Metallomics

Cite this: *Metallomics*, 2011, **3**, 1098–1108

www.rsc.org/metallomics

MINIREVIEW

Bacterial ATP-driven transporters of transition metals: physiological roles, mechanisms of action, and roles in bacterial virulence†

Joshua S. Klein and Oded Lewinson*

Received 21st June 2011, Accepted 15th August 2011
DOI: 10.1039/c1mt00073j

Maintaining adequate intracellular levels of transition metals is fundamental to the survival of all organisms. While all transition metals are toxic at elevated intracellular concentrations, metals such as iron, zinc, copper, and manganese are essential to many cellular functions. In prokaryotes, the concerted action of a battery of membrane-embedded transport proteins controls a delicate balance between sufficient acquisition and overload. Representatives from all major families of transporters participate in this task, including ion-gradient driven systems and ATP-utilizing pumps. P-type ATPases and ABC transporters both utilize the free energy of ATP hydrolysis to drive transport. Each of these very different families of transport proteins has a distinct role in maintaining transition metal homeostasis: P-type ATPases prevent intracellular overloading of both essential and toxic metals through efflux while ABC transporters import solely the essential ones. In the present review we discuss how each system is adapted to perform its specific task from mechanistic and structural perspectives. Despite the mechanistic and structural differences between P-type ATPases and ABC transporters, there is one important commonality: in many clinically relevant bacterial pathogens, transporters of transition metals are essential for virulence. Here we present several such examples and discuss how these may be exploited for future antibacterial drug development.

Introduction

It is estimated that 30–45% of known enzymes are metalloproteins that depend on a metal co-factor for their function.[1,2] Often, the co-factor is a transition metal such as iron, manganese, zinc, or copper. As a result, many essential physiological processes including respiration, photosynthesis, replication, transcription, translation, signal transduction, and cell division depend on the presence of transition metals.[1] However, transition metals are toxic at elevated intracellular concentrations as they can perturb the cellular redox potential, produce highly reactive hydroxyl radicals, and displace functionally important metal co-factors from their physiological locations.

Department of Microbiology, The Ruth and Bruce Rappaport Faculty of Medicine, The Technion-Israel Institute of Technology, Haifa, Israel. E-mail: lewinson@tx.technion.ac.il; Fax: +972-4-829-5205; Tel: +972-4-829-5428

† This article is published as part of a themed issue on Metal Toxicity, Guest Edited by Gregor Grass and Christopher Rensing.

Joshua S. Klein

Joshua Klein received his PhD in Biochemistry and Molecular Biophysics at the California Institute of Technology with Prof. Pamela Bjorkman and later worked jointly with Profs. Bjorkman and David Baltimore, where his research focused on characterizing the relationship between antigen density and antibody avidity in the neutralization of HIV. In 2011, Josh joined Oded Lewinson's group to focus on developing inhibitors of bacterial metal transporters. In his spare time, Josh is an avid cyclist.

Oded Lewinson

Oded Lewinson is a biochemist in the Department of Microbiology at the Technion (Israel Institute of Science). His longstanding interest is to understand the molecular mechanisms of transport proteins. His lab currently focuses on ABC transporters and P-type ATPases of transition metals. Outside of the lab, Oded Lewinson is a climber and a mountain biker, and raises his three young children.

Moreover, other non-essential transition metals (*e.g.*, cadmium, lead, silver, and mercury) exhibit acute toxicity by binding to macromolecules and perturbing their physiological interactions/functions.[3,4]

In both eukaryotes and prokaryotes, a diverse ensemble of membrane-embedded transporters participates in metal translocation across cell membranes. In gram-negative bacteria, these included ion gradient- and ATP-driven transport systems belonging to the RND, ABC, CDF, and P-type ATPase superfamilies.[5] As depicted in Fig. 1, each of these superfamilies has a unique architecture and composition: RND transporters are comprised of multiple subunits spanning the inner membrane, periplasm, and the outer membrane. A substrate may enter the translocation pathway either at the cytoplasm or at the periplasm[6] (Fig. 1), but in both cases the substrate will be expelled to the cell exterior. ABC transporters are embedded in the inner membrane, and through interactions with their cognate substrate-binding proteins,[7] outer membrane receptors[8] (*e.g.*, BtuB in Fig. 1), and the ExbB/ExbD/TonB system[9,10] they participate in metal uptake through the outer membrane, the periplasm, and the inner membrane, delivering transition metals to the cytoplasm. P-type ATPases and CDF transporters catalyze metal translocation across the inner membrane, and in both systems the substrate is transported from the cytoplasm to the periplasm.

In this review, we compare the two ATP-driven superfamilies: ABC transporters and P-type ATPases. As discussed below, these two superfamilies exhibit complementary physiological functions in maintaining transition metal homeostasis, diverge in their structural and mechanistic organization, and play distinct roles in bacterial virulence.

Physiological roles of bacterial transition metal transporters

In many enzymatic reactions, the limited chemical reactivity of the side chains of the 20 amino acids is supplemented by the heightened reactivity of transition metal co-factors.[11–14] It is however the same heightened reactivity that makes transition metals potentially toxic. As such, a delicate intracellular balance is required to maintain the essential supply of these metals while preventing toxic overload. One mechanism by which bacterial (and mammalian) cells maintain this balance is through a combination of sequestration and controlled release. Since the toxic form of transition metals is their unliganded ionic form, the metals may be stored as bound moieties by specialized sequestering proteins. Examples of such storage proteins include the iron-storage protein ferritin[15] and the zinc and copper-storing metallothioneins.[16] Metal coordination by

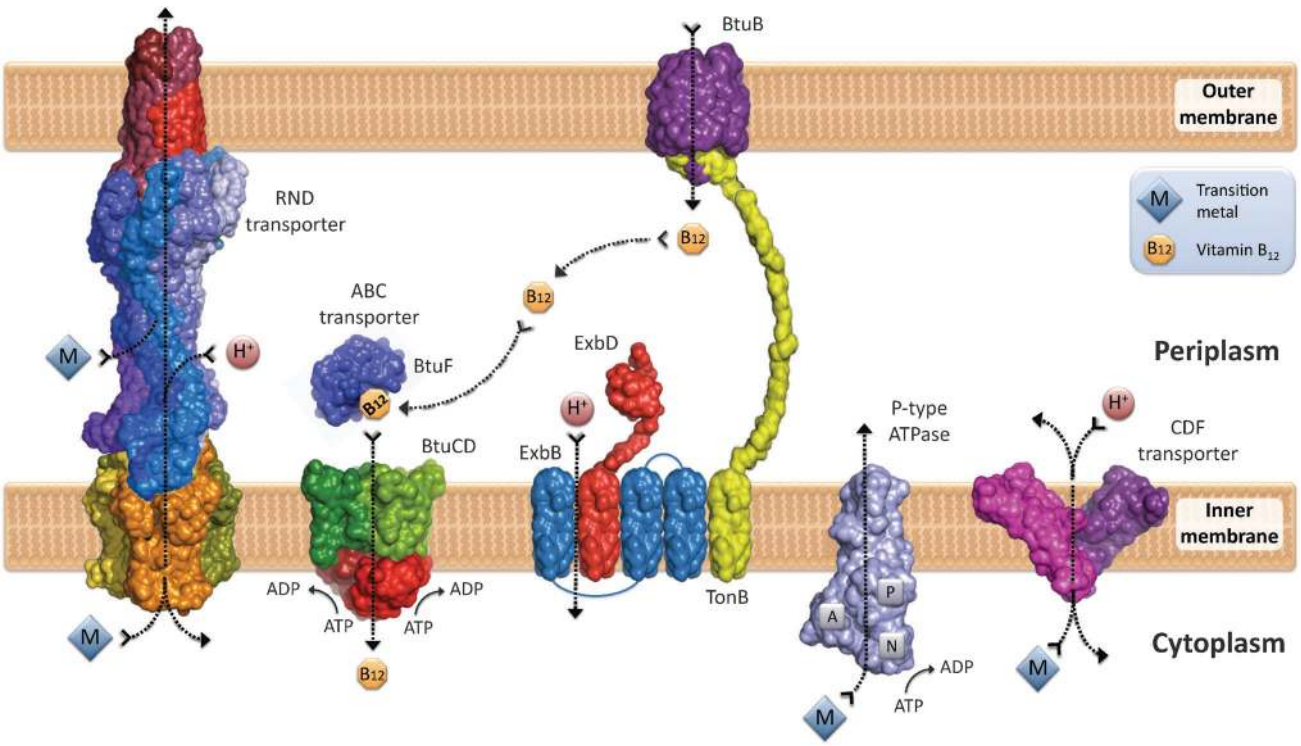

Fig. 1 The different transporter types participating in transition metal translocation in gram-negative bacteria. Arrows indicate the directionality of transport of protons and transition metals, and proteins are colored according to chains. From left to right: RND transporters have a trimeric organization and traverse both the inner and outer membranes. They utilize the energetically downhill movement of protons across the inner membrane ($\Delta\mu H^+$) to drive metal efflux from the cytosol and the periplasm to the cell exterior. ABC transporters have a dimeric organization and are embedded in the inner membrane. They use the energy of ATP hydrolysis and require a cognate substrate binding protein for their function (*e.g.*, BtuF). ABC transporters partner with the energy-transducing ExbB/ExbD/TonB complex (schematic model composed of PDB IDs 2PFU and 1ENV) and high affinity outer membrane transporters (*e.g.*, BtuB) to deliver essential trace elements from the environment to the cytosol. P-type ATPases are monomers (schematic model from PDB ID 3RFU), and like ABC transporters, are powered by ATP to catalyze metal efflux from the cytosol, across the inner membrane, to the periplasm. A similar task is performed by the dimeric CDF transporters (PDB ID 2QFI) that utilize the energy of $\Delta\mu H^+$ to drive metal efflux across the inner membrane.

the sequestering protein neutralizes the metals' reactivity. The sequestered metals are then released in a controlled manner as the need arises, thereby providing the mechanisms of both protection and storage.

