 3 ECF modules. Whether the different modules in one organism interact with specific subsets of S-components is unknown. Both group I and group II ECF transporters may be present within a given organism. For example, *Lactobacillus delbrueckii* subsp. *bulgaricus* 2038 carries 3 group I ECF transporters as well as a group II ECF module that can interact with 8 different S-components. Finally, apart from the transition metal ion importers that exclusively belong to group I, there seems to be no apparent linkage between the nature of the transported substrate and grouping into I or II. For example, group I CbrTUV and group II ECF-CbrT transporters exist, both specific for vitamin B₁₂ (32) (Table 1).

Solitary S-components are defined as S-components present in organisms that do not encode an ECF module (28–30). The absence of an ECF module can be deduced from failure to identify a T-component or from analysis of the ABC-type ATPases. In organisms with solitary S-components, the latter are all associated with ABC transporters from different types than ECF

Figure 2

Phylogeny of ECF-type ABC transporters based on the SEED database. The color coding for the different genera in all panels can be found in the inset; the phylogeny was generated using phyloT (<https://phylot.biobyte.de>). Phylogeny of (a) group I and (b) group II ECF-type transporters. (c) Phylogeny of solitary S-components [BruM is not annotated in the SEED database (30)]. Abbreviations: ABC, ATP-binding cassette; ECF, energy-coupling factor.

transporters (30). In contrast to full ECF transporters, solitary S-components are not widespread in Firmicutes. There are 304 solitary S-components, which are mainly distributed across 2 families (**Figure 2c**). The 141 solitary BioY proteins (specific for biotin) are most prevalent in Proteobacteria, Actinobacteria, and Cyanobacteria. The 131 solitary S-components that belong to the BtuM family (specific for vitamin B₁₂) are almost exclusively found in Proteobacteria (30). Sequence analysis failed to recognize that BtuM is an S-component, but a recent crystal structure showed that it does have the S-component fold (30). BtuM is not yet annotated as S-component in the SEED database (21, 30).

Twenty-seven different families of S-components are currently known; they are either associated with group I or II transporters or are solitary and are predicted or confirmed to be specific for different substrates (**Table 1**). S-components that are specific for different substrates often share very little sequence identity (~15%), which raises the possibility that additional families of S-components have escaped detection. This makes identification of new S-components difficult, as exemplified by the characterization of BtuM (13, 27, 28, 30).

Subunits of ECF transporters may also be involved in different functions than substrate uptake. *Listeria* spp. contain an ECF-T protein that is predicted to facilitate flavin export in combinations with the S-component RibU but without using the ATPase subunits (33). Some Cyanobacteria (**Table 1**) and plants encode an ECF-T protein but carry genes for neither ECF-A nor S-components. These ECF-T proteins, termed cyano_T, either are nonfunctional, are broken ECF transporters, or have acquired a new function that may be unrelated to nutrient transport (see section titled Future Issues) (27).

Besides the differences in genetic organization, regulation of expression also differs between group I and II transporters. Group I ECF transporter levels are often regulated by the intracellular substrate concentration, most commonly by riboswitches (13, 28). Likewise, levels of S-components from group II ECF transporters are strongly regulated by the cellular needs, with more protein produced when the substrates are scarce (13, 28). In contrast, the expression of the group II ECF module is constitutive, giving rise to a constant pool of units available to interact with substrate-bound S-components (13, 28). The differentially regulated expression of the group II ECF module and associated S-component can lead to an imbalance, in which a great surplus of S-components exists. These excess S-components may act as substrate scavengers remaining bound to their substrate until an ECF module becomes available (28).

CRYSTAL STRUCTURES OF ECF TRANSPORTERS

Currently, five crystal structures of complete group II complexes and one structure of a group I transporter are available (32–38). Additionally, structures of isolated S-components (group I, II, and solitary) and of the ATPase subunits have been reported (25, 30, 38–46) (see **Supplemental Table 1**).

Collectively, the structures support the notion that the S-component, ECF-T, and two homologous or identical ATPases are present in a 1:1:1:1 stoichiometry for both group I and II transporters (13, 28, 47). In some cases, two or three subunits of the transporters may be fused in various combinations, resulting in multiple-domain proteins with fusion of the two ATPases being most common (13, 28). S-components from group II ECF transporters can dissociate from the complex dynamically and most likely exist as monomers until they associate again with an ECF module (25, 40, 41, 48). The group I ECF transporters for Ni²⁺ and Co²⁺ appear to require one or two additional small integral membrane subunits for transport (CbiN, NikN, or NikKL), but the exact role of these subunits is unclear (13, 15, 38).

Deviations from the generic 1:1:1:1 subunit stoichiometry in ECF transporter and monomeric S-components have been postulated for BioMNY and ECF-RibU on the basis of in vivo analyses

and cross-linking studies (46, 49–51). These data are difficult to reconcile with the crystal structures and other biochemical data (28, 47, 52). It is possible that supramolecular complexes of different subunit stoichiometry are formed in some conditions (e.g., in vivo), but the apparent differences in stoichiometry could also stem from experimental artefacts (46, 49–51). In our discussion of the proposed transport mechanism, we adhere to the 1:1:1 subunit stoichiometry.

