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Poolman, Berend; Slotboom, Dirk

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Bert Poolman and Dirk-Jan Slotboom
Department of Biochemistry, University of Groningen, Groningen, The Netherlands

Definition

- ABC = ATP-binding cassette. ABC proteins are defined by conserved sequence motifs in the nucleotide-binding domain ("the ATP-binding cassette"), which are critical for the binding and hydrolysis of Mg-ATP.

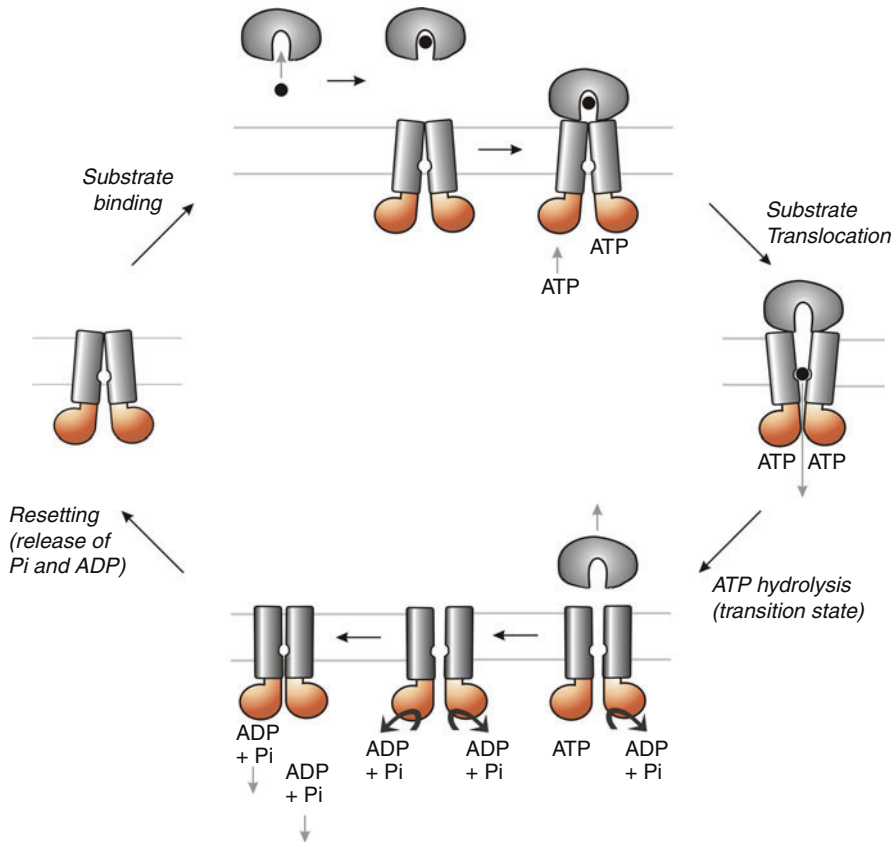
  ATP-binding cassette transporters = ABC transporters = ABC importers and exporters
  Before 1992, ABC transporters were also referred to as traffic-ATPases.
- ABC exporter catalyzes ATP-driven export of compounds from the cytoplasm to the external medium or organelle lumen.
- ABC importer catalyzes ATP-driven uptake of solutes from the medium into the cytoplasm of prokaryotes.
- SBP = Substrate-binding protein, originally referred to as periplasmic binding protein or substrate receptor. Some ABC transporters have the SBP fused to the transmembrane domain (TMD) and here the receptor is referred as substrate-binding domain (SBD).
- TMD = Transmembrane domain. Two TMDs are present per functional ABC transporter; the TMDs can be separate proteins, fused to each other, fused to the NBD, and/or fused to the SBD.
- NBD = Nucleotide-binding domain. Two NBDs are present per functional ABC transporter.
- ECF = Energy coupling factor. A subset of ABC transporters (named ECF-type) does not require an SBP/SBD for solute import. Instead, they use an integral membrane substrate-binding protein (S-component).
- ECF module = a tripartite complex with which the S-component associates to form a complete transporter. The module consists of a membrane-embedded subunit (EcfT or T-component) and two NBDs (EcfA or A-component).
- Translocator = TMD plus NBD that facilitates the movement of substrate across the membrane.
- Atypical ABC transporters are neither importers nor exporters, but fulfill different functions, e.g., ATP-gated channels like CFTR.