An additional mechanism that bacteria have evolved to control their intracellular transition metal concentrations is by active uptake and extrusion. Given the low passive membrane permeation of transition metals, the net change in intracellular metal concentrations is determined by the rates and directionality of active transport. In this respect, the roles of P-type ATPases and ABC transporters are readily distinguished: transition metal P-type ATPases function as detoxifiers through their efflux activity[17,18] while their ABC counterparts function exclusively as high-affinity importers.[7,19,20] P-type ATPases export both essential transition metals (*e.g.*, Zn, Cu, and Co) and metals that are exclusively toxic (*e.g.*, Ag, Cd, Pb, and Hg).[21] Such a recognition spectrum is beneficial since all transition metals (including the essential ones) are toxic at elevated intra-cellular concentrations. In contrast, ABC transporters of transition metals import only the essential ones (*e.g.*, Fe, Zn, Mn, Ni, and Co). Clearly, it is disadvantageous to invest energy in the import of non-essential toxic compounds. Several reports have implicated P-type ATPases as having roles in influx, rather than efflux, of transition metals.[22–24] However, P-type ATPases that function as importers are uncommon, and the great majority of P-type ATPases characterized to date operate as efflux pumps.

In addition to their role in maintaining the balance between influx and efflux, prokaryotic transition metal transporters appear to be involved in other cellular processes. Many bacterial genomes contain more than one gene encoding a Cu^{+}-transporting P-type ATPase.[21,25] Of these, usually only the *copA1* gene is essential for copper tolerance. In *Rhizobia*, *copA*2 (*fixI*) has been proposed to be important for nitrogen fixation,[26] through its role in supplying copper to copper-containing enzymes such as cytochrome c oxidase and nitrous oxide.[27–29] In *P. aeruginosa copA*2 has been shown to be involved in cuproprotein assembly: disruption of *copA*2 had no effect on copper tolerance yet resulted in an increased sensitivity to oxidative stress (as a result of decreased cellular oxidase activity) and reduced virulence in a plant model.[30] In the photosynthetic bacteria *Synechocystis*, the complementary action of two P-type ATPases has been shown to be important to respiration and photosynthesis[24,25] through the supply of copper to plastocyanin.

Substrate recognition by transition metal transporters

Bacterial P-type ATPases contain a single membrane-embedded metal recognition site and 1 to 3 soluble **M**etal-**B**inding **D**omains (MBDs) located in the cytoplasm.[21] The membrane-embedded site is essential for transport activity, and mutations of conserved residues in this site inhibit transport.[31–33] The soluble MBDs are not essential for transport *per se*, as P-type ATPases where only the membranous site is functional are still transport-competent.[34,35] Substrate specificity appears to be fully dictated by the membrane-embedded recognition site: when the soluble copper-binding MBD of a Cu^{+1} P-type ATPase was fused to a Zn^{+2} P-type ATPase (replacing its endogenous MBD), the resulting chimera retained its activity towards Zn^{+2} but displayed no transport activity towards Cu^{+1}.[36] The role of the soluble MBDs seems to be regulatory, controlling the kinetics of the transport cycle, or serving as intermediate metal binding sites shuttling the substrate to the membranous site.[34,35,37,38]

Metal transport by a Cu^{+1}-transporting P-type ATPase is often facilitated by soluble cytoplasmic metal (mostly copper) chaperones. This phenomenon has been recognized in both eukaryotes[39] and prokaryotes. In most cases, the copper chaperones deliver their substrate to the soluble MBDs of the transporter. Such an interaction between human Atox1 (chaperone) and Wilson's disease protein (P-type ATPase) results not only in transfer of the metal but also in the stimulation of the pump's ATP hydrolysis activity.[40] The chaperones are substrate specific and pump specific: the bacterial copper chaperone Atx1 interacts with two copper-transporting P-type ATPases but not with zinc or cobalt P-type ATPases.[24] A somewhat different mode of interaction has been described between CopZ (copper chaperone) and CopA (copper pump) of *A. fulgidus*. Here, CopZ delivers Cu^{+1} not only to the soluble MBDs, but perhaps more importantly it delivers copper directly to the transport-competent, trans-membrane metal binding domain.[41]

The regulation of transport kinetics by cytoplasmic substrate binding domains has also been observed in ABC transporters: In both the methionine and the molybdate/tungstate transporters a regulatory cytoplasmic substrate-binding domain is fused to the ATPase domain of the transporter.[42,43] When the intracellular substrate concentration exceeds a certain threshold, the substrate binds to the regulatory domain and inhibits both ATP hydrolysis and transport.

Unlike P-type ATPases, substrate recognition by ABC importers of transition metals does not occur in the membrane but rather by a soluble, periplasmic **S**ubstrate **B**inding **P**rotein (SBP). It is the SBP that defines the system's substrate specificity and conveys it with its characteristic high affinity.[44–46] The trans-membrane domains offer little in terms of substrate recognition: from observations of BtuCD–F,[47] the intrinsic affinity of the membrane-embedded permease towards the substrate is very low (*i.e.*, in the mM range as compared to the nM range of the SBP). This weak binding likely explains why ABC importers are unable to transport their substrate in the absence of their cognate SBP (*e.g.*, ref. 48).

Almost without exception, P-type ATPases will transport either monovalent metals (*e.g.*, Cu^{+1} and Ag^{+1}) or the divalent ones (*e.g.*, Zn^{+2} and Cd^{+2}).[21] A highly conserved C–P–C motif in trans-membrane (TM) helix 6 of Cu^{+1}/Ag^{+1} and Zn^{+2}/Cd^{+2} P-type ATPases is essential for metal binding and transport. In addition to this central motif, residues engaged in metal coordination have also been identified in TM helices 4–8. The recently solved structure of the *L. pneumophila* copper pump CopA[49] reveals that residues involved in metal binding are clustered in three main sites of the membrane domain: an entry site accessible from the cytoplasm, a central, high-affinity site centered around the conserved CPC motif, and an exit site accessible to the periplasm.

Several key residues engaged in metal coordination are conserved within each substrate specific subgroup (*i.e.*, mono- or divalent recognition) but differ between them. For example, a conserved Asn in TM7 of Cu^{+1}/Ag^{+1} pumps is essential for their function.[50] This Asn is replaced by an essential Lys in Zn^{+2}/Cd^{+2} pumps. Similarly, a conserved and essential Asp in TM8 of Zn^{+2}/Cd^{+2} [51] pumps is replaced by a Pro in pumps of Cu^{+1}/Ag^{+1}.

A blend of such conserved residues from both mono- and divalent recognizing subgroups is observed in HmtA of *Pseudomonas aeruginosa*. This pump has been proposed to function as an importer, rather than an exporter.[23] HmtA's substrate recognition profile is unique in the sense that it recognizes Cu^{+1} and Zn^{+2} but not Ag^{+1} or Cd^{+2}, thus importing only the essential transition metals and not their toxic counterparts. In accordance with its distinctive substrate selectivity, HmtA harbors metal recognition motifs borrowed from both Cu^{+1}/Ag^{+1} and Zn^{+2}/Cd^{+2} pumps.

Whether an importer or an exporter, P-type ATPases are able to recognize and transport metals in their free, ionic form. Cysteines and other thiols have been repeatedly reported to stimulate ATP hydrolysis and transport by transition metal P-type ATPases, presumably due to metal chelation.[17,32,52] Nevertheless, P-type ATPases will also efficiently transport metals in the complete absence of any thiols.

Conversely, ABC transporters often transport a complex of a transition metal bound to a chelator.[7,20,53] Examples of such substrates are the cobalt-containing vitamin B_{12} and various chelated iron species (Fig. 2). An important class of iron chelators is the siderophores: these low molecular weight cyclic peptides are synthesized and secreted by many bacteria. Siderophores bind Fe^{+3} with some of the highest affinities found in nature ($K_d \approx 10^{-30}$ M,[54]), and are thus able to scavenge iron under the most dilute conditions and strip host's iron-containing proteins of their cargo. Other important iron chelates are heme and hemin, often harvested by symbionts and pathogens from their hosts. In addition to recognition and transport of chelated metals, substrate-binding proteins of ABC transporters can bind Zn^{+2} and Mn^{+2} in their ionic form.[55,56] In these cases the transported moiety is the metal itself and not a metal–chelate complex.

