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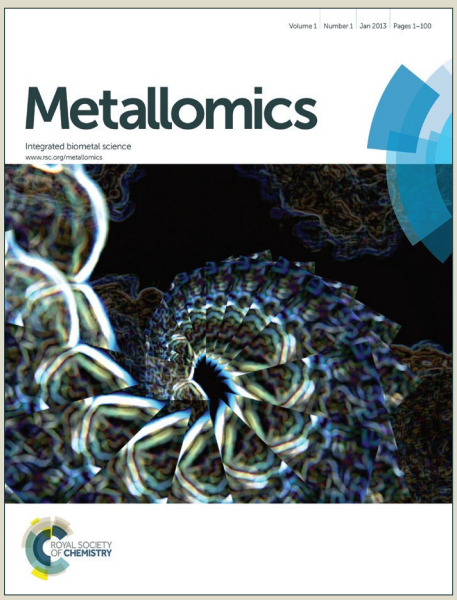

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Bacterial Cu^+-ATPases: Models for molecular structure-function studies

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José M. Argüello,[a] Sarju J. Patel[a] and Julia Quintana[a]

The early discovery of the human Cu^+-ATPases and their link to Menkes and Wilson's diseases brought attention to the unique role of these transporters in copper homeostasis. The characterization of bacterial Cu^+-ATPases has significantly furthered our understanding on the structure, selectivity and transport mechanism of these enzymes, as well as their interplay with other elements of Cu^+ distribution networks. This review focuses on the structural-functional insights that have emerged from studies of bacterial Cu^+-ATPase at the molecular level and how these observations have contributed to draw up a comprehensive picture of cellular copper homeostasis.

Distribution of Cu^+-ATPases in biological systems and their relevance for copper homeostasis

Rhizobium meliloti fixI/copA2, identified as a P-type ATPase associated with N_2 fixation, was probably the first gene encoding for a Cu^+-ATPase mentioned in the literature [1]. It was later established that FixI/CopA2 translocates cytoplasmic Cu^+ for metallation of the cbb_3-type cytochrome complex [1-4]. The early observation of the role of *Enterococcus hirae* CopA and CopB in copper tolerance linked the large family of P-type ATPases with the transmembrane copper transport [5, 6]. The contemporary discovery of the human Cu^+-ATPases associated with Menkes and Wilson's diseases, ATP7A and ATP7B respectively, generated significant interest and further drove the study of this subfamily of proteins [7-9].

From a functional-phylogenetic standpoint, Cu^+-ATPases are classified within the P_{1B} subfamily of P-type ATPases. Members of this subfamily transport various heavy metals including Cu^+, Cu^{2+}, Zn^{2+}, Mn^{2+}, Co^{2+} and Fe^{2+} [10-14]. Cu^+-ATPases are widely distributed in all kingdoms of life [12, 15, 16]. Their key role in copper tolerance is well established in bacteria and archea [17-19]. They contribute to maintain the cellular Cu quota (i.e. total Cu in the cell) by driving the cytoplasmic Cu^+ efflux. Several Cu^+-sensing transcriptional regulators (CueR, CopY, CsoR) control Cu^+-ATPase levels [17-19]. In recent years, the involvement of bacterial Cu^+-ATPases in the metallation of secreted, periplasmic, and plasma membrane cuproproteins has been uncovered[20-24]. Again, this is directly linked to the molecular function of all Cu^+-ATPases, that is, the energy dependent efflux of cytoplasmic Cu^+.

These essential biological functions; i.e., Cu^+ tolerance and enzyme metallation, are also common to the eukaryote Cu^+-ATPases. For instance, studies of *Saccharomyces cerevisiae* CCC2 provided the initial evidence for the ATP-mediated Cu^+ transport into the secretory pathway for the metallation of iron oxidase FET3 that is required for iron uptake [25, 26]. The genomes of yeasts, insects and nematodes encode a single Cu^+-ATPase; however, gene duplication occurred in chordates (birds, fish and mammals) [27]. Interestingly, the genomes of photosynthetic organisms have an array of genes coding for Cu^+-ATPases [28, 29]. The model plant *Arabidopsis thaliana*, for example, contains four Cu^+-ATPase isoforms (AtHMA5–AtHMA8). AtHMA5 is involved in copper compartmentalization and detoxification within root cells [30]. Localized in the post-Golgi network, AtHMA7 is responsible for metallation of ethylene receptors [31]. AtHMA6 transport Cu^+ across the chloroplast inner envelope membrane into the stroma and is required for Cu^+ loading into Cu/Zn-SOD. AtHMA6 also works in tandem with AtHMA8, located in the thylakoid membrane, providing Cu^+ to plastocyanin [32, 33].