POWERING TRANSPORT BY ATP HYDROLYSIS

ATP hydrolysis is essential for substrate transport by ECF transporters (47). The ATPases of ECF transporters have all the hallmarks of ABC transporter ATPases (**Figure 1**), which have been extensively reviewed elsewhere (17, 53, 54). The ~31-kDa proteins form dimeric assemblies with (pseudo) two-fold symmetry. Two catalytic sites for ATP hydrolysis are located at the dimer interface (27, 34, 35, 46). The ATPase dimer consists of either two identical (homodimer, ECF-AA) or two homologous (heterodimer, ECF-AA') ATPases (13). ECF transporter ATPases do not contain degenerate sites, a feature that is sometimes observed with heterodimeric ATPases of ABC transporters (55–62). Structurally, each ATPase is a three-domain protein consisting of a RecA-like domain, an α -helical domain, and a C-terminal extension (46). The RecA-like and α -helical domain are universal to all ABC transporter ATPases (27, 53).

Structural and biochemical studies indicate that the ATPases of ECF transporters function in a similar manner as those of other ABC transporters (17, 27, 28, 38, 39, 46, 63, 64). In the absence of ATP, the two subunits are separated from each other and adopt an open conformation (46, 53). ATP binding leads to tight association of the dimer by bringing the RecA-like domain from one subunit in close contact with the α -helical domain of the other subunit. In the resulting closed conformation, the catalytic sites are now complete, ready for ATP hydrolysis to proceed (38, 53). Upon ATP hydrolysis and release of ADP and orthophosphate, the dimer opens again. The open and closed conformations have been observed structurally in isolated ATPase dimers in the absence of the membrane subunits (38, 46). For the complete group I transporter BioMNY, it has also been shown that open and closed states are visited by electron paramagnetic resonance (EPR) experiments (63). In addition, intermediate states may also exist (63). In all crystal structures of complete ECF transporter complexes determined to date, the ATPases are in the open conformation, which limits our understanding of how conformational changes in the ATPases are propagated to the membrane subunits (32–38). A structure of ECF-FoIT2 from *L. delbrueckii* in complex with the slowly hydrolyzable ATP analog AMP-PNP has been solved (37), but the ATPases remained in an open conformation, with the nucleotides exclusively contacting the RecA-like domains (**Figure 3**). In other ABC transporter systems, nonhydrolyzable ATP analogs can lead to closure but sometimes fail to do so (55, 62, 65). In BioMNY the ATPase activity is only moderately affected by inhibitors such as AMP-PNP or orthovanadate. Taken together, ECF transporters may be less sensitive compared with other ABC transporters to ATPase inhibitors (63).

Within each ATPase subunit, a groove exists between the two domains where interaction with the ECF-T subunit takes place. Specific for the ATPases of ECF transporters, the groove between the domains contains a conserved acidic residue that makes contact with a conserved arginine residue in ECF-T (**Figure 3**; see section titled The Scaffold Subunit ECF-T) (46, 49, 66). The groove of the ATPases from ECF transporters also contains a specific element, termed the Q-helix, comprised of six residues with sequence X-P-D/E-X-Q- ϕ (X is any and ϕ is a hydrophobic residue). The Q-helix is located toward the dimer interface and is essential for transport activity, although it is not directly involved in ATP binding or hydrolysis (46).

The group II ECF transporter ATPases possess an additional C-terminal helical extension (**Figure 3**) that is absent in many group I ECF transporter ATPases (13). It likely mediates dimer

Figure 3

Overview of structural features in ECF-type ABC transporters. From left to right, viewed from the plane of the membrane: The folate-bound S-component FolT (PDB code 5D0Y), the ECF transporter ECF-FolT2 (PDB code 5D3M), and the ECF module of ECF-FolT2 (rotated 120° relative to the middle panel). FolT and FolT2 are colored yellow, ECF-T is cyan, and the ATPases are magenta (ECF-A') or gray (ECF-A). Coupling helices are dark blue, helices 1 and 6 in FolT and FolT2 are red, and substrates (folate in FolT and AMP-PNP in ECF-AA') are light brown. Helix 1 in FolT and FolT2 is highlighted with a dashed line and shows the ~90° toppling between the upright and toppled states. (a) The folate-bound substrate binding pocket in FolT. Residues involved in substrate binding, located in loop 1 and 3 and helix 6 (L1, L3, and H6), are highlighted. (c) This panel highlights one of the two ATP binding sites, occupied with an AMP-PNP molecule. All residues involved in nucleotide binding and all motifs present in canonical ABC transporter ATPases are highlighted. (b) Helix 3 of ECF-T is highlighted by a dashed line. The kink in the helix at Pro71 is the hinge point for conformational flexibility of the membrane domain relative to the coupling domain. (ef) These panels show the residue interaction between the conserved Arg residues in ECF-T with Asp residues in ECF-A and ECF-A'. This interaction anchors the ends of the two coupling helices to the ATPases. (d) The C-terminal domain of the two ATPases is highlighted with a dashed circle. Abbreviations: ABC, ATP-binding cassette; ECF, energy-coupling factor; PDB, Protein Data Bank.

formation in the absence of nucleotides (46). C-terminal extensions at the NBDs can also be found in type I ABC importers (**Figure 1**), in which they usually have regulatory functions (67–69). Whether the C-terminal domains in the ATPases of ECF transporters also exert regulatory function over transport activity remains to be shown.

