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Metal transport across biomembranes: Emerging models for a distinct chemistry.

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Transition metals are essential components of important biomolecules and their homeostasis is central to many life processes. Transmembrane transporters are key elements controlling the distribution of metals in various compartments. However, the chemical properties of transition elements require functional and structural characteristics distinct from previously described alkali and alkali earth ion transport mechanisms. Emerging structural information and functional studies have revealed distinctive features of metal transport. Among these are the relevance of multifaceted events involving metal transfer among participating proteins, the importance of coordination geometry at transmembrane transport sites, and the presence of the largely irreversible steps associated with vectorial transport. Here, we discuss how these characteristics shape novel transition metal ion transport models.

Micronutrient transition metals (Mn, Fe, Co, Ni, Cu, Zn, Mo, W) serve catalytic and structural functions as prosthetic groups in metalloproteins. In these roles, they are required for a number of diverse physiological processes ranging from gene transcription to respiration (1). However, in spite of their essential roles and ubiquitous presence, metals can cause deleterious effects by catalyzing the production of free radicals, simply impairing metalloenzyme functions by substituting for the optimal metal cofactors. Consequently, organisms strive to maintain a tightly controlled homeostasis of these elements through the coordinated action of transmembrane transporters, chaperone, complexing and storage molecules, and metal responsive transcriptional regulators (2-4). These components distribute the ions to appropriate targets and maintain adequate metal quotas, keeping the cellular compartments essentially free of unsequestered metals (3,5,6).

This review focuses on structural and functional aspects of transmembrane transporters that participate in the homeostasis of transition metals. Current understanding of ion transmembrane transport is rooted in six decades of research characterizing alkali (H^+, Na^+, K^+) and alkali earth (Mg^{2+}, Ca^{2+}) channels, carriers and pumps. These ions are free (hydrated) and abundant in biological systems. Therefore, their transport mechanisms are shaped by electrochemical gradients and governed by their reversible interaction with transmembrane transport sites constituted by polar amino acid side chains (7). Consideration of the physico-chemical differences between alkali/alkali earth and transition metal ions quickly reveals that the existing models describing ion transmembrane translocation cannot explain the mechanism of transport of transition metals. In this context, emerging paradigms for the transport of uncomplexed metal ions are discussed here. Transporters of metal-complexes (siderophore-metal, heme, etc.) will not be considered, since their selectivity and mechanism might not be determined by the bound metal, but rather by the coordinating molecules (8-10).

General characteristics of transition metal transport

A number of families of carriers and pumps responsible for metal influx and efflux from various subcellular compartments have been identified: ABC-type ATPases (ATP binding cassette), P_{IB}-type ATPases, ZIP (Zrt/Irt-like protein), Ctr (copper uptake), Nramp (natural resistance-associated macrophage proteins), RND (resistance-nodulation-cell division), and CDF (cation diffusion facilitators) transporters, among others (6,11-13). These are polytopic membrane proteins that show diverse structural arrangements of transmembrane segments (TM), combined in some cases with regulatory and catalytic hydrophilic domains. High-resolution structures of model members of some of these families have been reported (14,15) (Fig. 1). While the presence of an ion path across the membrane is a logical feature of these transporters, additional distinctive characteristics are surfacing; for instance, the presence of putative docking regions where chaperone proteins and/or chelating molecules might deliver the metal substrate to the transporter or alternatively receive the metal subsequently after its translocation (14,16-19) (see below). However, while this docking will contribute to the *in vivo* substrate selectivity of transporters, the metal coordination during transport is the defining

feature that determines the functional capabilities of these proteins (20) (21).

Biologically relevant transition metals (Mn, Fe, Co, Cu, Zn) are located in the d-block of the periodic table (most in period 4, groups 7-12), i.e. their electronic d shell is incomplete (except for Zn). These elements are considered soft Lewis acids (22). They present high binding stability constants in aqueous media (fM^{-1} range) when coordinated by soft Lewis base ligands such as thiolate (sulfur) and imidazolium (nitrogen). These differ from alkali/alkali earth metals that behave as hard Lewis acids. These rather prefer coordination by smaller hard Lewis bases such as carboxylate (oxygen), forming ionic adducts that have lower binding stability constants (1). Consequently, it is expected that transition metal binding sites involved in the transient association during translocation across the membrane would be mostly constituted by fitting intermediate (N) or soft (S) bases (1). Furthermore, the outer shell electronic configurations would favor particular coordination geometries for the various metal substrates. Well-known metal coordination architectures are largely based on the characterization of organometallic complexes and of metal sites within soluble metalloproteins where the static prosthetic group remains bound for the life of the protein. In contrast, transmembrane transport sites although binding the metal with very high affinities, only transiently interact with the substrate during transport. Moreover, these sites must present the flexibility to allow the vectorial ion release (i.e. across the permeability barrier) upon minimal coordination shifts (17,20,23). Consequently, novel ligands and metal coordination architectures might be expected at the transmembrane transport/binding sites (TM-MBS) of transition metal transporters. An example of these features is the coordination of Cu^+ by P_{1B}-ATPases or Ctr proteins. While this metal has a tetragonal (e.g., superoxide dismutase) or tetrahedral (e.g., plastocyanin) coordination in metalloproteins, and a linear coordination in regulatory cytosolic metal binding domains (e.g., N-MBD Cu^+-ATPases), it has a trigonal planar coordination during transport (17,24,25). Moreover, the unexpected coordination by one or more oxygen containing side chains is observed in these sites. Consequently, we hypothesize that a specific metal-coordination in the TM-MBs is required for transport and that evolution has selected some coordination states over others. An additional outcome of a selectivity based on acid-base Lewis chemistry and coordination geometry is the capability of these transporters to bind and, in some cases, translocate non-physiological ligands at a greater extent than alkali/alkali earth transporters. Cu^+ transporters can translocate Ag^+ and probably Au^+, Pb^{2+} and Cd^{2+} function as substrates of Zn^{2+}-ATPases, and these can also bind non-transported metals (Cu^{2+}, Co^{2+}, Ni^{2+}) with similar affinity (26-30).