The human genome encodes only two Cu^+-ATPases, ATP7A and ATP7B, and mutations in the corresponding genes lead to Menkes and Wilson's diseases, respectively [7-9]. *ATP7A* and *ATP7B* display tissue and cell type-specific expression patterns generating distinct physiological functions. ATP7A is present in most extrahepatic tissues, whereas ATP7B is preferentially found in liver [34-36]. Cu^+ efflux by ATP7A located in the basolateral membrane of the intestinal epithelia is responsible for copper absorption in humans [34]. Consequently, dysfunction of ATP7A leads to a severe systemic copper deficiency associated with Menkes disease, occipital horn syndrome and ATP7A-related distal motor neuropathy [34, 35]. In particular, Menkes disease is characterized by severe developmental abnormalities, dramatic neurologic impairment, and death during early childhood [37, 38]. ATP7B-deficiency results in Wilson

[a] Department of Chemistry and Biochemistry, Worcester Polytechnic Institute, 100 Institute Road, Worcester, MA 01609, USA.
E-mail: arguello@wpi.edu

disease. This is associated with copper overload in the liver and brain, manifested as progressive neurological and hepatic abnormalities as well as neuropsychiatric disorders[35, 39-41]. ATP7A and ATP7B are located in the trans-Golgi network and transport Cu^+ into the secretory pathway for incorporation into cuproenzymes such as dopamine-β-hydroxylase, tyrosinase, lysyl oxidase, peptidylglycine-α-amidating monooxygenase and extracellular SOD3 (ATP7A) or ceruloplasmin (ATP7B) [34, 42, 43]. Importantly, in response to changes in the intracellular copper quota, ATP7A and ATP7B traffics to the plasma membrane to extrude ion excess [42, 44]. Upon restoring physiological levels, these enzymes are relocated to the trans-Golgi network [45 46]. Cu^+-ATPases might also play an important role at the host-pathogen interface. It has been proposed that macrophages recruit ATP7A to phagosomes containing encapsulated bacteria inducing toxic Cu^+ stress as part of the immune response [47]. The importance of bacterial Cu^+-ATPases in virulence, further supports this attractive hypothesis [48].

The molecular structure responsible for copper transmembrane translocation

The hallmark of P-type ATPases is the catalytic mechanism driven by phosphorylation of the Asp residue in the invariant DKTGT sequence. Cu^+-ATPases, as all P-type ATPases, have three large cytoplasmic domains (Fig. 1) [49-53]. The nucleotide (N) domain binds and positions ATP for phosphorylation of the DKTGT region located in the phosphorylation (P) domain. This drives the movement of the actuator (A) domain, leading to the rearrangement of transmembrane segments (TMs) necessary for substrate translocation across the permeability barrier [54, 55]. Most P-type enzymes have 6-12 TMs with the substrate-binding TMs flanking the P and N domains [12, 54, 56]. Cu^+-ATPases have eight TMs (Fig. 1). Metal binding assays performed in *Archaeoglobus fulgidus* CopA [57], *Escherichia coli* CopA[23] and *A. thaliana* HMA6 and HMA8[22] have shown the presence of two metal binding sites in the transmembrane region of Cu^+-ATPases. Transmembrane fragments 6^{th}, 7^{th} and 8^{th} contain conserved residues for the two transmembrane metal binding sites (TM-MBS) that coordinate Cu^+ during transport (Fig. 1) [12, 57, 58]. Site I is constituted by two Cys in TM6 and a Tyr in TM7. An Asn in TM7 and Met and Ser in TM8 form Site II. Except for the Asn amino group, the absence of side chains capable of acid/base protonation/deprotonation is conspicuous in these sites. On the other hand, coordination by intermediate (N) or soft (S) Lewis base ligands is congruent with Cu^+ soft Lewis acid attributes [59]. Interestingly, a single transmembrane Cu^+ binding site has been observed in *Legionella pneumophila* CopA [60]. While this enzyme has the six invariant residues in the 6^{th}, 7^{th} and 8^{th} transmembrane segments, only the two Cys in the 4^{th} and the Met in the 8^{th} appear to coordinate the ion.

