Minireview

The structures of BtuCD and MscS and their implications for transporter and channel function

Randal B. Bass[a,1], Kaspar P. Locher[a,b,2], Elizabeth Borths[a], Yan Poon[a], Pavel Strop[a,3], Allen Lee[a,b], Douglas C. Rees[a,b,*]

[a] *Division of Chemistry and Chemical Engineering, 114-96, California Institute of Technology, Pasadena, CA 91125, USA*
[b] *Howard Hughes Medical Institute, California Institute of Technology, Pasadena, CA 91125, USA*

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Abstract The passage of most molecules across biological membranes is mediated by specialized integral membrane proteins known as channels and transporters. Although these transport families encompass a wide range of functions, molecular architectures and mechanisms, there are common elements that must be incorporated within their structures, namely the translocation pathway, ligand specificity elements and regulatory sensors to control the rate of ligand flow across the membrane. This minireview discusses aspects of the structure and mechanism of two bacterial transport systems, the stretch-activated mechanosensitive channel of small conductance (MscS) and the ATP-dependent vitamin B12 uptake system (BtuCD), emphasizing their general implications for transporter function.

Key words: Transport protein; Mechanosensitive channel; ATP binding cassette transporter; Membrane protein structure

1. Introduction

All cells are surrounded by one or more membranes that create a permeability barrier separating the inside of the cell from the extracellular environment. Consequently, passage of most biologically relevant molecules across these membranes must be mediated by specialized proteins known as transporters. Currently, over 360 families of transporters have been identified through biochemical and genomic analyses [1], highlighting the significance of transport processes in cellular metabolism. The integral membrane proteins that mediate these transport processes may be divided into two general categories: channels that facilitate the movement of molecules across the membrane in their thermodynamically favorable direction, and active transporters that function as molecular pumps to translocate their substrates across the membrane against a chemical potential gradient. For the latter category of transporters, this thermodynamically unfavorable process is powered by coupling to a second, energetically favorable process such as ATP hydrolysis, oxidation-reduction reactions, or the movement of a second solute down a transmembrane concentration gradient.

Although these transport families encompass a wide range of functions, molecular architectures and mechanisms, there are several common elements that must be incorporated within their structures [2]. These include a translocation or permeation pathway that enables the transmembrane passage of substrates, specificity elements to ensure that the proper substrate is transported, and regulatory sensors that control the flow of molecules across the membrane. As described in this issue, the past five years have witnessed an explosion of progress in the structural characterization of channels and transporters that has started to establish the structural organization of these basic elements. In this minireview, we describe aspects of the structure and mechanism of two bacterial transport systems, the stretch-activated mechanosensitive channel of small conductance (MscS) and the ATP-dependent vitamin B12 uptake system (BtuCD), emphasizing general implications for the transporter function.

2. The mechanosensitive channel MscS

The characterization and identification of intrinsically mechanosensitive (stretch-activated) channels in bacteria reflect the pioneering efforts of C. Kung and co-workers, who identified the first mechanosensitive channel (MscS) activity in *Escherichia coli* in 1987 [3,4]. Recent reviews of mechanosensitive channels emphasizing their structures and gating mechanisms may be found in [5–9]. The conductance of MscS is approximately 1 nS (several orders of magnitude larger than voltage-gated K^+ channels, for example), with a slight preference for anions. In the absence of applied tension, the closed state is stabilized by 28 kJ/mol [10]. Intriguingly, MscS also exhibits voltage-dependent gating, with the channel opening more easily as the membrane is depolarized [3,11]; unfortunately, the conductance of MscS exhibits desensitization, which has so far precluded a quantitative analysis of this voltage sensitivity. In 1999, Booth and co-workers established

*Corresponding author. Fax: (1)-626-744 9524.
E-mail address: dcrees@caltech.edu (D.C. Rees).

[1] Present address: Amgen, Seattle, WA, USA.
[2] Present address: Institut für Molekularbiologie und Biophysik, Eidgenössische Technische Hochschule Zürich, 8093 Zürich, Switzerland.
[3] Present address: Department of Molecular and Cellular Physiology, Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, CA 94305, USA.