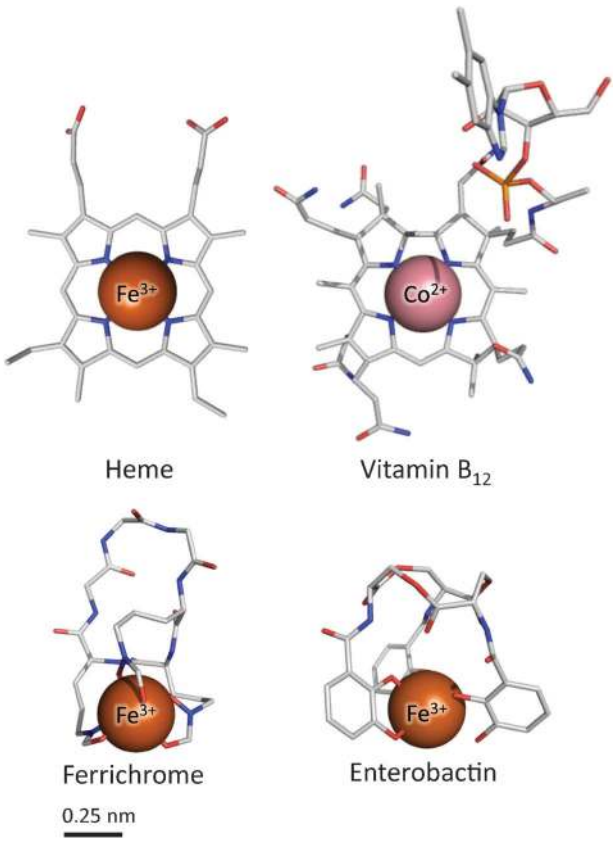

Fig. 2 Structures of metal–chelate complexes that are substrates of ABC importers. Shown are the siderophores Fe-ferrichrome (PDB ID 1FCP) and Fe-enterobactin (PDB ID 3CMP), heme (PDB ID 1EHJ), and vitamin B_{12} (PDB ID 2YCL), as indicated. Scale bar is 0.25 nm.

Molecular organization of ATP-driven transporters

The molecular organization and structural fold of prokaryotic P-type ATPases and ABC transporters are very different (Fig. 3). The functional unit of prokaryotic transition metal transporting P-type ATPases (also referred to as heavy metal or type P_{1B} ATPases) is a monomer, composed of a single polypeptide chain, encoded by a single gene. They contain a membrane-spanning domain and three cytoplasmic domains: A (actuator), P (phosphorylation) and N (nucleotide) domains (Fig. 3). The N-domain binds the nucleotide and phosphorylates the conserved aspartic residue in the canonical DKTGT sequence of the P-domain. The role of the A domain is to dephosphorylate the P-domain, thus completing the ATPase cycle. The membrane-spanning domain serves two functions: it creates the permeation pathway through the membrane and is responsible for substrate binding and selectivity.

ABC transporters of transition metals are minimally comprised of three subunits, each encoded by a different gene: a *n*ucleotide *b*inding *d*omain (NBD), a trans-membrane permease (TM), and a soluble, periplasmic **S**ubstrate-**B**inding **P**rotein (SBP). The three genes are transcribed as an operon, sometimes with overlapping open reading frames. At the protein level, both the NBD's and TM's function as homodimers, resulting in a functional unit that is composed of two copies each of the ATPase and permease, and one copy of the substrate-binding protein (Fig. 3). Unlike P-type ATPases, the membrane translocation pathway, formed by the homodimeric permease, offers little in terms of substrate selectivity, which is almost entirely determined by the periplasmic substrate binding protein.

Mechanisms of transport and ATP hydrolysis

The catalytic cycle of P-type ATPases is traditionally described by the Albers-Post model.[57,58] The model defines two conformations/states that have high or low affinity toward the transported substrate (E1 and E2, respectively). Both states also have a high-energy phosphor-enzyme intermediate, denoted E1P and E2P. Phosphorylation (and de-phosphorylation) of the pump drive conformational changes that result in vectorial substrate translocation.[52,59] The structure of CopA (Cu^{+1} P-type ATPase) shows that key catalytic and metal coordinating residues align very well with the corresponding

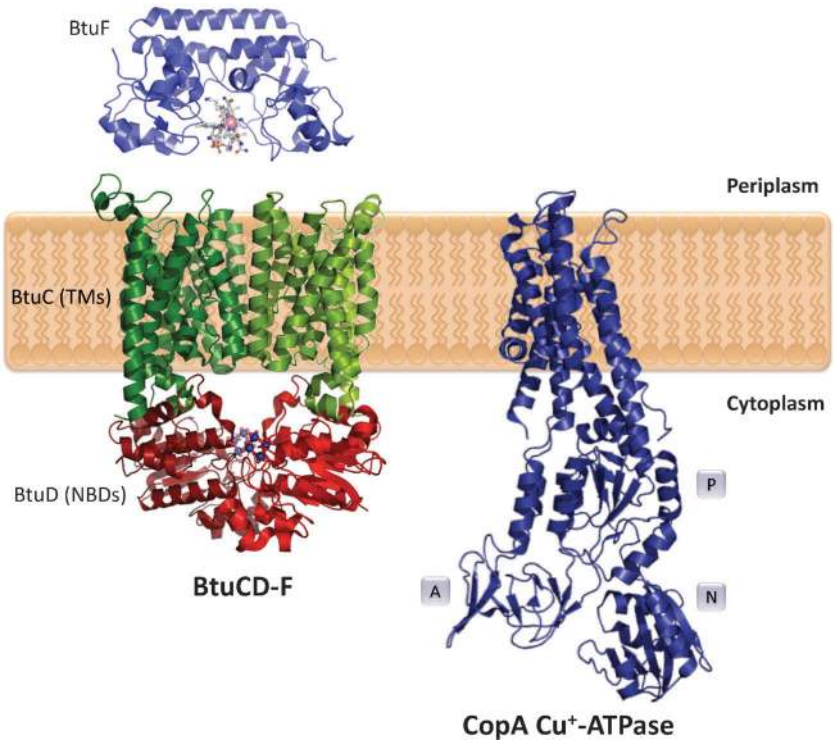

Fig. 3 Comparison of the structural folds of an ABC transporter (BtuCD–F) and a P-type ATPase (CopA Cu^{+}-ATPase 1). Proteins are colored by chain, and co-factors are shown as ball and stick models. Left: BtuCD (PDB ID 1L7V) displays a dimeric organization of the NBDs and TMs. One molecule of vitamin B_{12} is bound by the monomeric BtuF (PDB ID 1N4A). Two cyclo-tetrametavanadate (blue spheres) molecules are bound at the interface of the NBDs, indicating the location of the binding site for two ATP molecules. Right: P-type ATPases of transition metals are monomers, as exemplified by the class IB Cu^{+}-ATPase, CopA (PDB ID 3RFU).

residues of the SR Ca^{+2}-ATPase,[49] further suggesting conservation of the mechanism between P-type ATPases of alkali- and transition metals.

The single ATP hydrolysis site of P-type ATPases is absolutely essential for activity, and mutations in conserved residues completely abolish transport. Following ATP binding by the N domain, the γ-phosphate of ATP is transferred to a conserved aspartic residue in the P domain yielding a covalently phosphorylated transporter. The last step of the hydrolysis cycle is the de-phosphorylation of the P domain by residues of the A domain. ATP binding, phosphorylation, and de-phosphorylation are tightly coupled to conformational changes of the transporter that result in changes in affinity toward the substrate, and changes in substrate occlusion and inward/outward facing accessibilities. This mechanism is supported by a large body of experimental data and has been comprehensively described elsewhere (for detailed descriptions see ref. 60–62).

The ATPase/transport cycle of ABC transporters has not yet been as precisely described as it has for P-type ATPases. Nevertheless, available data strongly suggest considerable mechanistic variation among ABC transporters of different substrate specificities. For example, in the histidine ABC importer and in the osmoregulated ABC transporter for glycine betaine ATP hydrolysis and substrate translocation are coupled: efficient ATPase rates are only observed in the presence of substrate-loaded binding proteins.[63,64] In contrast, in BtuCD–F and in the *E. coli* D/L-methionine importer the substrate-loaded binding proteins have modest effects on ATP hydrolysis and substantial ATP hydrolysis is also observed in the complete absence of a substrate.[42,48] Considerable mechanistic variation among ABC transporters also exists with respect to the roles of the two ATP hydrolysis sites. In ABC transporters two symmetric ATPase sites are formed due to the dimeric, head-to-tail organization of the NBDs. ATP binding and hydrolysis occur at the dimer interface and necessitate dimer closure. It is unclear whether both sites are necessary for transport and whether both are functionally equivalent. Evidence from ABC transporters of transition metals is yet unavailable, but data from ABC transporters of other compounds suggest several different mechanisms: in the *Salmonella typhi* histidine ABC importer the two NBDs are functionally symmetric as both NBDs hydrolyze ATP and activate one another. However, when one of the NBDs is rendered non-functional by mutation, the remaining NBD will still hydrolyze ATP and support transport at half maximal rates.[65] The situation is quite the opposite in the archetypical human multi-drug exporter Pgp (MDR1): both NBDs are essential to support efficient transport, and the two NBDs are functionally asymmetric having different roles in the transport cycle.[66] Another variation is observed in the yeast multi-drug exporter Pdr5: Like in Pgp, the two NBDs are functionally non-equivalent, having different catalytic roles. However, unlike Pgp, efficient ATP hydrolysis occurs only at one of the NBDs and only this site is essential for transport.[67] It thus seems that within the large family of ABC transporters there is quite a variation in the mechanism and role of ATP hydrolysis.