Summary

Most ABC importers known to date employ a soluble substrate-binding protein to capture the ligand and donate the molecule to the translocator. The SBP can be a soluble periplasmic protein or tethered to the membrane via a lipid moiety or protein anchor or fused to the translocator. In the hybrid ABC transporters, multiple SBDs can be fused in tandem and provide several extracytoplasmic substrate-binding sites. A subset of ABC transporters employs a membrane-embedded S-component to capture the substrate. The S-component together with the ECF module also forms the translocation path for the substrate. Multiple S-components can associate consecutively with one and the same ECF module. An overview of the mechanism of substrate capture by different types of ABC transporters is presented, together with a scheme illustrating the alternating access mechanism for the overall transport process.

Introduction

ATP-binding cassette (ABC) proteins serve many functions, including the transport of nutrients into the cell, transport of compounds across organellar membranes, the secretion of proteins, antigen (peptide) presentation, cell volume regulation, regulation of protein synthesis, detoxification, and antibiotic resistance. The vast majority of ABC proteins are part of complexes that mediate vectorial translocation, i.e., the systems transport molecules across cellular or organellar membranes. A smaller group of ABC proteins is associated with soluble (supra)molecular complexes and involved in DNA repair, recombination, chromosome condensation and segregation, and translation elongation. Regardless of whether the ABC proteins are found in membrane transport or soluble (supra)molecular complexes, they provide a power stroke in which chemical energy is converted into mechanical energy (e.g., for a translocation or dislocation event). The mechanism of transport of



Substrate Capture by ABC Transporters, Fig. 1 Model of substrate capture and alternating access in ABC importers. A solute is captured by a soluble substrate-binding protein and transferred to the membrane-embedded translocator (*top*). Upon binding of ATP, the translocator is brought in the outward-facing conformation and is capable of accepting the substrate. Subsequent isomerization of the binding site from out to in allows the substrate to be released on the *trans* side of the membrane. Upon hydrolysis of ATP and release of the reaction products (*bottom*), the system is reset for another translocation cycle. Although both Type I and Type II ABC importers operate according to the alternating access mechanism, the nucleotide-induced conformational changes are opposite. In the maltose system (Type I) binding of ATP triggers the outward-facing conformation, whereas in the vitamin B12 transporter (Type II) ATP binding elicits a conformational change from outward- to inward facing (Joseph et al. 2011)

ABC importers involves the binding and release of substrate from a dedicated extracytoplasmic substrate-binding protein and alternating access of the substrate-binding site in the translocator domain. A simplified scheme of solute translocation by ABC importers is depicted in Fig. 1. In this entry, the emphasis is on ABC proteins that mediate transport of solutes into prokaryotic cells and the focus is on the proteins or protein domains (SBP, SBD, S-component) that capture the substrate from the medium and deliver it to the translocator.

Classification of ABC Transporters

ABC transporters have been subdivided into exporters and type I and type II importers. Exporters mediate transport of molecules from the cytoplasm to the external medium or organelle lumen. They bind their ligands directly within the TMD, without the need for accessory proteins. Access of the binding pocket is thought to be either from the membrane (lipophilic ligands) or cytoplasm (hydrophilic ligands), depending on the system (Bolhuis et al. 1996). Upon binding and

hydrolysis of Mg-ATP, the ligand is translocated across the membrane and released from the outward-facing conformation of the protein. Prototypical examples of ABC exporters are P-glycoprotein (Pgp), the transporter for antigen presentation (TAP), and the bacterial putative drug efflux system Sav1866 (Hollenstein et al. 2007).