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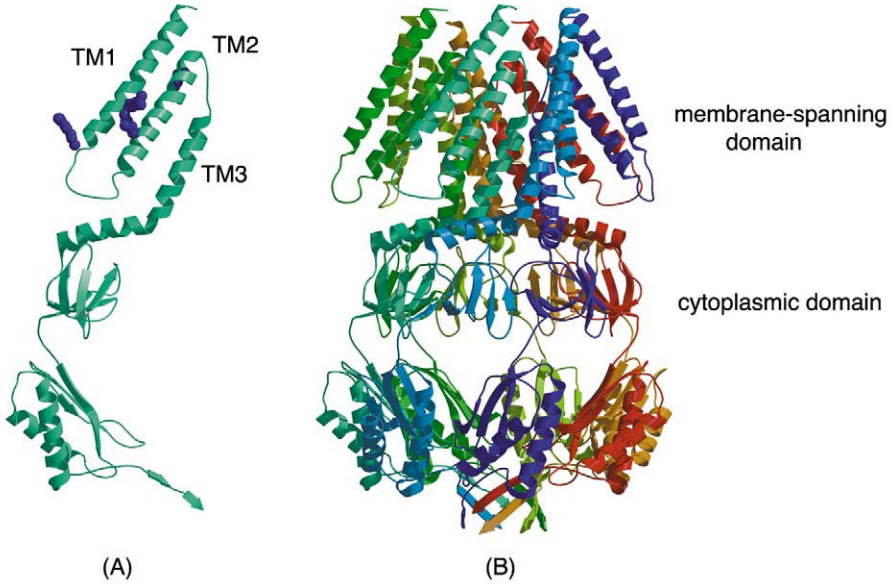

Fig. 1. Ribbon diagrams of the MscS channel [13]. A: The polypeptide fold of an individual MscS subunit, with the N- and C-termini of the protein at the top and bottom of the figure, respectively. The positions of the three membrane-spanning helices, TM1, TM2 and TM3, are indicated. Arginine residues located in the membrane-spanning region of this subunit are represented by CPK models. B: Side view of the MscS heptamer viewed from the same direction as in A, with each subunit represented in a separate color. Figures were prepared with MOLSCRIPT [40] and RASTER-3D [41].

that the 286 amino acid protein encoded by the yggB gene was associated with MscS activity [12].

The crystal structure of *E. coli* MscS [13] established that the channel is a homoheptamer that can be divided into transmembrane and cytoplasmic domains (Fig. 1). The transmembrane domain is organized around three membrane-spanning helices, designated TM1, TM2 and TM3, in each subunit. Starting from the periplasmic N-terminus, the TM1 and TM2 helices within one subunit are packed together in an antiparallel fashion that buries an extensive interface, but makes few contacts with other helices in the transmembrane domain. TM3 returns to the cytoplasm through the channel interior and generates the permeation pathway. Interestingly, the TM3 helix is kinked at Gly113, so that the axis of the C-terminal end of this helix is oriented nearly perpendicular to the membrane normal. The cytoplasmic domain of MscS is extensive, with the most notable structural feature being the presence a large, interior chamber of $\sim$40 Å diameter that connects to the cytoplasm through multiple openings ($\sim$14 Å diameter) formed between the component domains of each subunit. The entire heptameric assembly is linked at the carboxy-terminus by a seven-stranded parallel β barrel that contains one strand from each subunit.

The equilibrium between closed and open states of MscS can be shifted towards the open state by a combination of tension applied to the membrane and depolarization of the membrane potential [3]. The sensitivity to tension and voltage are coupled so that as the membrane is depolarized, less tension is required to open the channel, and vice versa. The diameter of the permeation pathway in the crystal structure is $\sim$12 Å, and probably represents the open state of the channel. In the absence of direct characterization, the structural changes associated with channel closing are not known, although it seems plausible that the transition involves changes in TM3 (perhaps through tilting or kinking of the helix near Gly113), by analogy to observations on the gating process in MscL [14,15] and K channels [16,17]. Biochemical studies have also implicated substantial rearrangements in the cytoplasmic domain of MscS during channel gating [18,19].