X-ray spectroscopic analysis showed the trigonal planar coordination of Cu^+ at both TM-MBS [57]. Cu^+ is relatively unique among transition metals in that it capable of adopting 2, 3 and 4-coordinate complexes [61]. Nevertheless, it could be considered that despite binding Cu^+ with very high affinity, TM-MBSs only transiently interact with the metal as in the presence of ATP they are transported across the membrane. Moreover, the vectorial ion release (i.e. across the permeability barrier) would occur upon minimal coordination shifts. Then, observation of novel ligands (various Lewis bases) and the trigonal coordination architecture in these proteins might be a functional requirement. Similar trigonal coordination is apparent in the Ctr and Cus Cu^+ transport systems [59, 62, 63].

Description of high-resolution structures of *L. pneumophila* CopA had a significant impact in our understanding of Cu^+ transport [52, 53] (Fig. 1). These confirmed the architecture of the A, N, and P domains and gave a detailed first view of the transmembrane region when the enzyme is an E2 conformation (Fig. 2). Although the obtained structures do not reveal the metal bound architecture, they show the proximity of residues proposed to form the two TM-MBSs. Importantly, the structures revealed a platform formed by the bending of the 2^{nd} TM and the proximity of invariant residues forming a putative entry site into the permeation path (Fig.1). Cu^+ ions access the ATPase bound to cytoplasmic Cu^+-chaperones (see below). Then, it was proposed that the platform might serve as a landing pad for the chaperone-ATPase interaction, as the invariant residues might be involved in ligand exchange and Cu^+ transfer to the TM-MBSs [52]. Subsequent studies yielded experimental evidence consistent with this hypothesis [64]. On the other hand, Cu^+ extrusion through a specific permeation path with participation of invariant Met and Glu amino acids has been proposed [53]. Different studies have also shown that similar to the cytoplasmic platform, periplasmic (luminal) loops provide a docking site for metal accepting apo-chaperones or alternative cuproproteins (Fig. 1) [23, 65].

Cu^+-ATPases also contain Cu^+ sensing regulatory domains (N-MBDs) in their cytoplasmic N-terminus. The presence of N- or C-terminal regulatory domains is a common feature of various P-type ATPases [66-68]. Bacterial Cu^+-ATPases contain one or two N-MBD while eukaryote enzymes contain up to six [69]. These 70 amino acids long domains present a classic βαββαβ ferredoxin-like folding with an invariant GXXCXXC Cu^+-binding motif [69-71]. Less common N-MBDs with cuprodoxin folding have been recently identified [72]. Mechanistic studies have uncovered the regulatory role of N-MBDs in bacteria [73]. These are not required for enzyme activity or transport; however, Cu^+ binding to N-MBDs increases enzyme velocity. The interaction of N-MBD and N domains in the absence of Cu^+ has been observed [51, 74, 75]. Then, if this interaction slows the nucleotide hydrolysis, it provides a structural mechanism by which N-MBD can affect the enzyme turnover rate. Studies in eukaryote systems have shown the importance of these domains in the trafficking and cellular roles of ATP7A and ATP7B [34, 76].

The transport mechanism of Cu^+-ATPases

Cu^+-ATPases drive the efflux of cytoplasmic Cu^+ using the energy provided by ATP hydrolysis. They perform this task following the classical Albers-Post cycle described for P-ATPases (Fig. 2) [77]. During transport, the enzyme adopts two basic conformations (E1↔E2). In the case of Cu^+-ATPases, these conformational changes were verified using partial proteolytic digestions while placing the enzyme in alternating conformations [78, 79]. The cornerstone of the catalysis is however the enzyme phosphorylation upon binding of ATP and the outward transported cytoplasmic substrate (Fig. 2, steps 1-4). This is, metal binding sites face the cytoplasm when ATP occupies the N domain. As a consequence of this structural/mechanistic constrain, all Cu^+ ATPases drive the efflux of cytoplasmic Cu^+. *In vivo* studies have repeatedly shown this phenomenon [17-19]. In addition, detailed transport studies using membrane vesicles and taking advantage of the sidedness of ATP hydrolysis have experimentally shown a consistent outward direction of transport [21, 80]. Alternatively, the necessary coupling of ATP hydrolysis and ion transport has enabled the study of the catalytic cycle by measuring ATPase activity. Cu^+-ATPases transport Cu^+ at a relatively slow rate (200 min^{-1}) when compared to the faster proton or alkali transporting P-type ATPases (10,000 min^{-1}). This appears to be a general characteristic of transition metal transporters [59].