Biochemical characterization of the ATPase activity has been carried out for ECF transporters from various organisms. **Supplemental Table 2** summarizes data on ATPase activity that has been determined for ECF transporters. The K_M values for ATP hydrolysis range from ~0.1 mM up to ~16 mM (**Supplemental Table 2**). In the full complexes, ATP hydrolysis is not stimulated by the

transported substrate, which contrasts with other ABC transporters (17, 37, 63). In the group I cobalt transporter CbiMNQO, ATPase activity is dependent on the presence of the S-component, but it is not clear whether this is a generic property of ECF transporters (38). Comparisons of transport kinetics and ATPase activity (**Supplemental Table 3**) have revealed that ECF-type ABC transporters, like group II ABC importers, exhibit ATPase activity without coupling to transport events (37, 70). Whether the futile ATPase activity is an artifact or mechanistically relevant is unclear (see section titled Transport Mechanisms).

THE SCAFFOLD SUBUNIT ECF-T

The integral membrane subunit ECF-T is the scaffold of the complex. It has an L shape with a peripheral coupling domain on the cytoplasmic side of the membrane that binds to the ATPase subunits and an integral membrane domain that keeps the ECF module associated with the lipid bilayer (**Figure 3**).

The transmembrane domain is poorly conserved and can vary in the number of transmembrane helices, ranging from four (e.g., in BioN QrtU), to five (most ECF-Ts), six (YkoC, CbrV, and some QrtU), seven (ECF-T in *Thermotogales* and some BioN), or eight (some CbrV) (27). In contrast, the coupling domain is better conserved and contains two long α -helices that are arranged in an X shape (**Figure 3**). These α -helices form the main interaction site for ATPases and are hence termed coupling helices, with each coupling helix making tight contact with a single ATPase (49). Both coupling helices contain a conserved arginine residue at their C-terminal end, which binds in the deep groove of the ATPase subunit near the Q-helix and anchors the subunits together (**Figure 3**). The conserved arginine residue is part of a short motif, X-Arg-X (most often Ala-Arg-Gly) (66). Because of the tight interaction between the C-terminal ends of the coupling helices and the ATPase subunits, it is expected that the coupling helices are forced to move jointly with the ATPases when the latter switch between open and closed conformations upon nucleotide binding and release, thereby transferring conformational changes in the ATPases fueled by ATP hydrolysis to the membrane subunit ECF-T (27, 28, 38, 39).

Coupling helices are a common structural feature of ABC transporters used to propagate conformational changes from the ATPases to the membrane domains (71). The main difference to ECF transporters is that other ABC transporters have one coupling helix per membrane domain, each interacting with one ATPase, whereas ECF-T harbors both helices and thus contacts two ATPases. Further, the coupling helices in other ABC transporters are much shorter than the ones in ECF transporters and are well separated from each other instead of forming an interacting X-shaped structure (**Figures 1 and 3**) (53, 71).

While the cytoplasmic surface of the coupling domain of ECF-T interacts with the ATPases, the opposite side of the domain, exposed toward the membrane interior, allows for the interaction with the S-component (**Figure 3**). The interacting surfaces of ECF-T and the S-component are hydrophobic and highly complementary in shape. They are also extensive, covering approximately one-third of the S-component surface, which explains the tight interaction between the membrane subunits observed in the nucleotide-free complexes (34, 35, 37). The coupling domain is located between the S-component and the ATPase subunits and precludes the S-component from interacting directly with the ATPases (34, 35). Nonetheless, movement of the coupling helices upon closing and opening of the ATPase dimer also affects the interaction interface of ECF-T with the S-component (35, 37). This may cause dissociation of the S-component (at least in group II transporters) and reorientation of the substrate binding site, leading to alternating access (37, 39).

In all crystal structures of ECF transporters, the coupling domains of ECF-T, the ATPase subunits, and the S-components have very similar conformations, regardless of the nature of the

S-component in the complex (FolT, FolT2, PanT, CbrT, PdxU2, or CbiM) or the presence or absence of the nucleotide analog AMP-PNP (32–38). In contrast, the membrane domains of ECF-T adopt different conformations in the various crystal structures. They pivot to different extents relative to the coupling domain, indicating that they can display considerable conformational flexibility, even in complexes trapped in the same state (**Figure 3**). Such conformational differences are observed in structures of the identical ECF modules complexes with different S-components (ECF-PanT, ECF-FolT, and ECF-PdxU2 from *Lactobacillus brevis*, and ECF-FolT2 and ECF-CbrT from *L. delbrueckii*) (32–37). In these cases, the flexibility may be required to accommodate specific structural features of the S-components, which are poorly related in sequence (13). The conformational differences are also observed in two crystal structures of the same ECF transporter (ECF-FolT2), suggesting that the flexibility may be an inherent feature of ECF-T proteins (37), which could be relevant for the mechanism of transport (see section titled Transport Mechanisms). Regardless of the conformational differences observed in the membrane domains of ECF-T in the various structures, they all are in contact with the bound S-component and may act as a flexible surface to allow movements of the S-components during transport (see the following section) (35).