Although a detailed molecular mechanism remains to be defined, the TM1–TM2 helical hairpins at the periphery of the membrane-spanning region are likely candidates for mediating the tension and voltage sensitivities of MscS [13]. A swinging of this hairpin with respect to the permeation pathway would generate a change in the cross-sectional area of the channel, coupling the protein conformation to applied membrane tension. Furthermore, TM1 and TM2 each contain several arginine residues, most notably Arg46 in TM1 and Arg74 in TM2, that are in the likely membrane-spanning region where they would be appropriately sited to respond to changes in membrane potential. Consequently, rearrangements of the TM1–TM2 hairpin would not only change the cross-sectional area, as required for the mechanosensitivity, but would also reposition the membrane-embedded arginines, conferring sensitivity of MscS to changes in membrane potential. The positive electrostatic potential arising from another arginine, residue 88, near the lip of the permeation pathway, may contribute to the preference for anion conductance noted for MscS.

2.1. Implications for voltage-dependent gating

The best-studied voltage-gated channels are members of the Kv potassium channel family. Each subunit of a tetrameric Kv channel contains six transmembrane helices, designated S1 to S6, with a series of arginine residues present in the S4 helix (see [20]). In the structure of KvAP, an archaeal homolog of Kv channels, determined by MacKinnon and co-workers [21], the S4 helix, together with the C-terminal region of the S3 helix, forms a helical hairpin termed the voltage-sensor paddle. This paddle is suggested to 'operate somewhat like hydro-

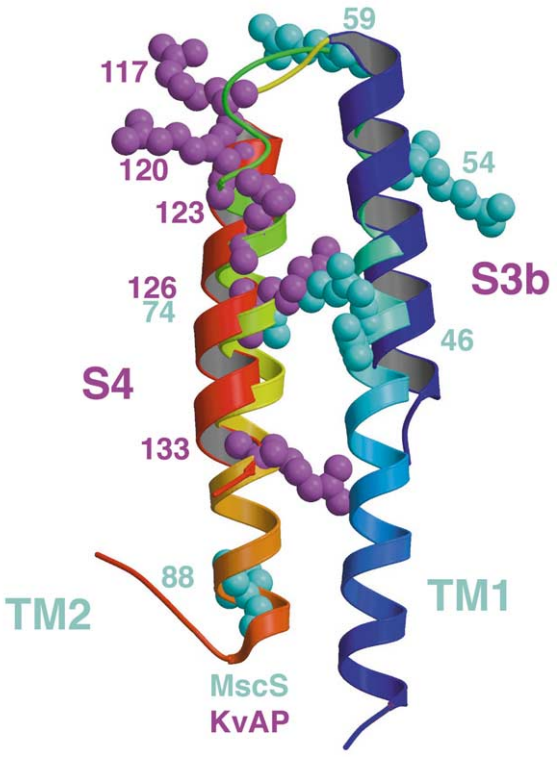

Fig. 2. Comparison of the helical-hairpin sensor elements for MscS [13] and the KvAP voltage-gated potassium channel from *Aeropyrum pernix* (KvAP) [21]. The superimposed TM1–TM2 of MscS (thin ribbons) and the S3b–S4 helices of KvAP (thick ribbons) are colored with a rainbow gradient scheme from the N-terminus (blue) to the C-terminus (red). Arginine residues within these helical hairpins are shaded cyan and magenta, for MscS and KvAP, respectively.

phobic cations attached to levers' [21], to open and close the pore in response to changes in membrane potential. This is the same general mechanism for voltage gating proposed previously for MscS [13]. Indeed, the structures of the corresponding regions in MscS and KvAP are quite similar (Fig. 2). The observation that Kv channels are also mechanosensitive further emphasizes the similarity in the conformational rearrangements proposed for gating by Kv-type and MscS channels [22]. Although the structures and functions of Kv and MscS channels are quite distinct, the sensor elements share 'the presence of positively charged groups (arginines) in the membrane-spanning region and the relatively loose packing against the remainder of the channel, so that voltage-mediated changes in the position of these helices can occur and be coupled to the opening and closing of the permeation pathway' [13].