In the absence of a substrate, P-type ATPases show very low ATP hydrolytic activity.[32,52] This is generally not true for ABC transporters that are notorious for their high basal ATP hydrolysis rates in the absence of a substrate.

Upon addition of a substrate, the ATPase activity of P-type ATPases is stimulated 20 to 30-fold.[17,32,52] Conversely, the maximal substrate-induced stimulation of BtuCD's basal ATP hydrolysis is only 1.5 to 2-fold.[48] Remarkably, despite these very different substrate-stimulatory effects, and despite the very different mechanisms for ATP hydrolysis, the maximal ATPase rates of P-type ATPases and ABC transporters are quite similar: CopA, the *E. coli* Cu^{+1} efflux ATPase, has a maximal ATPase rate of 0.2 μmol mg^{-1} min^{-1} in the presence of Cu^{+1}, very similar to its *A. fulgidus* homologue.[18,32,68] Roughly similar rates, 0.3–0.4 μmol mg^{-1} min^{-1}, were observed for the vitamin B_{12} ABC importer BtuCD.[48] The highest ATPase rates observed to date are 3 μmol mg^{-1} min^{-1} for the *E. coli* Zn^{+2}/Cd^{+2} efflux ATPase ZntA.[17]

BtuCD–F as a model for ABC transporters of transition metals

ABC transporters comprise one of the largest protein families of any proteome, with ~80 representatives in *E. coli* and human.[69–71] Sequence alignments and structural conservation divide this superfamily of proteins into several sub-groups, each with its distinct sequence divergence and unique structural elements.[72–74] ABC transporters of iron-siderophores and vitamin B_{12} share a high degree of sequence similarity, and comprise one of these subgroups. From a structural perspective, the fold of the *E. coli* vitamin B_{12} transporter BtuCD is highly comparable to the fold of the *H. influenza* putative metal transporter H11470/1,[75] and the structures of the substrate binding proteins (SBPs) for vitamin B_{12}, Fe^{+}-ferrichrome, and heme are very similar (Fig. 4). Another unique feature that is shared between BtuCD–F and H11470/1 is the formation of highly stable, high affinity transporter–SBP complexes, contrasting with the low affinity complexes formed by other types of ABC transporters.[47] ABC transporters for ionic Fe^{+}, dicitrate-Fe^{+}, Mn^{+2}, and Zn^{+2} form another distinct phylogenetic branch, closely related to the branch of BtuCD. Again, from a structural perspective, the fold of SBPs of these systems is quite similar to the fold of the vitamin B_{12} binding protein (Fig. 4 and ref. 72). Unfortunately, we currently have very little structural and mechanistic information on ABC transporters of Fe^{+}-siderophores, Fe^{+}, Mn^{+2}, and Zn^{+2}. Therefore, we use the data available from structure–function studies of BtuCD–F to construct a plausible mechanistic framework of transition metals ABC transporters.

Mechanism of vitamin B_{12} transport

The first step of active uptake of vitamin B_{12}, iron (and iron complexes), and zinc occurs across the outer membrane, and is mediated by specific, high affinity transporters (*i.e.*, BtuB in Fig. 1 and ref. 8). Regardless of their substrate specificity, these outer-membrane transporters share a common architecture, composed of a 22-stranded β-barrel plugged by a hatch domain.[76] A common mechanistic feature is their high substrate affinity, dictated by the scarcity (or insolubility in the case of iron) of the target substrate. For example, the outer-membrane transporters BtuB (vitamin B_{12}) and FepA (Fe-enterobactin) have a K_D of 0.2–0.3 nM towards their substrates.[77,78]

Since there is no ATP in the periplasm and no ion gradients across the outer membrane, conventional bacterial energy currencies cannot be used to drive active transport across the outer membrane. Therefore, the energy utilized by outer-membrane transporters is transduced from the inner membrane by the ExbB/ExbD/TonB complex[79] (Fig. 1), by a mechanism that is poorly understood. Once in the periplasm, the substrate is captured by the system's substrate binding protein, often with a K_D in the nM range (the K_D of BtuF to vitamin B_{12} 15 nM[45]).

To complete the uptake of vitamin B_{12} into the cytoplasm, BtuF must associate with its inner-membrane partner and

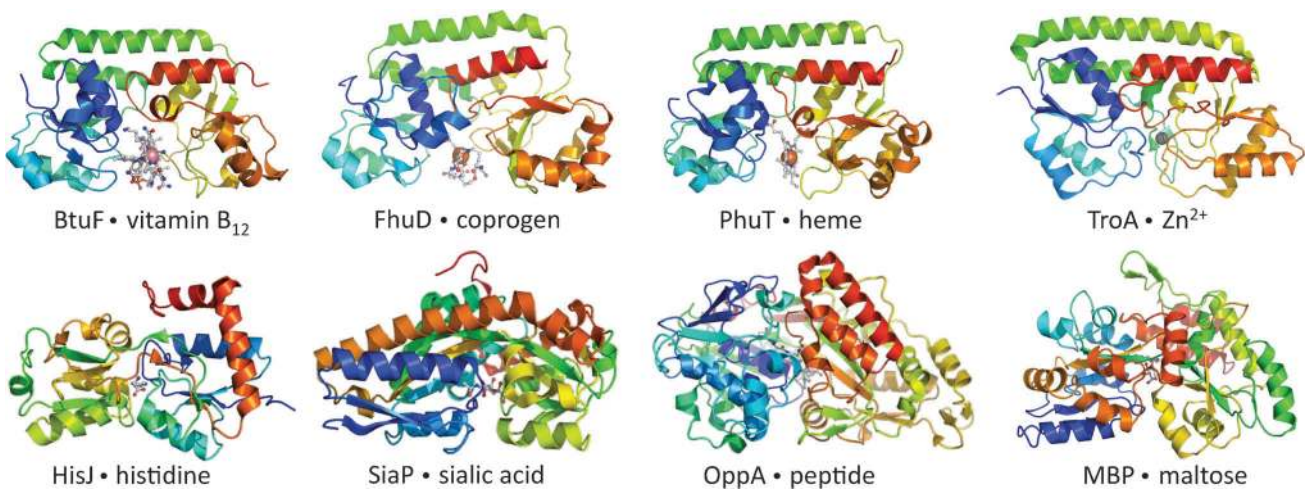

Fig. 4 Structural similarity between the periplasmic substrate binding proteins for vitamin B_{12} (BtuF, PDB ID 1N4A), for coprogen Fe-siderophore (FhuD, PDB ID 1ESZ), for heme (PhuT, PDB ID 2R7A), and for ionic zinc (TroA, PDB ID 3MFQ) (top row). Shown for comparison are the unrelated substrate binding proteins for histidine (HisJ, PDB ID 1HSL), sialic acid (SiaP, PDB ID 3B50), peptides (OppA, PDB ID 3DRF), and maltose (MBP, PDB ID 1ANF) (bottom row). Proteins are rainbow colored from blue (N-terminus) to red (C-terminus).

deliver its bound cargo. Formation of the BtuCD–F transport complex occurs spontaneously, and does not require an energy input.[47] Upon formation of the ultra-stable BtuCD–F complex, vitamin B_{12} is released into the cytoplasm, again in an energy-independent manner (Fig. 5, ref. 47). In the maltose ABC import system these two steps, complex formation and substrate release, are not spontaneous and do require energy.[80] In the vitamin B_{12} system the sub-nM affinity of the initial step of translocation across the outer membrane, followed by the spontaneity of the two down-stream events (complex formation and intracellular substrate release), provide a 'de-facto' unidirectional inwardly directed substrate flux. Only after the substrate is released to the cytoplasm does the system require energy (Fig. 5). Perhaps non-intuitively, the role of energy input (ATP binding) in BtuCD–F is to break the complex, rather than to stabilize it. Whether this phenomenon is specific to the vitamin B_{12} system or is a general mechanism of high-affinity uptake of trace metals has yet to be determined. However, it is noteworthy that in the absence of ATP, the *H. influenza* putative transition metal transporter Hi1470/1 also spontaneously forms a high-affinity complex with its substrate binding protein.[47]

Another unique (and counter-intuitive) feature of vitamin B_{12} import is that the substrate reduces the equilibrium affinity (K_D) between the transporter and the binding protein $\sim 10^5$-fold. The seeming paradox of this substrate effect is better understood by examining the effects of vitamin B_{12} on the non-equilibrium kinetics of complex formation: vitamin B_{12} accelerates both complex formation and complex dissociation rates, by 15- and $\sim 10^7$-fold respectively (resulting in the observed decreased equilibrium affinity). Such a substrate effect may prove detrimental to a receptor or an enzyme, but not for a transporter. In order for the transport reaction to proceed in a cyclic manner, the binding protein and the transporter must continuously associate and dissociate in a cyclic manner. Slow association rates are unfavorable as are slow dissociation rates. In a sense, vitamin B_{12} exerts a positive feedback on its own transport by increasing both the k_{on} and k_{off}. This effect is so pronounced that in the absence of a substrate, the dissociation rate (k_{off}) of the BtuCD–F complex is reduced to 10^{-8} s^{-1}, an infinity in terms of bacterial life cycle. Whether similar substrate effects occur in additional transition metal ABC import systems is unclear. However, it seems that also in the ABC import system for