MEMBRANE-EMBEDDED SUBSTRATE-BINDING PROTEINS: S-COMPONENTS

S-components bind the transported substrates of ECF transporters. S-components are integral membrane proteins of ~20–25 kDa and are not related to soluble, periplasmic, or extracellular SBPs in type I and type II ABC importers (**Figure 1**; also see the sidebar titled Overview of the ABC Transporter Superfamily) (53). Crystal structures have been determined of isolated S-components from group I (NikM, YkoE) and group II transporters (BioY, FolT, RibU, ThiT) and of solitary BtuM, all in substrate-bound states (25, 30, 40–45) (**Supplemental Table 1**). The core structure of all S-components is a bundle of six α -helices arranged like a cylinder. Some group I S-components have additional N- or C-terminal extensions (38, 42, 45). Even though S-component families for different substrates do not share significant sequence similarity, their structures are well conserved. For example, the surfaces of S-components that interact with the ECF-T subunit consist mostly of α -helices 1, 2, and 3 and are structurally very similar (37, 40).

The orientation of the isolated S-components in the membrane has been deduced from the positive inside rule and molecular dynamics simulations, indicating that the N terminus and C terminus of the six-helix bundles are located in the cytoplasm and that the six helices are membrane-spanning (45, 72). In this orientation, the binding site is located close to the extracellular side of the membrane in a deep pocket (30, 40, 45). Substrates are bound with high affinity, characterized by dissociation constants often in the low nanomolar or subnanomolar range (**Supplemental Table 4**), which can be explained from the structures, as in many cases every possible hydrogen bond interaction between substrate and protein is satisfied (44, 73–75). The group I S-components NikM and CbiM (specific for nickel and cobalt ion, respectively) contain an additional N-terminal α -helix, and their N termini are located on the extracellular side of the membrane. The first two amino acids of the extra helices participate in substrate binding (38, 42).

In all isolated S-components from group II transporters as well as in the group I S-component NikM, the binding pocket is occluded by extracellular loops (25, 40–44). Structures of isolated S-components in the *apo* state have not been determined, but in the structures of the full complexes, the S-components are in *apo* states (32–38). Comparison of the *apo* and substrate-bound structures suggests that loop 1 (connecting transmembrane helices 1 and 2) and loop 3 (connecting helices 3 and 4) act as the extracellular gate for substrate access (37, 40, 41, 44, 72, 76). Other conformational changes do not appear to occur upon substrate binding in S-components; thus, the helical core of

S-components appears rigid. This conclusion is consistent with EPR experiments showing that changes in the conformation of loop 1 only occur upon thiamine binding to isolated ThiT (72). Different from this gating mechanism, the group I S-component YkoE from *B. subtilis* does not possess such gating loops. Although the substrate thiamine is deeply buried inside the protein, it is not occluded from the environment. Also, in the crystal structure of the solitary S-component BtuM, the binding site is not occluded (30, 45).

An unprecedented structural feature of the S-components became apparent when the first structures of full complexes of ECF transporters were solved (34, 35): The S-components are toppled in the complex by almost 90° compared with the predicted orientation of the isolated S-components. The transmembrane helices 1–4 lay parallel to the membrane, close to the cytoplasmic side, instead of traversing the bilayer. In the toppled state, the substrate binding site is accessible from the cytoplasm (**Figure 3**). It is noteworthy that the positively charged cytoplasmic loops remain located on the cytoplasmic side of the membrane in the toppled state, and they likely prevent a full rotation of the protein. Loops 1 and 3, which are predicted to be located extracellularly in the isolated S-components, are located close to the cytoplasmic side of the membrane in the toppled state. These loops generally do not contain charged residues, which may facilitate the adoption of the toppled state (37). Loop 5 remains close to the extracellular side of the membrane in both states (**Figure 3**).

Toppling may be a new mechanism of transport, which allows alternate exposure of the binding site to either side of the membrane, thus following the generic alternating access model of membrane transport (77, 78). In the proposed toppling mechanism, the substrate binding pocket travels through the membrane but remains confined to the S-component (**Figure 3**). It has been hypothesized that ECF-T plays a key role in aiding with the toppling of the S-component by offering a surface for the S-component to glide along, although solitary S-components like BioY and BtuM may achieve transport also by a toppling mechanism without an ECF module (29, 30, 35, 37). The toppling mechanism of transport resembles the elevator mechanism found in secondary transporters (79). In transporters using the elevator mechanism, the transport domain (equivalent to the S-component) moves through the membrane to carry the substrate from one side to the other, and a scaffold domain (equivalent to ECF-T) provides a stable scaffold along which the movement can take place. In the glutamate transporter family, the transport domains occlude the substrate completely (as in S-components of group II), whereas in other cases (such as in CitS or vcINDY) the substrates are not completely occluded by the transport domain and face the scaffold domain during transport (as may be the case in YkoE) (45, 79–83). A difference between the elevator mechanism and the toppling mechanism is that toppling involves mostly rotation of the S-component, with a minor translational component, whereas the converse takes place in elevator-like movements (79, 80, 84, 85). A structural feature common to all S-components that may facilitate toppling is the long helix 6 that in both the upright and toppled states is tilted relative to the membrane by $\pm 45^\circ$. This tilt is likely required to match the width of the lipid bilayer, meaning that it would stick out of it in a perpendicular orientation. Thus, this helix might act as a switch locking the S-component in either state (**Figure 3**).