3. The vitamin B12 importer BtuCD, an ATP binding cassette (ABC) transporter

ABC transporters are a ubiquitous family of importer and exporter proteins that pump their ligands across the membrane against a concentration gradient (for overviews of this large field, see [23–25]). Members of this family invariably consist of two membrane-spanning domains that form the translocation pathway, and two cytoplasmic ABC domains that power the transport reaction through binding and hydrolysis of ATP. While most eukaryotic ABC transporters export hydrophobic molecules from the cytoplasm, the majority of bacterial ABC transporters import essential nutrients that are delivered to them by specific binding proteins. These periplasmically located binding proteins bind their substrates selectively and with high affinity, which is thought to ensure the specificity of the transport reaction. The membrane-spanning domains are poorly conserved, including a wide variability in the number of predicted transmembrane helices, which likely reflects the diversity of transported substrates. What ties the family together is a number of highly conserved motifs in the ABC cassettes, many of which are directly involved in the binding and hydrolysis of ATP. These motifs include the P-loop and a short polypeptide stretch of sequence ...LSGG... that is so specific to ABC cassettes that it is generally referred to as the 'ABC signature sequence'. Because of these similarities, it is generally assumed that all ABC cassettes bind and hydrolyze ATP in a similar fashion, and use a common mechanism to transport substrates through the membrane-spanning domains.

The crystal structure of the *E. coli* vitamin B_{12} importer BtuCD ([26], Fig. 3) provides a framework for addressing the mechanism of this widespread family of active transporters. The functional unit of this transporter consists of two

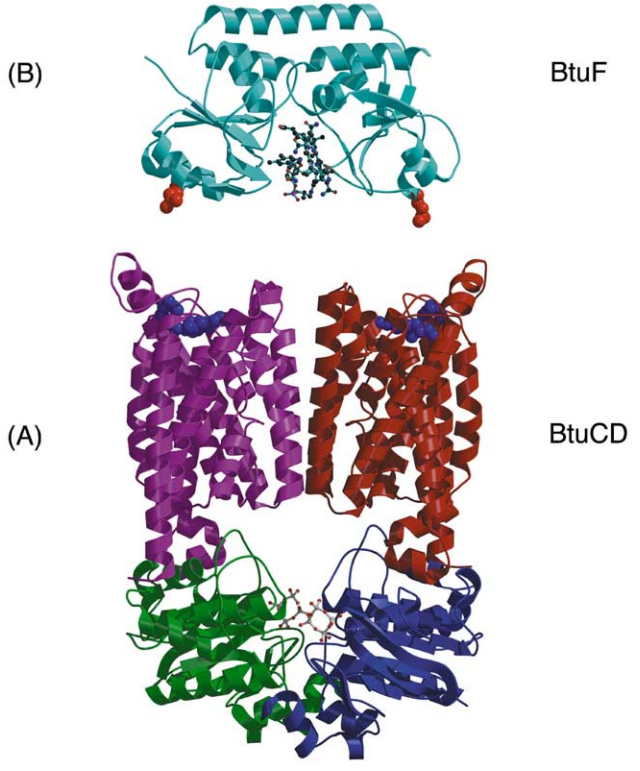

Fig. 3. A: Ribbon diagram of the polypeptide fold for the BtuCD vitamin B12 transporter [26]. The complete transporter is assembled from four subunits, two membrane-spanning BtuC subunits (purple and red) and two ABC cassette BtuD subunits (green and blue). B: Ribbon diagram of the BtuF vitamin B12 binding protein [28], illustrating the binding of the cobalamin ligand in the cleft between the two domains of this protein. The relative orientations of BtuCD and BtuF in A and B, respectively, are such that conserved arginines on BtuC and conserved glutamates on BtuF (blue and red CPK models, respectively) are juxtaposed in a proposed docking complex for these species.

copies each of the membrane-spanning BtuC subunits and the ABC subunit BtuD. These subunits assemble such that the two ABC cassettes are in close contact with each other, as are the membrane-spanning transport subunits. At the center of the heterotetramer, surrounded by all four subunits, there is a water-filled channel that connects with the cytoplasm. Each of the two BtuC subunits traverses the membrane 10 times for a total of 20 transmembrane helices in the transporter. The interface between the two BtuC subunits is formed primarily by the antiparallel packing of two pairs of helices that create a large cavity opening to the periplasmic space. Although no ligand is present in the structure, this cavity is of sufficient size to accommodate the corrin ring of vitamin B_{12}, and hence likely represents the translocation pathway. The cavity is closed to the cytoplasm by residues in two loops that appear to function as a gate. The overall fold of the BtuD subunit closely resembles that observed in other ABC cassettes. The dimeric arrangement of BtuD subunits is similar to that first observed in the DNA repair enzyme Rad50 [27]. Significantly, the subunits are aligned such that the nucleotide is bound at the dimer interface between the ABC signature of one subunit and the P-loop of the other, so that the nucleotide binding site is a property of dimeric ABC cassettes, and not of individual domains.