In vitro studies have shown that Cu^+-ATPases transport and are activated by free Cu^+ in solution[81]. These enzymes are also activated by non-cognate ions such as Ag^+ and Au^+ [81, 82]. These ions can apparently fit into the TM-MBSs and enable the required catalytic conformational changes. There is no evidence of the relative affinities of Cu^+ ATPases for Ag^+ or Au^+. However, kinetics characterization suggests that Ag^+ drives a higher enzyme turnover with a larger $K_{1/2}$ [81]. It can be then hypothesized that the non-cognate ions have a poorer interaction (lower binding affinity) with a faster efflux associated with poor metal coordination. Since the metal release appears as the rate-limiting step in the transport cycle [81], a higher Vmax for non-cognate ions could be expected. CopA binds two Cu^+ at the TM-MBSs with affinities in the 0.1-1 fM range [57]. Access of Cu^+ to these sites is not sequential but rather independent to either site [57]. The high affinity of these sites prevents the backward release of Cu^+ from inward facing sites, since this would lead to free Cu^+ in the cytoplasm with the ensuing toxic effects. Cu^+ occupancy of both sites is required to trigger enzyme phosphorylation by ATP (Fig. 2, step 4)[57, 58]. Protein phosphorylation is followed by E1P→E2P conformational change concomitant with the opening of TM-MBS to the periplasm ($gram^-$ bacteria) or extracellular milieu (Fig. 2, steps 5 & 6). In this conformation the TM-MBS have an undetermined but lower affinity for Cu^+ that is released from the TM-MBS to bind a soluble target protein [23].

Chaperone mediated ion uptake and release of Cu^+-ATPases

As aforementioned, Cu^+ ATPases can operate obtaining Cu^+ from the assay solution and releasing the ion into a vesicular compartment [21, 80, 83]. The high affinity of metal sensing transcriptional regulators for Cu^+ is congruent with the virtual absence of free Cu^+ in cells [84, 85]. Consequently, *in vivo* Cu^+ gains access and leaves the ion-transporting ATPases bound to soluble chaperones [23, 86]. Thus, metallochaperones are a major component of Cu^+ distribution networks, enabling accurate metal targeting to transmembrane transporters and metalloproteins. The cytoplasmic Cu^+ chaperone CopZ shares high sequence-structure homology with the N-MBD of Cu^+-ATPases [69, 71] (Fig. 1). As N-MBDs, the two Cys in the CXXC sequence linearly coordinate one Cu^+ ion. Nevertheless, it has been hypothesized that *in vivo* the participation of glutathione leading to a trigonal Cu^+ coordination might prevent the chaperone homodimer formation observed *in vitro* [69, 87]. The cytosolic concentration of CopZ appears in the 0.1 - 1 μM range with pM K_D for Cu^+ [88, 89]. Extensive studies have demonstrated that CopZ interact with the regulatory N-MBD of Cu^+-ATPases, exchanging Cu^+ with K_{eq}≈1 [69-71, 90]. This equilibrium of the Cu^+-N-MBD pool with Cu^+-CopZ is in line with the regulation of ATPase activity by N-MBDs upon "sensing" the Cu^+-CopZ pool. The N-MBD/CopZ recognition appears mediated by electrostatic interactions [69, 71]. Upon specific docking, Cu^+ is transferred via ligand exchange. This Cu^+ exchange between CopZ and N-MBDs is independent of the delivery of Cu^+ to the ATPase permeation path for transmembrane transport [64, 86] (see below).