Type I and type II importers capture their ligand via so-called accessory substrate-binding proteins (SBP). Upon substrate capture, the SBP changes conformation from open to closed and, subsequently, docks onto the translocator. Through the binding of Mg-ATP in the NBDs, the translocator transits from an inward- to an outward-facing conformation, which allows the transfer of the substrate from the SBP to the TMD. Subsequent hydrolysis of ATP and release of ADP and inorganic phosphate (Pi) completes the reaction cycle and releases the substrate on the *trans* side of the membrane. Type I and type II importers differ in their architecture of the SBP and TMD, but also mechanistically these transporters appear to operate differently (Davidson et al. 2008; Lewinson et al. 2010). The maltose transporter MalFGK2E from *E. coli* is the paradigm for type I import (Davidson et al. 2008; Oldham et al. 2007), the vitamin B12 transporter BtuCDF from *Escherichia coli* is prototypical of a type II importer (Locher et al. 2002). For type I importers, it has been shown that a limited number of related SBPs may associate with one and the same translocator, enabling the system to import multiple distinct substrates. There is evidence that type I importers hydrolyze two molecules of ATP per substrate translocated (Patzlaff et al. 2003). The power stroke for transport is elicited by the binding of ATP; the hydrolysis of ATP and release of reaction products resets the system to the ground state (Davidson et al. 2008; Hollenstein et al. 2007).

Here, the ECF transporters are defined as type III importers. ECF-type ABC transporters capture their substrates via membrane-embedded S-components, which associate with the ECF module (EcfT or T-component plus two NBDs, often referred to as A and A' component). The type III importers transport micronutrients such as vitamins and trace metal ions, and multiple different S-components can associate with a single ECF module (Rodionov et al. 2009). How the substrate, bound via the S-component, is subsequently transported is poorly understood compared to type I and type II importers. Crystal structures are available for riboflavin- and thiamine-specific S-components (Erkens et al. 2011; Zhang et al. 2010). Rearrangement of the membrane-embedded L1 loop of the S-component may open a lateral gate for the vitamin facing the EcfT subunit. Repositioning of L1 will perturb the binding site residues, which will reduce the binding affinity and allow the vitamin to leave the S-component. It has been hypothesized that the actual translocation step takes place on the interface between EcfT and the S-component, in line with the import of substrates via type I and II ABC transporters as well as exporters.

Structural Features of Substrate-Binding Proteins

Substrate-binding proteins were first discovered in the periplasm of *E. coli* (Berger and Heppel 1974), a Gram-negative bacterium, and even to date they are often referred to as periplasmic binding proteins. The first SBP crystal structure, the L-arabinose-binding protein (ABP), was solved in 1974 (Quiocho et al. 1974). In general, the soluble SBPs are present in large excess over the translocator complexes in the membrane, allowing efficient capture of substrates and initiation of the translocation reaction. In Gram-positive bacteria and archaea, i.e., microorganisms lacking an outer membrane and periplasm, SBPs are exposed on the cell surface and attached to the cytoplasmic membrane via a lipid-anchor or a transmembrane-peptide (to date, the latter has been only observed in archaea), or they can be fused to the TMDs resulting in two SBDs per functional complex. In some cases, two or even three SBDs fused in tandem are linked to the TMDs and these systems have a total of four or six extracytoplasmic substrate-binding sites. Systems with SBDs fused to the TMDs can be also found in Gram-negative bacteria but less frequently than in Gram-positives (van der Heide and Poolman 2002). The linking of SBDs to the membrane or the fusing of multiple SBDs to the TMD increases the effective concentration of the substrate-binding sites near the translocator and may increase the

efficiency of transport. The mechanism of substrate binding of periplasmic, membrane-anchored, and TMD-fused SBDs is similar and so is the mode of action of the corresponding ABC transporters.

The archetype SBP consists of two lobes connected by a linker or hinge (domain). The two lobes close and engulf the ligand upon substrate binding (mode of substrate capture: akin to that of a Venus's Flytrap) (Quiocho and Ledvina 1996). Structures are available of SBPs in the open-unliganded, closed-unliganded, open-liganded, and closed-liganded forms, of which the latter conformation is thought to productively interact with the translocator complex. In solution, the equilibrium state of the SBPs is toward the open-unliganded conformation in the absence of substrate and closed-liganded in the presence of substrate. Mutational and structural analyses indicate that each lobe of the SBP binds to one of the TMDs (Davidson et al. 2008; Hollenstein et al. 2007), and conformational changes in the NBDs upon binding of ATP are transmitted via the TMDs to the SBP. Thus, indirectly ATP is involved in the opening of the SBPs and substrate transfer to the translocator.