Although S-components for different substrates do not share significant sequence identity, in the transmembrane helix 1 of group II transporters, a A-X-X-X-A motif is found, which is essential for the tight interaction with the coupling domains in the ECF module and activity of the transporter (28, 40, 86). For group I transporters, there are variations of the motif, like A-A-X-X-X-A for BioMNY or S/A-X-X-X-I/V-V for YkoEDC (45, 63). This motif does not seem to be conserved in solitary S-components, which do not interact with an ECF module, at least in the case of BtuM (30). Therefore, the motif appears to be critical for nonsolitary S-components to interact with the ECF module, but given the more extensive interaction surface, the motif alone is

POWER STROKE AND THERMAL RATCHET MECHANISMS OF MEMBRANE TRANSPORT (115, 116)

The coupling between the chemical reaction of ATP hydrolysis and the vectorial process of substrate translocation is poorly understood in ABC transporters. ATP hydrolysis is often assumed to drive a power stroke that forces the substrate to move from one side of the membrane to the other via a series of conformational changes. These conformational changes would be highly unfavorable without the free energy released by interactions of the protein with ATP, ADP, or inorganic phosphate. Coupling is achieved if the conformational changes can take place only when the transported substrate is bound.

The thermal ratchet model provides an alternative conceptual framework to describe ATP-coupled substrate transport. In a thermal ratchet model, the substrate movement step across the membrane occurs by thermal motions (as in facilitated diffusion mechanisms), but interactions of the transport protein with another component stabilize the inward-facing conformation. The bias comes at a price: Free energy from ATP hydrolysis is needed to reset the transporter for the next round of transport. In a ratchet mechanism, the resetting of the substrate-free transporter, instead of substrate movement across the membrane, is coupled to ATP hydrolysis.

unlikely to be sufficient to mediate specificity of complex assembly. Structural data provide a clue because large hydrophobic patches are involved in mediating protein–protein interactions (34, 35, 37). It remains unclear how the affinity of different S-components for the same ECF-T is tuned in group II transporters (see section titled Competition Between Different S-Components for the ECF Module) (28).

TRANSPORT MECHANISMS

Two mechanistic interpretations of the structural and biochemical data are currently prevalent, and they can be classified roughly as the thermal ratchet and power stroke models (see the sidebar titled Power Stroke and Thermal Ratchet Mechanisms of Membrane Transport) (37, 39). We discuss these mechanistic models using **Figure 4**.

State 1 is the only conformation for which the full complexes have been structurally resolved to date (**Supplemental Table 1**). The ATPases are in the open conformation, and the *apo* S-component is toppled over with its binding pocket exposed to the cytosol. The side chains that constitute the high-affinity substrate binding site in the isolated S-components are displaced in this state, which suggests that substrate affinity is lost. This notion is consistent with the observation that binding of the transported substrate to the full complexes in the nucleotide-free state has not been observed (32, 39). Apparently, the free energy released by the interaction between the S-component and ECF-T is used to destroy the binding site. The mechanism by which this disruption occurs may differ. In group II transporters, the binding site destruction is mostly allosteric, with the gating loops 1 and 3 pried apart as a result of the association of the S-component with the ECF module (32, 34, 36, 37). In the group I transporter CbiMNQO, direct competition also plays a role, with a phenylalanine side chain of ECF-T entering the binding pocket (38, 42) (**Figure 5**). This competition resembles the scoop-loop mechanism used by the type I ABC transporter for maltose, and a similar mechanism is proposed for the type II ABC transporter for vitamin B₁₂, even though their transport mechanisms are unrelated to that of ECF transporters (70, 87).

Both the power stroke and thermal ratchet models postulate that Mg-ATP binding leads to reorientation of the *apo* S-component to an upright conformation, with the empty binding site exposed to the outside, ready to bind a substrate molecule from the environment. In group II

Figure 4

Proposed transport mechanisms for ECF-type ABC transporters. ATPases are shown as gray and purple triangles, ECF-T is shown in cyan with its transmembrane domain as a box and the two coupling helices as bars, an S-component is depicted in yellow, and transported substrate is represented by peach triangles. In state 1 of the transporter (*bottom, center*), the ATPases are nucleotide free and separated (open conformation) and the S-component is in its toppled state with the binding site disrupted and exposed to the cytoplasmic side. The membrane domain of ECF-T may be flexible, as indicated. Upon binding of ATP molecules (*top, center*) the ATPases move toward each other, which also results in a different conformation of the coupling helices potentially disrupting the interaction interface with the S-component. This disruption leads to dissociation of the S-component, which topples to the upright conformation with the binding site exposed to the exterior. In this conformation, the S-component can tightly bind the transported substrate. These steps are common to both proposed mechanisms. (a) In the thermal ratchet model, ATP is hydrolyzed and ADP + P_i are released, which results in separated ATPases. The substrate-bound S-component can now associate with the complex, and flexibility in the membrane domain of ECF-T allows for interaction with different S-components. The S-component topples over and clicks onto the coupling domain, thereby disrupting its substrate-binding site and releasing the substrate into the cytosol. (b) In the power stroke mechanism, the substrate-bound S-component can associate only with the nucleotide-bound ECF module, and hydrolysis of ATP triggers toppling. This results in binding-site disruption and substrate release. Abbreviations: ABC, ATP-binding cassette; ECF, energy-coupling factor.

transporters the S-component may dissociate from the ECF module in this step. ATP binding to the ATPase subunits likely leads to repositioning of the coupling helices in ECF-T, which would modify the tight interface with the S-component, located on the opposite face of the coupling helices, and thus can lead to reorientation the S-component (37, 39).