CopZ loads the Cu^+ substrate into TM-MBS of Cu^+-ATPases [48, 64, 75, 86] (Fig. 2, steps 2 & 3). Experiments using truncated *A. fulgidus* CopA lacking the cytoplasmic MBD, support the idea that the chaperone mediated Cu^+ loading of TM-MBS is independent of the presence of Cu^+ transfer to the N-MBD[86]. However, the sequence of events in the full length ATPase, this is, what site is loaded first N-NMD or TM-MBS, has not been established. Similar experiments performed with archaeal and eukaryote proteins have shown that MBD could not transfer Cu^+ to TM-MBS[21, 63]. Homology modeling to available chaperone structures and mutagenesis experiment suggest that the docking of the negatively charged surface of CopZ with the electropositive platform region of CopA appears to direct the chaperone Cu^+-binding residues toward the ion permeation path [52, 64] (Fig. 3). The electrostatic interaction seems quite specific, as no activation by homologous N-MBD/chaperone domains has been observed [22, 52, 64, 86]. Mutagenesis experiments have also shown the requirement for the Cu^+ transfer of three invariant Met, Glu and Asp residues located close to the platform [52, 64]. It has been proposed that these form a ligand exchange, "Cu^+ entrance site", which bridges the Cu^+ access to the TM-MBSs. This model for Cu^+ delivery by the chaperone and access the permeation path in the ATPase is however distinct in the case of *Streptococcus pneumoniae*[72, 91]. In this organism, the membrane associated chaperone CupA, as the N-MBDs in the ATPase CopA, have singular cupredoxin-like folds. While the specific protein-protein interaction is still required for

conferring Cu^+ tolerance, mutations of the platform structure or a His residue in the possible "Cu^+ entrance site" of the ATPase do not impair the copper tolerance. This is the chaperone docking and the Cu^+ transfer still occurs. These observations suggest an alternative Cu^+ delivery model for the chaperone/ATPase system of *S. pneumoniae.*

The proposed mechanism for the delivery of Cu^+ by CopZ would imply that Cu^+ access the transport path dehydrated. Interestingly, *in vitro,* the Cu^+-CopZ drives a higher V_{max} than free Cu^+ [86]. A possible explanation is that the ligand exchange site can strip off Cu^+ from the chaperone faster than from the hydration shell surrounding a free Cu^+. An alternative hypothesis is that the presence of the chaperone rebinding the released ion prevents the backward binding of Cu^+ to outward facing binding sites. The inhibition of transport at high Cu^+ concentration is well documented [92]. Cu^+ transfer from Cu^+-CopZ to CopA appears essentially stoichiometric [75]. This could be explained by a model where CopA has high affinity for Cu^+-CopZ such as total transfer will occur even when the ratio of Cu^+-CopZ to CopA is low. The underlying implication is the preponderance of apo-CopZ under physiological conditions. Alternatively, CopA appears to have very low affinity for apo-CopZ. This is a logic mechanistic requirement as CopZ carry a single Cu^+ and the enzyme requires the binding of two Cu^+ to undergo catalysis and transport.

A similar Cu^+ transfer is observed at the exit site of Cu^+-ATPase. Using *E. coli* CopA and CusF, direct Cu^+ transfer from the ATPase to the periplasmic chaperone was observed [23] (Fig. 3). CusF is the periplasmic soluble chaperone (Fig. 1), part of CusCBA system responsible for periplasmic Cu^+ efflux to the extracellular milieu. CusF binds Cu^+ via conserved His^{36}, Trp^{44}, Met^{47}, and Met^{49} with binding affinities in the nM/pM range [93, 94]. *In silico* analysis and biochemical assays have shown the specific interaction of the Cu^+-bound form of CopA with apo-CusF for subsequent metal transfer upon ATP hydrolysis (Fig. 2, step 5 & 6). That is, the Cu^+ bound CopA has a higher affinity for the apo-CusF than for the holo-CusF (Fig. 3). It is apparent that the conserved electropositive surface of CusF interact with the 3rd extracellular loop of the Cu^+-ATPase (Fig. 1), as replacement of polar amino acids in these surfaces leads to a decrease in Cu^+ transfer [23, 52, 53]. The presence of the chaperone does not seem to affect the transport stoichiometry because both Cu^+ leave the ATPase to load two CusF equivalents, provided that the chaperone is in excess [23].