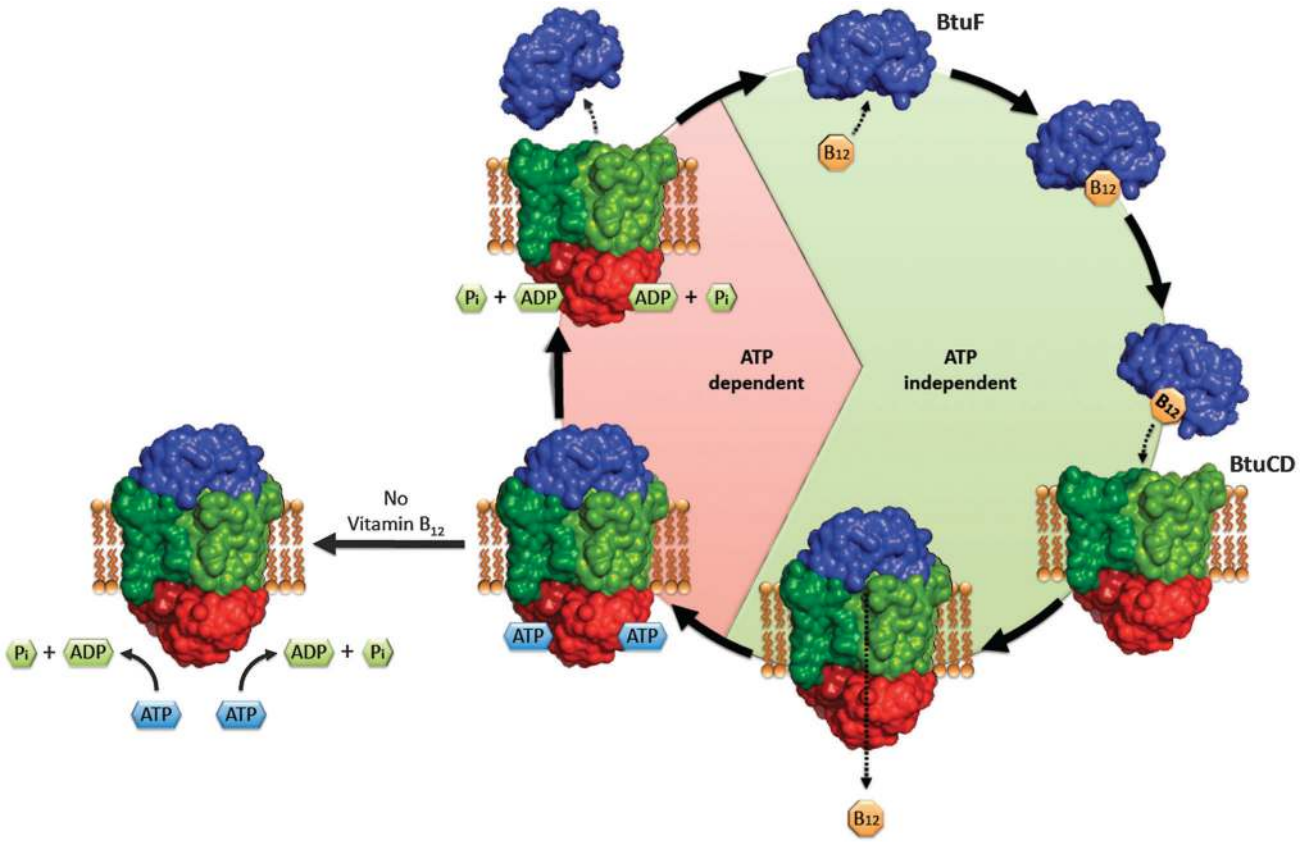

Fig. 5 Proposed catalytic cycle of vitamin B_{12} transport by BtuCD–F. The pie chart in the background indicates which part of the cycle is ATP-dependent. As vitamin B_{12} is transported into the periplasm by the combined action of BtuB and the ExbB/ExbD/TonB complex, it is captured by BtuF. BtuF has the highest affinity towards the nucleotide-free BtuCD, and once the BtuCD–F complex is formed, vitamin B_{12} is released from BtuF and is not retained by the complex. The now substrate- and nucleotide-free complex is extremely stable ($K_D \approx 0.1$ pM) and will only dissociate in the presence of ATP and free vitamin B_{12}, with both the nucleotide and the substrate contributing towards complex dissociation. ATP binding, hydrolysis, and release of ADP and phosphate reset the system for a subsequent cycle of transport. In the absence of periplasmic vitamin B_{12}, BtuF will remain bound to BtuCD, essentially plugging the permeation pathway.

iron-siderophores FhuBC-D, and in the Fe-citrate system FecCD-B substrate reduces the equilibrium affinity between the transporter and the binding protein (Fig. 6 and 7 in ref. 46 and 81).

Transporters of transition metals and bacterial virulence

When present at elevated concentrations, transition metals are toxic to bacteria. As a defense mechanism, the innate immune system employs transition metal overload in a tissue-specific manner. Accordingly, several examples of bacterial P-type ATPases with roles in virulence have been reported: CueA, a putative P-type copper ATPase (exporter), has been demonstrated to have a role in *Pseudomonas aeruginosa* virulence. Strains with mutations of the *cueA* gene exhibited a 20-fold attenuated virulence (compared to wild-type strains) in spleens of mice.[82] Similar findings were reported for the *Mycobacterium tuberculosis* putative P-type ATPase (copper efflux) CtpV. Mice infected with the Δ*ctpV* mutant displayed decreased lung damage, reduced immune response to the bacteria, and a significant increase in survival time.[83]

In contrast to the small number of examples of P-type ATPase transition metal *exporters* with roles in bacterial virulence, the involvement of transition metal ABC *importers* appears to be a very common phenomenon[84–95] (Table 1).

In order to propagate in the host's environment a bacterial pathogen must acquire the essential transition metals. Consequently, to fight infection, one of the first lines of defense of the innate immune system is to sequester transition metals in specialized storage proteins. Perhaps the best-characterized example of such a protein is ferritin, a 450 kDa complex capable of sequestering approximately 4500 Fe^{3+} ions. Ferritin is constitutively used for iron storage; however, its human serum levels rise dramatically upon infection.[96] Elaborate interactions between hosts and pathogens surrounding transition metal limitation also occur within macrophages. Following phagocytosis, bacteria are confined in the phagosome, where acidic pH, reactive oxygen species (ROS), and iron/manganese depletion combine to create bacteriostatic/bacteriolytic conditions. To deplete iron and manganese (the latter is essential for bacterial defense against ROS attacks), the mammalian transporters Nramp1 and Nramp2 pump these metals out from the phagosome.[97] To compete for phagosomal manganese and iron, bacteria employ high affinity transporters, predominantly ABC importers. Since many bacterial pathogens pass through the phagosome, their ABC import systems of transition metals play a key role in their intracellular survival, and hence in

Table 1 Examples of P-type ATPases and ABC transporters of transition metals with roles in bacterial virulence and pathogenesis

Superfamily	Organism	Direction of transport	Transport system	Substrate specificity	Role in virulence and pathogenesis
P-type ATPase	*Pseudomonas aeruginosa*	Efflux	CueA	Cu	In a murine model of systemic infection, the Δ*cueA* strain had a 50-fold higher LD_{50} relative to the wild-type strain.[82]
P-type ATPase	*Listeria monocytogenes*	Efflux	CtpA	Cu	The Δ*ctpA* strain showed restricted growth in mouse liver.[22]
P-type ATPase	*Mycobacterium tuberculosis*	Efflux	CtpV	Cu	Mice infected with the Δ*ctpV* strain had a 16-week increase in time-to-death and displayed consistently lower levels of tissue damage.[83]
ABC transporter	*Mycobacterium tuberculosis*	Uptake	IrtA/B	Fe-siderophores	The knockout strain has ~100-fold reduction in its ability to multiply in mice lungs.[94]
ABC transporter	*Bacillus anthracis*	Uptake	MntABC	Mn, Fe	The *ΔmntA* strain has $\sim 10^4$ reduction in lethality relative to the wild-type strain in guinea pigs infection model.[89]
ABC transporter	*Streptococcus pneumoniae*	Uptake	pit1	Fe	The double knockout strain has an $\sim 10^3$ reduced lethality in a murine pulmonary infection model.[87]
ABC transporter	*Streptococcus pneumoniae*	Uptake	pit2	Fe	
ABC transporter	*Streptococcus pneumoniae*	Uptake	psaBCA	Mn	Deletion of any component of the transport system results in a 10^4 higher LD_{50}.[91]
ABC transporter	*Streptococcus mutans*	Uptake	sloABC	Mn, Fe	The Δ*sloA* strain is a-virulent in a rat model for endocarditis.[92]
ABC transporter	*Streptococcus pyogenes*	Uptake	MtsABC	Mn, Fe	Deletion strain has 30-fold higher LD_{50} relative to the wild-type strain in a murine model of infection.[90]
ABC transporter	*Yersinia enterocolitica*	Uptake	irp6/7	Fe-siderophores	Deletion strain had a 10^5 and 10^3 reduction in its ability to propagate in mice spleens and livers, respectively.[86]
ABC transporter	*Yersinia pestis*	Uptake	YbtP/Q	Fe-siderophores	Deletion strain is a-virulent in a murine model of infection.[88]
ABC transporter	*Yersinia pestis*	Uptake	yfeABCD	Mn, Fe	Deletion strain is a-virulent in mice infected intravenously.[85]
ABC transporter	*Salmonella enterica*	Uptake	ZnuABC	Zn	The deletion strain has an $\sim 10^3$ higher LD_{50} relative to the wild-type strain in a murine model of infection.[84]
ABC transporter	*Salmonella enterica*	Uptake	sitABCD	Mn, Fe	Both transport systems are required for full virulence in mice.[95]
ABC transporter	*Salmonella enterica*	Uptake	MntH	Mn, Fe	
ABC transporter	*Salmonella enterica*	Uptake	sfaABC	Fe	Deletion strain is a-virulent in a mice model.[93]