SBPs are not only associated with ABC transporters but with a wide variety of translocation and signal transduction systems in both prokaryotic and eukaryotic organisms, including tripartite ATP-independent periplasmic (TRAP)-transporters, two-component regulatory systems, guanylate cyclase-atrial natriuretic peptide receptors, G-protein-coupled receptors (GPCRs), and ligand-gated ion channels (Berntsson et al. 2010). In addition, SBP domains are part of prokaryotic DNA-binding proteins involved in gene regulation. SBPs are very diverse in sequence and phylogenetic analyses based on multiple sequence alignments do not yield stable alignments. (The sequence identity of the proteins is often <20%.) However, the structures of SBPs are remarkably similar, which has been used to cluster the SBPs based on structural similarity instead of sequence similarity (Berntsson et al. 2010). The structures of more 100 SBPs have been superimposed in pairwise fashion to produce a structural distance tree. The SBPs were found to group into six defined clusters (A–F), three of which (cluster A, D, and F) were further subdivided. The analyses have shown that the proteins within the six clusters can be discriminated on the basis of the linker (hinge) region that connects the two protein lobes (Fig. 1): The generic secondary structure is $(\beta)_{4/5}(\alpha)_n$-hinge-$(\beta)_{4/5}(\alpha)_n$ (Fukami-Kobayashi et al. 1999). There is little or no correlation between the structural clustering and functional classification, i.e., SBPs with very different substrate specificities are present in each cluster. Furthermore, in the individual clusters, the proteins are not necessarily homologous, as judged from the absence of significant sequence similarity. Clusters B and F contain the most diverse SBPs, associated with various types of transport and signal transduction systems; SBPs in cluster A are unique to type II ABC transporters; cluster C and D contain SBPs of type I ABC transporters; and SBPs in cluster E are found in TRAP transporters. Specific information on the six clusters is presented below (Fig. 2):

Cluster A

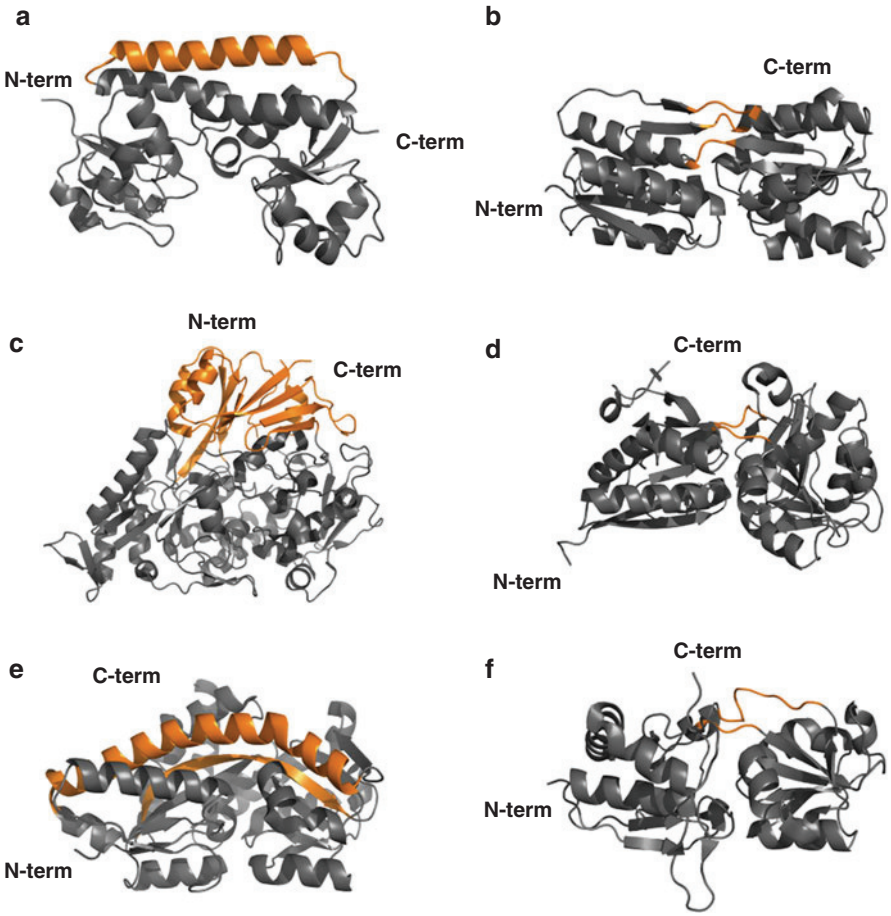
The distinguishing characteristic of cluster A is an α-helix serving as the hinge between the two domains. The rigidity of this helix is reflected in the small movement of both domains upon substrate binding. All of the SBPs in cluster A play a role in metal binding, either directly or as metal chelates. The cluster A SBPs associate with type II ABC importers.