Cu^+ transfer to and from chaperones is an integral part of the ATPase catalytic cycle. The conformations that the enzyme adopts during the catalytic cycle interact differentially with alternative chaperones. The Cu^+-CopZ interacts with the enzyme in the E1 conformation (cytoplasmic facing TM-MBSs) (Fig. 2, steps 1 & 3)[75]. Upon binding of two Cu^+, the ATPase is phosphorylated and transitions to the E1·P state, where Cu^+ ions are in an occluded state (Fig. 2, step 4). Subsequently, the enzyme moves into the E2 form, where Cu^+ is released (Fig. 2, step 5 and 6). Accordingly, the metal bound E1 and E1P forms of CopA interact with apo-CusF [23]. In line with these observations, there is no evident interaction of apo-CopZ or Cu^+-CusF with CopA. A gradient in the relative affinities of chaperones and transporters has been considered as a mechanism to direct the outward flux of cytoplasmic Cu^+. A central implication of the structural ligand exchange between chaperone and Cu^+-ATPase, the high affinity of CopZ-Cu^+/CopA or CopA-Cu^+/CusF and the stoichiometrical transfer is that the affinities of each protein for Cu^+ is not likely relevant for the transfer. Protein affinities for Cu^+ describe the preference of Cu^+ to be in water *vs* being bound and are likely determined by k_{off} rather than diffusion limited k_{on}. On the other hand, *in vivo* Cu^+ transfer occurs in an apparently dehydrate environment and it is dependent on the preference of Cu^+ for the alternating ligands. Extrapolation of the energetics provided by K_D to the ion partition between two different non-polar environments (compared to water) might not be straightforward.

Functional diversity of homologous Cu^+-ATPases

Bacterial genomes, with very few exceptions, encode at least one Cu^+-ATPase that maintains the cytoplasmic metal quota [12, 19]. Furthermore, the presence of multiple Cu^+-ATPase genes in bacterial genomes has long been recognized [12, 48]. Why do simple bacterial systems require multiple Cu^+-ATPases? The described specificity in the chaperone-transporter-chaperone relay provides a sound hypothesis where alternative ATPases function delivering Cu^+ to different targets. For instance, consider periplasmic chaperones that deliver Cu^+ either to efflux systems or target cuproenzymes. It seems then reasonable that distinct proteins might interact with specific partners. Moreover, while the "Cu^+-efflux network" might be under Cu^+ sensing regulators, alternative Cu^+ distribution networks might be under different control.

In support of these ideas, the metallation of membrane and periplasmic cuproproteins has been linked to the ATPase mediated Cu^+ transport [23, 24, 95]. For instance, *Pseudomonas aeruginosa* contains two homologous Cu^+-ATPases. CopA1 mediates copper detoxification and CopA2 is involved in cytochrome *c* oxidase metallation [80]. A second, perhaps more complex example is *Sinorhizobium meliloti,* a symbiotic organism that contains five homologous Cu^+-ATPases. CopA1a and CopA1b seem to maintain the cytoplasmic Cu^+ levels at different stages of symbiotic life-cycle. CopA2a and CopA2b are necessary for the assembly of two different cytochrome *c* oxidases which are synthesized at different life-stages. Finally, CopA3 is expressed in response to redox stress and shows low enzymatic activity. This suggest a role in metallation of periplasmic or membrane-bound cuproenzymes [4]. The pathogen *Salmonella typhimurium* contains two Cu^+-ATPases, CopA and GolT [96]. These are required to maintain cytosolic quota or deliver Cu^+ to the periplasmic chaperone CueP for metallation of periplasmic Cu/Zn- superoxide dismutase SodCII [24]. Strikingly, both ATPases are functionally redundant, which arises new questions about functional paralogs among Cu^+-ATPases.

Future directions

Significant progress has been made to understand the similarities, and differences, of Cu^+-ATPases with the very well characterized alkali metal transporting ATPases. Nevertheless, Cu^+ permeation along the protein is poorly understood. Upon enzyme phosphorylation, Cu^+ is released from the TM-MBS. However, it must travel a 7-12 Å short permeation path before exiting the protein. The *L. pneumophila* CopA structure suggest that the ions exit through a narrow, dehydrated channel. It has been proposed that conserved Met and Glu residues at the periplasmic exit suggest a mechanism of extrusion driven by hydrophilic interactions [53]. While mutation of the invariant residues impairs the function, this interesting hypothesis invites further experimental corroboration. Similarly, only the fundamental aspects of Cu^+ transfer between chaperones and ATPases has been explored. Further studies are required to understand the role of putative ligand exchange residues and specificity determinants.

While alternative periplasmic Cu^+-chaperones targeting the ion to different proteins have been described [24, 94, 97], this is not the case for the cytoplasmic equivalents. How is the Cu^+ distribution network configured? Clearly, Cu^+-ATPases are central to Cu^+ distribution and perhaps the best characterized elements of the proposed networks. The identification of nodes, links, forks, etc. is perhaps the challenge ahead.