The most direct evidence for this step comes from monitoring S-component dissociation from the ECF module in detergent solution upon Mg-ATP binding and associated substrate binding to the S-component (39).

Release of the S-component from the complex is a prerequisite for the observed competition of different S-components for the same ECF module (see section titled Competition Between Different S-Components for the ECF Module). Whether release also occurs in group I transporters is not clear. The group I transporter BioMNY does not release the S-component BioY

Figure 5

Mechanisms of substrate release by binding-site disruption by ECF-T. (a) In CbiMQO in the *apo* state, loop 1 (red) of the S-component CbiM (yellow) has moved away from the binding pocket, effectively disrupting the binding site, and the pocket is occupied by the side chain of Phe75 located in helix 3 of ECF-T (cyan). (b) When the Ni²⁺-bound S-component NikM is structurally aligned with CbiM, Phe75 would clash with the S-component; therefore, a substrate-bound state in the full complex is not possible. The S-component binds either the substrate or the ECF module, but not both. Abbreviations: ABC, ATP-binding cassette; ECF, energy-coupling factor.

when reconstituted in nanodiscs, whereas in detergent solution, they do dissociate. In the former case, possible release may have been obscured by the belt protein, which creates a confined bilayer patch. In the latter case, the authors state that the observation is not relevant because conditions were nonphysiological (29, 63).

Once the S-component has bound the transported substrate from the outside, the two models start deviating (Figure 4). In the power stroke model (39), the substrate translocation step is deterministic: The substrate-loaded S-component binds to the ATP-bound ECF module, which triggers ATP hydrolysis, thereby opening the ATPase subunits with concomitant rearrangement of the coupling helices of ECF-T, essentially pulling the S-component to the inward-facing toppled state observed in the crystal structures of the full complexes (Figure 3). This inward state has the disrupted substrate binding site, facilitating the release of the transported cargo and leading to accumulation of the substrate in the cytosol.

In the thermal ratchet model (37), the ECF module hydrolyzes ATP regardless of the presence of a substrate-loaded S-component. The substrate-loaded S-component can topple over spontaneously. It can then reach the toppled state by clicking into the interaction surface of ECF-T, again leading to disruption of the binding site and release of the substrate. In this mechanism, ATP hydrolysis is required not to translocate the substrate but to reset the ECF module and regenerate the binding interface for the S-component (Figure 4).

Figure 6

Membrane distortions caused by ECF-type ABC transporters. The conformation of the membrane around ECF-PdxU2 was simulated and taken from the MembProtMD database (<https://memprotmd.bioch.ox.ac.uk/home/>). The figure shows a slice taken from two angles through ECF-PdxU2 (surface representation, coloring for surface exposure to acyl-chains from *light yellow* to *light red*, not exposed to exposed) and the surrounding membrane boundaries (*black dots*). The transporter distorts, bends, and thins the membrane. Abbreviations: ABC, ATP-binding cassette; ECF, energy-coupling factor.

The thermal ratchet model postulates that S-components can topple over spontaneously once substrate is bound. Although substrate binding leads to burying of exposed hydrophilic and charged residues in the S-components, which might make toppling possible, it has been questioned if such spontaneous toppling can occur. Molecular dynamics simulations seem to argue against it, but it is noteworthy that such simulations were done in nonnatural, homogeneous bilayers (45). Bilayer imperfections *in vivo* could possibly facilitate toppling. In addition, the ECF module itself may cause bilayer wobbles, allowing the substrate-loaded S-component to topple over only in the vicinity of the ECF module. Indeed, the full ECF transporter complexes appear to wobble and twist the bilayer (**Figure 6**). In addition, the conformational flexibility of the membrane domain of ECF-T, as observed in the crystal structures (see section titled The Scaffold Subunit ECF-T), may also aid to reshape the membrane and hence facilitate toppling.

Arguing against the thermal ratchet model is data from Karpowich et al. (39), which showed that the substrate-bound S-component in detergent solution interacts with a hydrolysis-inactive mutant of the ECF module in the Mg-ATP-bound state with an affinity of $K_D \sim 8 \mu\text{M}$. However, it was not shown if the S-component still had substrate bound in the complex, what orientation the S-component adopted, and whether nucleotide was still bound (39). Further testing is required to show if this interaction is relevant in the wild-type protein and if it occurs in lipid bilayers. Future experiments should preferentially be done in as native conditions as possible, because the use of mutant proteins, detergent micelles, lipid nanodiscs, and proteoliposomes of unnatural lipid composition may obscure essential steps.