Cluster B

Cluster B consists of SBPs that bind carbohydrates (such as ribose, glucose, and arabinose), branched chain amino acids, natriuretic peptides, and autoinducer-2 (AI-2). The SBPs in cluster B interact with type I ABC importers, two-component histidine-sensory complexes, and guanylate cyclase-atrial natriuretic peptide receptors. The hinge of the SBPs in cluster B is built of three distinct regions connecting the lobes. Homologous to the proteins in cluster B are the *lac*-repressor type transcription factors, such as the LacR, PurR, and CcpA.

Cluster C

The cluster C SBPs interact with type I ABC transporters and bind diverse ligands including di- and oligopeptides, arginine, nickel ions, and cellobiose. They all have an extra domain. For AppA from *B. subtilis* and OppA from *L. lactis*, it has been shown that the extra domain extends the oligopeptide-binding cavity in order to accommodate very large ligands. OppA from *L. lactis* binds peptides



Substrate Capture by ABC Transporters, Fig. 2 The different clusters of SBPs are shown with their distinct structural feature colored in orange. (**a**) Cluster A contains proteins having a single connection between the two domains in the form of a rigid helix. (**b**) Cluster B contains SBPs with three interconnecting segments between the two domains. (**c**) Cluster C contains SBPs that have an extra domain and are significantly larger in size when compared with the others. (**d**) Cluster D contains SBPs with two relative short hinges (**e**) Cluster E contains SBP associated with TRAP transporters which all contain a large helix functioning as hinge region. (**f**) Cluster F contains SBPs with two hinges similar like cluster D; however, these hinges have almost double the length creating more flexibility inside the SBP. Please note that clusters A, D, and F can further be subdivided based on the substrate of the SBP (see text). The proteins used to illustrate the features in clusters A–F are BtuF (PDB code: 1N2Z), RBP (PDB code: 1DRJ), OppA (PDB code: 3DRF), ModA (PDB code: 1ONR), UehA (PDB code: 3FXB), and HisJ (PDB code: 1HSL), respectively (Figure was taken from Berntsson et al. 2010)

with widely varying length (up to at least 35 residues) and sequence. The function of the extra domain in other SBPs is not known.

Cluster D

The discernible feature of these proteins is that their hinge-region consists of two short stretches, 4–5 amino acids long. This large group of SBPs binds a large variety of substrates: carbohydrates, putrescine, thiamine, tetrahedral oxyanion as well as ferric or ferrous iron. The subclusters found in this cluster correspond to the substrate specificity of the proteins (for details, see Berntsson et al. 2010).

Cluster E

In this cluster, all substrate-binding proteins are part of the TRAP transporter (tripartite ATP-independent periplasmic transporter) family. In contrast to ABC

transporters, the TRAP transporters use an electrochemical ion gradient to fuel the uphill translocation of substrates. The remarkable feature of TRAP SBPs is a large single ß-strand that is part of the two five-stranded ß-sheets of both lobes. All TRAP-dependent SBPs structurally characterized so far have conserved features, such as the strand order, typical of class II SBPs ($\beta_2\beta_1\beta_3\beta_n\beta_4$), an additional ß-strand connecting both domains as well as the number and positioning of the flanking α-helices. A second distinguishing feature is a long helix that spans both domains. Such a long helix is found in all crystal structures of SBP proteins reported for TRAP transporters, although in some structures, this helix is interrupted by a kink. The known substrates of cluster E proteins are limited to ectoine, pyroglutamic acid, lactate, 2-keto acids, and sialic acid.