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Notes and references

‡ Bacterial Cu^+-ATPases have received various names (CopA, PacS, CueA, SilP, ActP, CtpV, etc.). For simplicity, we will refer to them as CopA.

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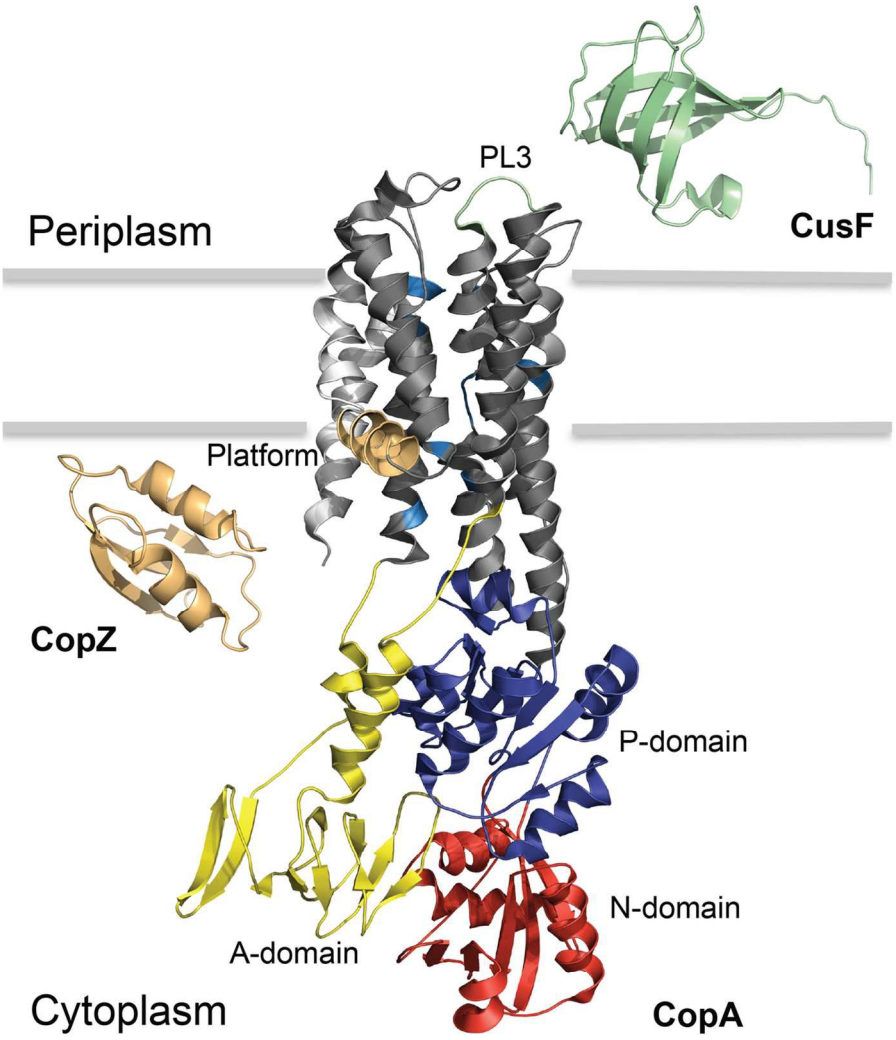
Figure legends

Figure 1. Molecular organization of Cu^+ transport across bacterial plasma membrane. Crystal structures and subcellular localization of *Enterococcus hirae* CopZ (PDB entry 1CPZ; light orange), *Legionella pneumophila* CopA (PDB entry 3RFU) and *Escherichia coli* CusF (PDB entry 1ZEQ; light green) are presented. Specific domains and features within the CopA structure are described. The actuator (yellow), nucleotide binding (red) and phosphorylation (dark blue) domains are shown. The transmembrane segments (TM1-TM2, light grey) leading to the platform helix (orange; involved in CopZ docking) and the periplasmic loop 3 (light green; involved in CusF docking) are emphasized. Within the TM3-TM8 membrane region (dark grey), residues implicated in Cu^+ entry site (M148/E205/D337), high-affinity binding sites: site-1 (C382/C384/Y688) and site-2 (N689/M717/S721) and exit site (E189) are highlighted in sky blue.