The power stroke model postulates that a specific interaction of the substrate-bound S-component with ECF-T triggers ATP hydrolysis. ECF-T must therefore recognize structural differences between substrate-free and substrate-bound S-components, which are small and confined largely to differences in the position of loop 1, located extracellularly (see section titled Membrane-Embedded Substrate-Binding Proteins: S-Components) (72). Such discriminative interaction has not yet been observed. For group II transporters, this mechanism implies that S-components for different substrates all can induce ATP hydrolysis. However, loop 1 is not conserved between different S-components in either length, conformation, or sequence (13, 25, 40, 41, 43, 44).

Possibly, the power stroke and thermal ratchet mechanisms are not as different as they seem. The observed futile ATPase activity of ECF transporters may lead to continuous remodeling and wobbling of the bilayer, which would facilitate toppling in the thermal ratchet model (37). In that case, ATP hydrolysis would facilitate toppling without necessarily being deterministic. Both models differ from proposed mechanisms for type I and type II ABC importers, in which the binding of ATP is associated with substrate release from the soluble binding protein (53).

Much less is known about the transport mechanism used by solitary S-components. So far, transport of biotin and cobalamin by members of the two largest families of solitary S-components (BioY and BtuM, respectively) has been assayed *in vivo*, by complementation studies of specifically engineered, recombinant *Escherichia coli* strains that are auxotrophic for the respective substrates (29, 30, 88). Although such *in vivo* transport assays can be regarded as physiologically relevant, further testing is required to corroborate the transport claims (30).

Mechanistically, how solitary transporters work is unclear. Although it cannot be ruled out that solitary S-components interact with a yet-to-be-identified protein, it is unlikely that BioY and BtuM encounter a specific partner in the heterologous *E. coli* expression host used for the complementation assays (29, 30). On the basis of the predicament that S-components can topple spontaneously, a facilitated diffusion mechanism with intracellular trapping was suggested for BtuM in which transport of cobalamin through the membrane could be achieved by toppling (30). For BioY, the possibility of S-component dimerization as part of the transport mechanism has been proposed, but conclusive data to support this hypothesis are lacking (51). It is noteworthy that cobalamin and biotin are required only in minute quantities by the cell, and thus a slow transport rate might be acceptable (89, 90).

Whether solitary BioY and BtuM have acquired a transport function that is absent from the S-components in group I and II transporters needs to be established. It appears that most group I and II S-components cannot support transport in similar complementation assays without the expression of an ECF module (32, 36, 40, 41). For instance, whereas solitary BtuM does support transport of cobalamin, the isolated group II S-component CbrT for the same substrate does not. In the latter case, complementation occurs only when the ECF module is expressed simultaneously (32). Isolated BioY from the group I transporter BioMNY from *R. capsulatus* was initially thought to be able to transport biotin, but later complementation studies with engineered *E. coli* strains showed that only real solitary BioY proteins can transport the substrate (16, 29, 41, 51). The cobalt-specific S-component CbiM, together with auxiliary protein CbiN and the nickel-specific counterpart NikMN, may display solitary transport activity. The additional components CbiN and NikN were required for this transport to occur (38, 91). These components could possibly facilitate toppling. Notably, potential substrate transport by isolated S-components from group I ECF transporters is not likely to be physiologically relevant, because these transporters form dedicated complexes, from which the S-components may not dissociate (63, 64).

COMPETITION BETWEEN DIFFERENT S-COMPONENTS FOR THE ECF MODULE

The structures of the group II ECF transporters containing identical ECF modules but different S-components have revealed how different S-components can interact with the same ECF module (see section titled The Scaffold Subunit ECF-T). The shapes of the interaction surfaces of the S-components are well conserved despite lack of sequence similarity (34, 35, 37, 41). Intriguingly, S-components compete more effectively for the same ECF module during transport catalysis in the presence of the transported substrate than in the *apo* state. This observation was made originally by Henderson et al. (7) and later confirmed in recombinantly expressed

ECF transporters (92). Thus, during turnover, ECF transporters must be able to distinguish between the *apo* and substrate-bound states of the different S-components (92). In the power stroke model, such distinction is direct, as only the interaction with the substrate-bound S-components leads to ATP hydrolysis. In the thermal ratchet model, the distinction is indirect. Toppling of the S-component is more efficient in the bound state than in the *apo* state, likely because hydrophilic loops 1 and 3 are exposed in the *apo* state, making toppling energetically highly unfavorable (37). In either case, dissociation of the S-component from the ECF module is required to explain competition, something that was demonstrated directly for Group II ECF transporters (39).

Not all S-components are equally good competitors for the ECF module. In the work by Henderson et al. (7), biotin had only minor effect on transport of folate or thiamin, whereas biotin uptake was greatly affected by the addition of both of the other vitamins. Thus, it appeared that transport of different substrates follows different kinetics, although the ECF module is the same (7). In more recent studies, these findings were corroborated in a recombinant system (92). The group II ECF transporter from *L. lactis* catalyzes import of thiamin and niacin (S-components ThiT and NiaX, respectively). When expressed in *E. coli*, niacin transport was approximately 100-fold faster than thiamine transport (40, 92), indicating that transport kinetics are strongly dependent on the nature of the S-component, even if the ECF module is the same. (7, 92). This difference in transport rate points toward the possibility that the two S-components have different modes of interaction with the same ECF module, although other factors may come into play, such as propensity to topple, ability to diffuse away from the ECF module, or ease by which the gates can be pried open by the coupling domain.