Biochemical studies with purified components have demonstrated the formation of a stable complex between BtuCD and its cognate binding protein BtuF of stoichiometry $BtuC_2D_2F$ [28]. Although the details of this interaction remain to be experimentally established, the structures of the individual proteins provide some clues. In common with other periplasmic binding proteins [29], the substrate binding site for vitamin B12 is located in a deep cleft between the two lobes of BtuF [28,30]. The B12 ligand is bound to BtuF in the 'base-on' conformation, with the directly bonded A and D pyrrole rings of the corrin exposed to the solvent. Conserved glutamate residues on the surface of BtuF adjacent to the exposed ligand surface may be important for interaction with a set of conserved arginine residues in BtuCD. A model of the $BtuC_2D_2F$ complex, generated from the individual crystal structures through alignment of the conserved charges, positions the bound B12 over the entrance to the translocation pathway of BtuC. The ability to form a stable complex between the binding protein and transporter should facilitate crystallographic characterization of this docking interaction.

The BtuCD and BtuF structures provide a framework for the architecture of ABC transporters and a plausible mechanistic scheme for ATP-powered import of vitamin B_{12} into *E. coli* [26]. The close contact between the BtuD subunits suggests that the power stroke in ABC transporters consists of a cooperative modulation of the ABC cassette interface upon ATP binding and hydrolysis, triggering rearrangements in the gate region of the membrane-spanning subunits. In view of the tight complexes observed between the binding protein and transporter for several importers [28,31], it is likely that one role of ATP hydrolysis is to dissociate the ternary complex and return the system to the resting state. The single translocation pathway at the interface of the membrane-spanning BtuC subunits is likely a common feature of ABC transporters of water-soluble substrates, although the size and the chemical nature of the internal surface will probably vary considerably. In essence, the transporter appears to function as an airlock, with nucleotide binding and hydrolysis orchestrating a sequence of conformational changes that open and close the appropriate gates so that unidirectional translocation of a ligand is achieved without ever creating a continuous channel across the membrane.

4. Concluding remarks

The MscS and BtuCD structures exemplify facets of the general structural organization of the basic functional elements of channels and transporters. The simplest mechanism for the passage of molecules down their chemical potential gradient through channels such as MscS is to have a continuous, open, translocation pathway across the membrane. Control of channel conductance could be achieved by the presence of a minimum of one gate that would block permeation in the closed state. Transporters, in contrast, pump molecules against a chemical potential gradient, and hence cannot have a completely continuous, open pathway at any point in their catalytic cycle. Rather, a set of two or more gates would seem to be required that alternatively block access to one side of the membrane or the other [32–34]; the role of the energy-dependent steps would be to control the proper sequence of gate opening and closing. In the BtuCD structure, the translocation pathway is closed at the cytoplasmic side, and it seems plausible that one function of the binding protein is to block access from the periplasmic side once the ligand has been delivered to the transporter.

For proper biological function, opening and closing of the translocation gates must be sensitive to environmental signals, and hence channels and transporters must have functional elements that can respond to the appropriate presence of ligands, membrane potential change, or membrane tension. In the case of MscS, the TM1–TM2 helices appear to function as integrated voltage–tension sensors that are coupled to conformational changes in the permeation pathway formed by the TM3 helices. The transport cycle of BtuCD requires association with the BtuF binding protein; these interactions must be transmitted to the membrane-spanning domains and ABC cassettes to trigger the transport cycle. Specificity elements can be incorporated into channels and transporters to select the proper substrate for membrane translocation; in the case of importer-type ABC transporters such as BtuCD, this function is provided by extra-membrane binding proteins, such as BtuF. In other systems, such as K^+ channels [35], chloride channels [36], and aquaporin-type channels [37,38], the selectivity element is an integral part of the membrane protein. Specificity elements are not absolutely required, however; MscS lacks this component and is primarily non-specific.

With representative structures now available for some of the major families of mechanosensitive, ligand- and voltage-gated channels, and for primary (ATP-dependent) and secondary transporters, this is an exciting time in the channel and transporter field [39]. These structures will undoubtedly serve as the foundation for significant and rapid progress in establishing the detailed molecular mechanisms of getting molecules across the membrane.

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