virulence. A central role for transition metal ABC importers has been demonstrated for numerous important human pathogens (Table 1). In most cases, these are Mn^{+2} and $Fe^{+2/3}$ (in its chelated or ionic form) transporters, although there are also examples for Zn^{+2} importers. In many cases, the effects of deleting the genes encoding the transport system have been quite dramatic, ranging from 10^3 to 10^6 increases in LD_{50} (Table 1). In several cases, the deletion strains are practically a-virulent,[85,88,89,91–93] even less virulent than the strain presently used for vaccination. These studies point to the potential of using such deletion strains for vaccine development, an approach that is being actively pursued.[98]

ABC importers of transition metals play a role in the virulence of gram-positive and gram-negative bacteria, of bacilli, enterococci, firmicutes, blood-born, extra-cellular, and intra-cellular pathogens (Table 1). In light of the generality of this phenomenon, and in conjunction with the present-day antibiotic crisis, it is tempting to speculate that perhaps we can use this dependence of pathogens on transition metal ABC transporters to develop novel antibiotics. If we are able to identify/develop inhibitors toward these transport systems, we may be able to generate similar effects to the ones achieved in the deletion studies, *i.e.* dramatic attenuation in virulence. In our group, we are currently pursuing this goal by trying to develop small-molecule inhibitors that will prevent the association between the membrane-embedded transporter and its cognate substrate binding protein. Since complex formation between these two components is essential for transport, such an inhibitory molecule is expected to inhibit transition metal uptake and as a direct result reduce bacterial virulence.

References

1 C. Andreini, I. Bertini, G. Cavallaro, G. Holliday and J. Thornton, Metal ions in biological catalysis: from enzyme databases to general principles, *JBIC, J. Biol. Inorg. Chem.*, 2008, **13**(8), 1205–1218.
2 K. J. Waldron, J. C. Rutherford, D. Ford and N. J. Robinson, Metalloproteins and metal sensing, *Nature*, 2009, **460**(7257), 823–830.
3 K. Helbig, C. Grosse and D. H. Nies, Cadmium toxicity in glutathione mutants of *Escherichia coli*, *J. Bacteriol.*, 2008, **190**(15), 5439–5454.
4 K. H. Thompson and C. Orvig, Boon and bane of metal ions in medicine, *Science*, 2003, **300**(5621), 936–939.
5 D. H. Nies, Efflux-mediated heavy metal resistance in prokaryotes, *FEMS Microbiol. Rev.*, 2003, **27**(2–3), 313–339.
6 E.-H. Kim, D. H. Nies, M. M. McEvoy and C. Rensing, Switch or Funnel: How RND-Type Transport Systems Control Periplasmic Metal Homeostasis, *J. Bacteriol.*, 2011, **193**(10), 2381–2387.
7 E. L. Borths, K. P. Locher, A. T. Lee and D. C. Rees, The structure of *Escherichia coli* BtuF and binding to its cognate ATP binding cassette transporter, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**(26), 16642–16647.
8 V. Braun and F. Endriss, Energy-coupled outer membrane transport proteins and regulatory proteins, *BioMetals*, 2007, **20**(3), 219–231.
9 D. M. Carter, I. R. Miousse, J.-N. Gagnon, â. Martinez, A. Clements, J. Lee, M. A. Hancock, H. Gagnon, P. D. Pawelek and J. W. Coulton, Interactions between TonB from *Escherichia coli* and the Periplasmic Protein FhuD, *J. Biol. Chem.*, 2006, **281**(46), 35413–35424.
10 K. J. James, M. A. Hancock, J.-N. Gagnon and J. W. Coulton, TonB Interacts with BtuF, the *Escherichia coli* Periplasmic Binding Protein for Cyanocobalamin, *Biochemistry*, 2009, **48**(39), 9212–9220.
11 D. W. Christianson and J. D. Cox, Catalysis by metal-activated hydroxide in zinc and manganese metalloenzymes, *Annu. Rev. Biochem.*, 1999, **68**(1), 33–57.
12 D. L. Huffman and T. V. O'Halloran, Function, structure, and mechanism of copper trafficking proteins, *Annu. Rev. Biochem.*, 2001, **70**(1), 677–701.
13 K. D. Karlin, Metalloenzymes, structural motifs, and inorganic models, *Science*, 1993, **261**(5122), 701–708.
14 A. F. Parisi and B. L. Vallee, Zinc Metalloenzymes: Characteristics and Significance in Biology and Medicine, *Am. J. Clin. Nutr.*, 1969, **22**(9), 1222–1239.
15 K. Orino, L. Lehman, Y. Tsuji, H. Ayaki, S. V. Torti and F. M. Torti, Ferritin and the response to oxidative stress, *Biochem. J.*, 2001, **357**(1), 241–247.
16 C. Blindauer, Bacterial metallothioneins: past, present, and questions for the future, *J. Biol. Inorg. Chem*, 2011, 1–14.
17 R. Sharma, C. Rensing, B. P. Rosen and B. Mitra, The ATP hydrolytic activity of purified ZntA, a Pb(II)/Cd(II)/Zn(II)-translocating ATPase from *Escherichia coli*, *J. Biol. Chem.*, 2000, **275**(6), 3873–3878.
18 C. Rensing, B. Fan, R. Sharma, B. Mitra and B. P. Rosen, CopA: An *Escherichia coli* Cu(I)-translocating P-type ATPase, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**(2), 652–656.
19 K. Hollenstein, D. C. Frei and K. P. Locher, Structure of an ABC transporter in complex with its binding protein, *Nature*, 2007, **446**(7132), 213–216.
20 M. T. Sebulsky, B. H. Shilton, C. D. Speziali and D. E. Heinrichs, The Role of FhuD2 in Iron(III)-Hydroxamate Transport in *Staphylococcus aureus*, *J. Biol. Chem.*, 2003, **278**(50), 49890–49900.
21 J. M. Arguello, Identification of ion-selectivity determinants in heavy-metal transport P1B-type ATPases, *J. Membr. Biol.*, 2003, **195**(2), 93–108.
22 M. S. Francis and C. J. Thomas, Mutants in the CtpA copper transporting P-type ATPase reduce virulence of *Listeria monocytogenes*, *Microb. Pathog.*, 1997, **22**(2), 67–78.
23 O. Lewinson, A. T. Lee and D. C. Rees, A P-type ATPase importer that discriminates between essential and toxic transition metals, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**(12), 4677–4682.
24 S. Tottey, S. A. Rondet, G. P. Borrelly, P. J. Robinson, P. R. Rich and N. J. Robinson, A copper metallochaperone for photosynthesis and respiration reveals metal-specific targets, interaction with an importer, and alternative sites for copper acquisition, *J. Biol. Chem.*, 2002, **277**(7), 5490–7.
25 S. Tottey, P. R. Rich, S. A. Rondet and N. J. Robinson, Two Menkes-type atpases supply copper for photosynthesis in Synechocystis PCC 6803, *J. Biol. Chem.*, 2001, **276**(23), 19999–20004.
26 D. Kahn, M. David, O. Domergue, M. L. Daveran, J. Ghai, P. R. Hirsch and J. Batut, Rhizobium meliloti fixGHI sequence predicts involvement of a specific cation pump in symbiotic nitrogen fixation, *J. Bacteriol.*, 1989, **171**(2), 929–939.
27 B. K. Hassani, C. Astier, W. Nitschke and S. Ouchane, CtpA, a Copper-translocating P-type ATPase Involved in the Biogenesis of Multiple Copper-requiring Enzymes, *J. Biol. Chem.*, 2010, **285**(25), 19330–19337.
28 H.-G. Koch, C. Winterstein, A. S. Saribas, J. O. Alben and F. Daldal, Roles of the ccoGHIS gene products in the biogenesis of the cbb3-type cytochrome c oxidase, *J. Mol. Biol.*, 2000, **297**(1), 49–65.
29 O. Preisig, R. Zufferey and H. Hennecke, The Bradyrhizobium japonicum fixGHIS genes are required for the formation of the high-affinity cbb3-type cytochrome oxidase, *Arch. Microbiol.*, 1996, **165**(5), 297–305.
30 M. González-Guerrero, D. Raimunda, X. Cheng and J. M. Argüello, Distinct functional roles of homologous Cu^+ efflux ATPases in *Pseudomonas aeruginosa*, *Mol. Microbiol.*, 2010, **78**(5), 1246–1258.
31 S. J. Dutta, J. Liu, A. J. Stemmler and B. Mitra, Conservative and Nonconservative Mutations of the Transmembrane CPC Motif in ZntA: Effect on Metal Selectivity and Activity, *Biochemistry*, 2007, **46**(12), 3692–3703.
32 B. Fan and B. P. Rosen, Biochemical characterization of CopA, the *Escherichia coli* Cu(I)-translocating P-type ATPase, *J. Biol. Chem.*, 2002, **277**(49), 46987–46992.
33 C. C. Wu, A. Gardarin, A. Martel, E. Mintz, F. Guillain and P. Catty, The cadmium transport sites of CadA, the Cd^{2+}-ATPase