Cluster F

The distinguishing feature of the cluster F proteins is a hinge consisting of two segments connecting the two lobes. The linker stretches of cluster F proteins are significantly longer (8–10 amino acids) than the hinges of 4–5 amino acids observed in SBPs of cluster D. Possibly the longer linker provides more flexibility between the open and closed conformation. Cluster F SBPs bind a large variety of substrates ranging from trigonal planar anions (nitrate, bicarbonate) to amino acids and compatible solutes such as glycine betaine. The overall structure of the proteins within cluster F is similar, but they can be subdivided based on their substrates (Berntsson et al. 2010). Noteworthy is the difference in primary sequence between OpuAC from *B. subtilis* and closely related proteins in cluster F: A domain swap has taken place.

Structural Features of S-Components

The crystal structures of the riboflavin-specific S-component from *Staphylococcus aureus* and thiamine-specific S-component from *Lactococcus lactis* have been determined (Zhang et al. 2010; Erkens et al. 2011). In both cases, the substrate was present in the protein. The overall fold of the two proteins is very similar (RMSD = 3.5 Å for 145 C_α atoms), although at the sequence level, the two proteins are unrelated (14% sequence identity). The lack of sequence conservation between S-components of the ECF transporters is remarkable, because these proteins interact with a common partner, the shared ECF energizing module. ThiT and RibU contain six hydrophobic helical segments that cross the membrane. A part of the L1 loop is also embedded in the lipid bilayer, which is needed because α-helix 2 is too short to span the entire thickness of the membrane. The position of L1 is thought to play an important mechanistic role in the translocation of substrates across the membrane.

The substrate-binding site is located in a pocket near the extracellular side of the membrane and lined by helices 4, 5, and 6 and the loops L1 and L5. In the crystal structures of both RibU and ThiT, the substrates are almost completely occluded. Entrance of a substrate into the binding site from the external side of the membrane would require conformational changes of loops L1, L3, and L5, which form lids on top of the substrate. On the basis of the structures of ThiT and RibU, it seems unlikely that the S-components by themselves line a translocation path. The translocation pore is most likely formed at the interface of the ThiT and EcfT subunits, requiring the repositioning of L1.

In conclusion, type I and type II ABC importers employ extramembranous substrate-binding proteins to capture substrate and deliver the molecule to the translocator. Type III ABC importers use an integral membrane protein (S-component) for the initial binding of substrate; transport is thought to take place at the interface of the S-component and another integral membrane protein. Whereas full structures of type I and type II ABC importers w/wo SBPs are available, the translocation mechanism by type III importers awaits further structural analysis.

Cross-References

- ▶ CFTR, Ion Channel Evolved from ABC Transporter
- ▶ Membrane Protein Function
- ▶ Membrane Proteins: Structure and Organization
- ▶ Membrane Transport, Energetics and Overview

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Subunit Composition of Protein Complexes

▶ Heteromeric Versus Homomeric Association of Protein Complexes

Succinate Dehydrogenase (Complex II)

Gary Cecchini
Molecular Biology, San Francisco VA Medical Center, San Francisco, CA, USA
Department of Biochemistry and Biophysics, University of California San Francisco, San Francisco, CA, USA

Synonyms

Menaquinol-fumarate reductase; Succinate-quinone oxidoreductase

Definition

Succinate dehydrogenase often referred to as complex II or succinate-ubiquinone oxidoreductase is the only membrane-bound member of the citric acid cycle (also called the tricarboxylic acid (TCA) cycle or the Krebs cycle).

Basic Characteristics

Succinate dehydrogenase or complex II in most organisms is composed of four distinct protein subunits (Cecchini 2003). These subunits are arranged in a hydrophilic domain exposed to the cytoplasm in bacteria or the matrix of mitochondria. The hydrophilic subunits are bound to the membrane through