Figure 2. The catalytic mechanism of Cu^+-ATPases. The scheme emphasizes the two enzyme conformations (E_1 dark grey) and (E_2 light), The Cu^+ transfer from cytoplasmic chaperones (CopZ, orange), binding of ATP and the subsequent enzyme phosphorylation. This is followed by Cu^+ release to the periplasmic chaperone (CusF, green) and the return of the enzyme to the E_2 form. The scheme includes the Cu^+-ATPase transmembrane region (grey), P (blue), N (red) and A (yellow) domains, Cu^+ ions (orange), CopZ (orange) and CusF (green).

Figure 3. Chaperone mediated Cu^+ uptake and release of Cu^+-ATPase. The model represents the docking of cytoplasmic Cu^+-CopZ and periplasmic apo-CusF with CopA and the release of apo-CopZ and Cu^+-CusF during the transmembrane translocation of Cu^+. The scheme was built using the available 3D structures of *Enterococcus hirae* CopZ (PDB entry 1CPZ; light orange), *Legionella pneumophila* CopA (PDB entry 3RFU; colour pattern explained in Figure 1), *Escherichia coli* apo-CusF (PDB entry 1ZEQ; light green, bound to CopA) and *Escherichia coli* holo-CusF (PDB entry 2VB2; light green, unbound) and reported docking calculations [23, 48].



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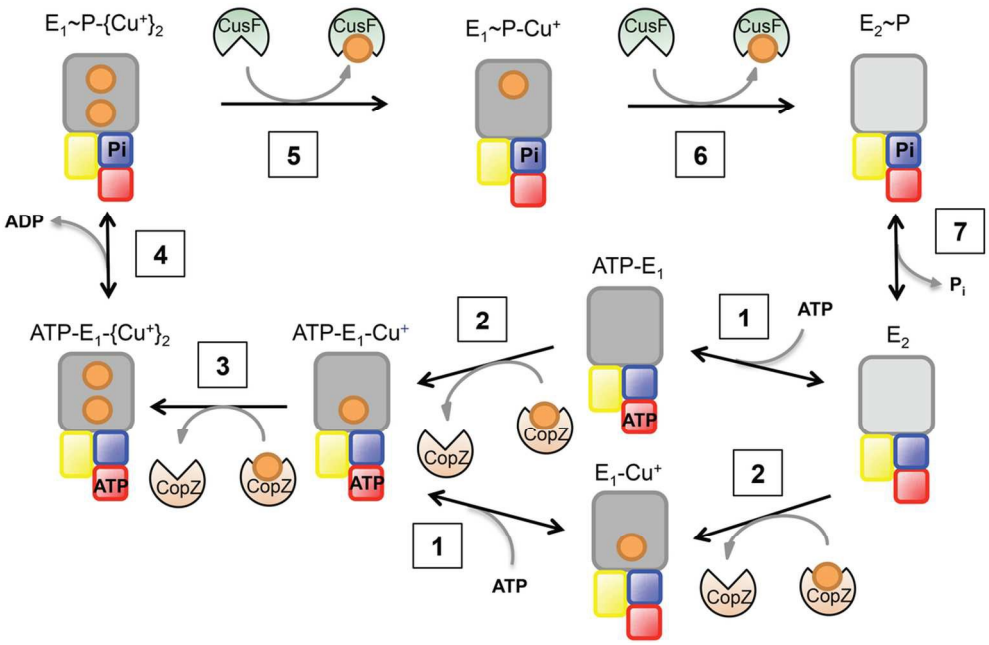

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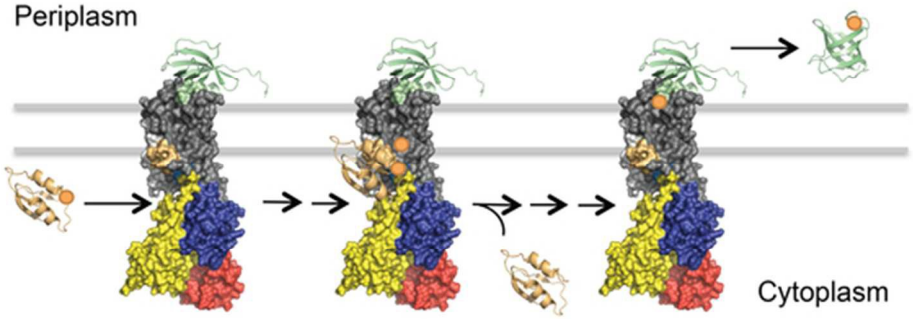

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