from *Listeria monocytogenes*, *J. Biol. Chem.*, 2006, **281**(40), 29533–29541.
34 N. Bal, C. C. Wu, P. Catty, F. Guillain and E. Mintz, Cd^{2+} and the N-terminal metal-binding domain protect the putative membranous CPC motif of the Cd^{2+}-ATPase of *Listeria monocytogenes*, *Biochem. J.*, 2003, **369**(3), 681–685.
35 B. Mitra and R. Sharma, The cysteine-rich amino-terminal domain of ZntA, a Pb(II)/Zn(II)/Cd(II)-translocating ATPase from *Escherichia coli*, is not essential for its function, *Biochemistry*, 2001, **40**(25), 7694–7699.
36 Z.-j. Hou, S. Narindrasorasak, B. Bhushan, B. Sarkar and B. Mitra, Functional Analysis of Chimeric Proteins of the Wilson Cu(I)-ATPase (ATP7B) and ZntA, a Pb(II)/Zn(II)/Cd(II)-ATPase from *Escherichia coli*, *J. Biol. Chem.*, 2001, **276**(44), 40858–40863.
37 B. Fan, G. Grass, C. Rensing and B. P. Rosen, *Escherichia coli* CopA N-Terminal Cys(X)2Cys Motifs Are Not Required for Copper Resistance or Transport, *Biochem. Biophys. Res. Commun.*, 2001, **286**(2), 414–418.
38 C.-C. Wu, W. J. Rice and D. L. Stokes, Structure of a Copper Pump Suggests a Regulatory Role for Its Metal-Binding Domain, *Structure (London)*, 2008, **16**(6), 976–985.
39 R. A. Pufahl, C. P. Singer, K. L. Peariso, S. J. Lin, P. J. Schmidt, C. J. Fahrni, V. C. Culotta, J. E. Penner-Hahn and T. V. O'Halloran, Metal Ion Chaperone Function of the Soluble Cu(I) Receptor Atx1, *Science*, 1997, **278**(5339), 853–856.
40 J. M. Walker, R. Tsivkovskii and S. Lutsenko, Metallochaperone Atox1 Transfers Copper to the NH2-terminal Domain of the Wilson's Disease Protein and Regulates Its Catalytic Activity, *J. Biol. Chem.*, 2002, **277**(31), 27953–27959.
41 M. Gonzalez-Guerrero and J. M. Arguello, Mechanism of Cu^{+}-transporting ATPases: Soluble Cu^{+} chaperones directly transfer Cu^{+} to transmembrane transport sites, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**(16), 5992–5997.
42 N. S. Kadaba, J. T. Kaiser, E. Johnson, A. Lee and D. C. Rees, The high-affinity *E. coli* methionine ABC transporter: structure and allosteric regulation, *Science*, 2008, **321**, 250–253.
43 S. Gerber, M. Comellas-Bigler, B. A. Goetz and K. P. Locher, Structural basis of trans-inhibition in a molybdate/tungstate ABC transporter, *Science*, 2008, **321**, 246–250.
44 M. K. Doeven, R. Abele, R. Tampe and B. Poolman, The binding specificity of OppA determines the selectivity of the oligopeptide ATP-binding cassette transporter, *J. Biol. Chem.*, 2004, **279**(31), 32301–32307.
45 N. Cadieux, C. Bradbeer, E. Reeger-Schneider, W. Koster, A. K. Mohanty, M. C. Wiener and R. J. Kadner, Identification of the Periplasmic Cobalamin-binding Protein BtuF of *Escherichia coli*, *J. Bacteriol.*, 2002, **184**(3), 706–717.
46 M. R. Rohrbach, V. Braun and W. Koster, Ferrichrome transport in *Escherichia coli* K-12: altered substrate specificity of mutated periplasmic FhuD and interaction of FhuD with the integral membrane protein FhuB, *J. Bacteriol.*, 1995, **177**(24), 7186–7193.
47 O. Lewinson, A. T. Lee, K. P. Locher and D. C. Rees, A distinct mechanism for the ABC transporter BtuCD-BtuF revealed by the dynamics of complex formation, *Nat. Struct. Mol. Biol.*, 2010, **17**(3), 332–338.
48 E. L. Borths, B. Poolman, R. N. Hvorup, K. P. Locher and D. C. Rees, *In vitro* Functional Characterization of BtuCD–F, the *Escherichia coli* ABC Transporter for Vitamin B12 Uptake, *Biochemistry*, 2005, **44**(49), 16301–16309.
49 P. Gourdon, X.-Y. Liu, T. Skjorringe, J. P. Morth, L. B. Moller, B. P. Pedersen and P. Nissen, Crystal structure of a copper-transporting PIB-type ATPase, *Nature*, 2011, **475**(7354), 59–64.
50 A. K. Mandal, Y. Yang, T. M. Kertesz and J. M. Arguello, Identification of the transmembrane metal binding site in Cu^{+}-transporting PIB-type ATPases, *J. Biol. Chem.*, 2004, **279**(52), 54802–54807.
51 S. J. Dutta, J. Liu, Z. Hou and B. Mitra, Conserved aspartic acid 714 in transmembrane segment 8 of the ZntA subgroup of P1B-type ATPases is a metal-binding residue, *Biochemistry*, 2006, **45**(18), 5923–5931.
52 Y. Hatori, E. Majima, T. Tsuda and C. Toyoshima, Domain organization and movements in heavy metal ion pumps: papain digestion of CopA, a Cu^{+}-transporting ATPase, *J. Biol. Chem.*, 2007, **282**(35), 25213–25221.
53 W. W. Ho, H. Li, S. Eakanunkul, Y. Tong, A. Wilks, M. Guo and T. L. Poulos, Holo- and apo-boundstructures of bacterial periplasmic heme-binding proteins, *J. Biol. Chem.*, 2007, **282**(49), 35796–35802.
54 J. B. Neilands, Siderophores: Structure and Function of Microbial Iron Transport Compounds, *J. Biol. Chem.*, 1995, **270**(45), 26723–26726.
55 C. Linke, T. T. Caradoc-Davies, P. G. Young, T. Proft and E. N. Baker, The Laminin-Binding Protein Lbp from *Streptococcus pyogenes* is a Zinc Receptor, *J. Bacteriol.*, 2009, **191**(18), 5814–5823.
56 V. Rukhman, R. Anati, M. Melamed-Frank and N. Adir, The MntC Crystal Structure Suggests that Import of Mn^{2+} in Cyanobacteria is Redox Controlled, *J. Mol. Biol.*, 2005, **348**(4), 961–969.
57 R. Albers, Biochemical aspects of active transport, *Annu. Rev. Biochem.*, 1967, **36**, 727–756.
58 R. L. Post, C. Hegyvary and S. Kume, Activation by Adenosine Triphosphate in the Phosphorylation Kinetics of Sodium and Potassium Ion Transport Adenosine Triphosphatase, *J. Biol. Chem.*, 1972, **247**(20), 6530–6540.
59 J. Argüello, E. Eren and M. González-Guerrero, The structure and function of heavy metal transport P1B-ATPases, *BioMetals*, 2007, **20**(3), 233–248.
60 M. Bublitz, H. Poulsen, J. P. Morth and P. Nissen, In and out of the cation pumps: P-Type ATPase structure revisited, *Curr. Opin. Struct. Biol.*, 2010, **20**(4), 431–439.
61 P. L. Jorgensen, K. O. Håkansson and S. J. D. Karlish, Structure and Mechanism of Na, K-ATPASE: Functional Sites and Their Interactions, *Annu. Rev. Physiol.*, 2003, **65**(1), 817–849.
62 C. Toyoshima, How Ca^{2+}-ATPase pumps ions across the sarcoplasmic reticulum membrane, *Biochim. Biophys. Acta, Mol. Cell Res.*, 2009, **1793**(6), 941–946.
63 G. F.-L. Ames, C. E. Liu, A. K. Joshi and K. Nikaido, Liganded and Unliganded Receptors Interact with Equal Affinity with the Membrane Complex of Periplasmic Permeases, a Subfamily of Traffic ATPases, *J. Biol. Chem.*, 1996, **271**(24), 14264–14270.
64 J. S. Patzlaff, T. van der Heide and B. Poolman, The ATP/substrate stoichiometry of the ATP-binding cassette (ABC) transporter OpuA, *J. Biol. Chem.*, 2003, **278**(32), 29546–29551.
65 K. Nikaido and G. F.-L. Ames, One Intact ATP-binding Subunit is Sufficient to Support ATP Hydrolysis and Translocation in an ABC Transporter, the Histidine Permease, *J. Biol. Chem.*, 1999, **274**(38), 26727–26735.
66 I. L. Urbatsch, L. Beaudet, I. Carrier and P. Gros, Mutations in Either Nucleotide-Binding Site of P-glycoprotein (Mdr3) prevent Vanadate Trapping of Nucleotide at Both SitesÄ†, *Biochemistry*, 1998, **37**(13), 4592–4602.
67 R. Ernst, P. Kueppers, C. M. Klein, T. Schwarzmueller, K. Kuchler and L. Schmitt, A mutation of the H-loop selectively affects rhodamine transport by the yeast multidrug ABC transporter Pdr5, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**(13), 5069–5074.
68 A. K. Mandal, W. D. Cheung and J. M. Argüello, Characterization of a Thermophilic P-type Ag^{+}/Cu^{+}-ATPase from the Extremophile *Archaeoglobus fulgidus*, *J. Biol. Chem.*, 2002, **277**(9), 7201–7208.
69 A. Bateman, E. Birney, R. Durbin, S. R. Eddy, K. L. Howe and E. L. Sonnhammer, The Pfam protein families database, *Nucleic Acids Res.*, 2000, **28**(1), 263–266.
70 H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, I. N. Shindyalov and P. E. Bourne, The Protein Data Bank, *Nucleic Acids Res.*, 2000, **28**(1), 235–242.
71 S. Henikoff, E. A. Greene, S. Pietrokovski, P. Bork, T. K. Attwood and L. Hood, Gene families: the taxonomy of protein paralogs and chimeras, *Science*, 1997, **278**(5338), 609–614.
72 R. P. Berntsson, S. H. Smits, L. Schmitt, D. J. Slotboom and B. Poolman, A structural classification of substrate-binding proteins, *FEBS Lett.*, 2010, **584**(12), 2606–2617.
73 E. Dassa and P. Bouige, The ABC of ABCS: a phylogenetic and functional classification of ABC systems in living organisms, *Res. Microbiol.*, 2001, **152**(3–4), 211–229.
74 A. L. Davidson, E. Dassa, C. Orelle and J. Chen, Structure, function, and evolution of bacterial ATP-binding cassette systems, *Microbiol. Mol. Biol. Rev.*, 2008, **72**(2), 317–364, table of contents.
75 D. C. Rees, E. Johnson and O. Lewinson, ABC transporters: the power to change, *Nat. Rev. Mol. Cell Biol.*, 2009, **10**(3), 218–227.
76 K. P. Locher, B. Rees, R. Koebnik, A. Mitschler, L. Moulinier, J. P. Rosenbusch and D. Moras, Transmembrane Signaling across