There are also examples from type I ABC transporters that can interact with different SBPs. For example, in *S. typhimurium*, two SBPs (HisJ and ArgT) share one ABC importer for the uptake of histidine and arginine, respectively (93). The glutamine and asparagine ABC transporter GlnPQ uses two different SBPs that are fused to the transmembrane part in a tandem configuration and, hence, compete for the same translocation channel for their substrates (94). The latter system was used to elucidate in detail the dynamics and kinetics of the interaction between the SBPs and the transmembrane part on the single-molecule level, a technique that will also be useful to investigate ECF transporter assembly in the future (95). In another example, in the *Thermotoga maritima* mannose ABC transporter, variants of the same SBP with different affinities but the same substrate specificity interact with the transporter, increasing its dynamic range for substrate recognition. This adaptation allows the organism to optimally react to changing environmental concentrations of the substrate (96). Because the various substrates of ECF transporters are required in different quantities, the apparent difference in competition kinetics could mirror the requirements of different substrates of the cell.

CONCLUDING REMARKS

The relationship between ECF-type ABC transporters and conventional ABC transporters and the mechanism used by the latter have been reviewed extensively in the past (17, 27, 28, 53, 54, 97–99). In this section, we place focus on two aspects of ECF transporters.

First, ECF transporters most likely function by dynamic toppling of S-components. The α -helical segments 1–4 in the S-components can therefore be oriented as membrane spanning (upright) or horizontal (parallel to the membrane plane). Horizontal helices have recently also been found in unrelated membrane proteins. Two prominent examples are the eukaryotic retinol transporter STRA6 and the rotary ATP synthases (100, 101). A single-particle cryo-electron microscopy (cryo-EM) structure of STRA6 showed that the dimeric assembly has a large outer

cleft with two layers of horizontal α -helices at the bottom in the center of the membrane. The horizontal helices are likely the location of the substrate binding site. The translocation of the substrate by STRA6 would not require rearrangement of these helices; thus, they can be considered static (100). The ATP synthase uses two long horizontal helices in the a-subunit in interaction with the c-ring for separating entry and exit pathways for the protons. This structural feature is also static (101, 102). In contrast, the horizontal helices in the S-component are predicted to undergo dynamic transitions between horizontal and transmembrane orientations (28). Reorientation of helices may also occur occasionally in membrane proteins during biogenesis and folding; however, in these cases the transition happens only once, whereas in ECF transporters the transitions are expected to occur during each turnover (103). To a lesser extent than in ECF transporters, dynamic transitions of the orientation of membrane helices occur in other transporters, too—for instance, in the domain movements during elevator-like transport (79, 80, 84). Hence, ECF transporters may be on the extreme end of the scale, but it appears that horizontal helices and dynamic transitions in the orientation of membrane-spanning α -helices are more general (103, 104).

Second, ECF transporters may be suitable protein complexes to study integral membrane protein–protein interactions (105, 106). A few other membrane protein model systems have been established for the same purpose. Dimerization was studied using glycophorin A, which consists of a single α -helix. A common interaction motif was identified: the G/S/A-X-X-X-G/S/A (X mostly hydrophobic in nature) motif and multiples thereof (86, 107–109). A more complex model system is the stable dimer of the *E. coli* chloride transporter ClC-ec1. Mutations allowed for destabilization of the interface, which made this protein amenable to study the homodimerization of a polytopic membrane protein (110, 111). However, because ClC-ec1 has a single interface, this system does not allow for the study of dynamic reorientation of membrane protein interfaces, which frequently occurs in transport proteins—for example, in the glutamate transporter homolog Glt_{TL}, the citrate transporter CitS, and the succinate transporter vcINDY—in which a transport domain slides along a static scaffold domain (80–83, 112). Glt_{ph} has been used to study the kinetics of intraprotein movement by a single-molecule fluorescence approach, but the molecular determinants that allow these dynamic transitions are not understood (113). ECF transporters may be a suitable system to address questions of kinetics of dynamic complex assembly in a lipid environment (S-component association/dissociation), structure–function relation [greasy van der Waals surface provided by ECF-T (35)], and molecular determinants of dynamic membrane protein interactions. The latter may be of a more general interest, as it would show how a natural modular system achieves tight interaction and polyspecificity at the same time.

FUTURE ISSUES

1. The distinction between power stroke and thermal ratchet models of transport may be made by using single-molecule methods that can monitor the timing of ATP hydrolysis and substrate translocation.
2. Structures of full ECF transporter complexes in other states need to be solved, possibly using single-particle cryo-EM.
3. The function of lone ECF-T proteins in plants and Cyanobacteria must be determined and possibly could involve lipid toppling (scrambling).
4. Specific lipids and lipid composition of membranes have strong effects on transporter activity, and these effects need to be tested with ECF transporters.

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5. ECF transporters can be used to understand dynamics of membrane protein–protein interactions in the lipid environment.
6. Future work is needed to understand the role of the ATPases' C-terminal domain.
7. How solitary S-components mediate transport needs to be established.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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