the Ligand-Gated FhuA Receptor: Crystal Structures of Free and Ferrichrome-Bound States Reveal Allosteric Changes, *Cell*, 1998, **95**(6), 771–778.
77 C. Bradbeer, P. R. Reynolds, G. M. Bauler and M. T. Fernandez, A requirement for calcium in the transport of cobalamin across the outer membrane of *Escherichia coli*, *J. Biol. Chem.*, 1986, **261**(6), 2520–2523.
78 R. Annamalai, B. Jin, Z. Cao, S. M. Newton and P. E. Klebba, Recognition of ferric catecholates by FepA, *J. Bacteriol.*, 2004, **186**(11), 3578–3589.
79 K. Postle and R. A. Larsen, TonB-dependent energy transduction between outer and cytoplasmic membranes, *BioMetals*, 2007, **20**(3–4), 453–465.
80 J. Chen, S. Sharma, F. A. Quiocho and A. L. Davidson, Trapping the transition state of an ATP-binding cassette transporter: evidence for a concerted mechanism of maltose transport, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**(4), 1525–1530.
81 V. Braun and C. Herrmann, Docking of the periplasmic FecB binding protein to the FecCD transmembrane proteins in the ferric citrate transport system of *Escherichia coli*, *J. Bacteriol.*, 2007, **189**(19), 6913–6918.
82 W. R. Schwan, P. Warrener, E. Keunz, C. K. Stover and K. R. Folger, Mutations in the cueA gene encoding a copper homeostasis P-type ATPase reduce the pathogenicity of *Pseudomonas aeruginosa* in mice, *Int. J. Med. Microbiol.*, 2005, **295**(4), 237–242.
83 S. K. Ward, B. Abomoelak, E. A. Hoye, H. Steinberg and A. M. Talaat, CtpV: a putative copper exporter required for full virulence of *Mycobacterium tuberculosis*, *Mol. Microbiol.*, 2010, **77**(5), 1096–1110.
84 S. Ammendola, P. Pasquali, C. Pistoia, P. Petrucci, P. Petrarca, G. Rotilio and A. Battistoni, High-Affinity Zn^{2+} Uptake System ZnuABC is Required for Bacterial Zinc Homeostasis in Intracellular Environments and Contributes to the Virulence of *Salmonella enterica*, *Infect. Immun.*, 2007, **75**(12), 5867–5876.
85 S. W. Bearden and R. D. Perry, The Yfe system of *Yersinia pestis* transports iron and manganese and is required for full virulence of plague, *Mol. Microbiol.*, 1999, **32**(2), 403–414.
86 D. Brem, C. Pelludat, A. Rakin, C. A. Jacobi and J. Heesemann, Functional analysis of yersiniabactin transport genes of *Yersinia enterocolitica*, *Microbiology (Reading, U. K.)*, 2001, **147**(Pt 5), 1115–1127.
87 J. S. Brown, S. M. Gilliland and D. W. Holden, A *Streptococcus pneumoniae* pathogenicity island encoding an ABC transporter involved in iron uptake and virulence, *Mol. Microbiol.*, 2001, **40**(3), 572–585.
88 J. D. Fetherston, V. J. Bertolino and R. D. Perry, YbtP and YbtQ: two ABC transporters required for iron uptake in *Yersinia pestis*, *Mol. Microbiol.*, 1999, **32**(2), 289–299.
89 O. Gat, I. Mendelson, T. Chitlaru, N. Ariel, Z. Altboum, H. Levy, S. Weiss, H. Grosfeld, S. Cohen and A. Shafferman, The solute-binding component of a putative Mn(II) ABC transporter (MntA) is a novel *Bacillus anthracis* virulence determinant, *Mol. Microbiol.*, 2005, **58**(2), 533–551.
90 R. Janulczyk, S. Ricci and L. Bjorck, MtsABC is Important for Manganese and Iron Transport, *Oxidative Stress Resistance, and Virulence of* Streptococcus pyogenes*, Infection and Immunity*, 2003, **71**(5), 2656–2664.
91 A. Marra, S. Lawson, J. S. Asundi, D. Brigham and A. E. Hromockyj, *In vivo* characterization of the psa genes from *Streptococcus pneumoniae* in multiple models of infection, *Microbiology*, 2002, **148**(Pt 5), 1483–1491.
92 S. Paik, A. Brown, C. L. Munro, C. N. Cornelissen and T. Kitten, The sloABCR operon of Streptococcus mutans encodes an Mn and Fe transport system required for endocarditis virulence and its Mn-dependent repressor, *J. Bacteriol.*, 2003, **185**(20), 5967–5975.
93 T. Pattery, J. P. Hernalsteens and H. De Greve, Identification and molecular characterization of a novel *Salmonella enteritidis* pathogenicity islet encoding an ABC transporter, *Mol. Microbiol.*, 1999, **33**(4), 791–805.
94 G. M. Rodriguez and I. Smith, Identification of an ABC transporter required for iron acquisition and virulence in *Mycobacterium tuberculosis*, *J. Bacteriol.*, 2006, **188**(2), 424–430.
95 M. L. Zaharik, V. L. Cullen, A. M. Fung, S. J. Libby, S. L. Kujat Choy, B. Coburn, D. G. Kehres, M. E. Maguire, F. C. Fang and B. B. Finlay, The *Salmonella enterica serovar typhimurium* divalent cation transport systems MntH and SitABCD are essential for virulence in an Nramp1G169 murine typhoid model, *Infect. Immun.*, 2004, **72**(9), 5522–5525.
96 D. S. Ong, L. Wang, Y. Zhu, B. Ho and J. L. Ding, The response of ferritin to LPS and acute phase of *Pseudomonas* infection, *J. Endotoxin Res.*, 2005, **11**(5), 267–280.
97 M. F. Cellier, P. Courville and C. Campion, Nramp1 phagocyte intracellular metal withdrawal defense, *Microbes Infect.*, 2007, **9**(14–15), 1662–1670.
98 H. S. Garmory and R. W. Titball, ATP-binding cassette transporters are targets for the development of antibacterial vaccines and therapies, *Infect. Immun.*, 2004, **72**(12), 6757–6763.