The tight metal binding to the transport sites has as an immediate consequence, which is the observed slow transmembrane transport rates. Alkali/alkali earth ions transport rates range from 10^9-10^7 ions/sec in the classical Na^+ or K^+ channels (31) to around 200 ions/sec for ion pumps (32). In contrast, estimated turnover rates for Cu^+-ATPases, Zn^{2+} transporting CDFs, or Cu^+ carrier CTR1 are <10 ions/sec (26,28,33) (Jack H. Kaplan, University of Illinois at Chicago, personal communication). Available functional determinations suggest that these low transport rates are associated with the slow release of metal by the transporter. However, it might be argued that in some cases reported metal transport rates are underestimated, since metal transport assays are frequently performed in the absence of a post-transport "receiving" molecule; i.e., an accepting metal sequestering molecule preventing release of free metal. Although this might be the case, it appears unlikely that the presence of accepting molecules in the reaction would substantially increase the transport rates (>10 fold) to levels comparable to those of alkali/alkali earth transporters.

Toward explaining the slow transport, it can be speculated that the minimal cellular requirements for transition metals has not represented a significant selective pressure for the evolution of faster metal transporters. Alternatively, alkali/alkali earth metals participate in dynamic events requiring significant mass/charge redistribution (signal transduction, osmotic and electrical balance). This has probably driven the selection of fast transporting molecules. Moreover, the necessary absence of free metals in cellular compartments constrains the overall

transport rate to the availability of chaperone/sequestering molecules. These characteristics of metal homeostasis explain the absence of transition metal "channels" where the ions travel at rates close to that of diffusion.

These common features of metal transport (substrate transfer through protein-protein interaction, specificity of substrate, and relatively slow transport rates) are best illustrated by discussing recent developments in the structural analysis of some of these transporters. Although in most instances the structures have been obtained from bacterial transporters, it is expected that their characteristic features are also shared with eukaryotic ones albeit with minor changes. Similarly, most of the information considered in this review refers to Cu^+-transporters, since most of the structural/mechanistic work has been done in Cu^+ transporting systems.

Metal transport by P_{IB}-ATPases

P_{IB}-ATPases are polytopic membrane proteins (Fig. 1A). Sharing a common core structure and catalytic mechanism, they belong to the superfamily of P-ATPases (21). They are present in all life kingdoms and most sequenced genomes contain several members of the P_{IB}-family with different substrate specificities or distinct functional roles (18,21,34). In eukaryotic cells, P_{IB}-ATPases are present in almost all organelles (vacuole, trans-Golgi network, chloroplast, or plasma membrane, among others) (35-37), where they assist in metal detoxification and metalloproteins synthesis (37,38), as also occurs in bacteria (34). Early studies based on bioinformatic, genetic, and biochemical analyses suggested the following distinct metal specificities: Cu^+ (P_{IB-1}), Zn^{2+} (P_{IB-2}), Cu^{2+} (P_{IB-3}), and Co^{2+} (P_{IB-4}) (18,21,39). These are determined by highly conserved amino acids present in three TMs constituting the TM-MBSs. The involved TMs flank the catalytic cytosolic loop where ATP binding and hydrolysis occur (21,39), providing a structural link for cytosolic metal export coupled to ATP hydrolysis and enzyme phosphorylation as described for well-characterized members of the P-ATPases superfamily (21,40,41).

The metal selectivity of P_{IB}-ATPases is the outcome of hierarchical multifaceted events. Ultimately these yield the binding of the correct substrate to the TM-MBS (Fig. 2). Functional and structural studies of *Archaeoglobus fulgidus* CopA, a Cu^+-ATPase, indicated that in a first step substrate specificity is determined *in vivo* by the interaction between a soluble metal loaded chaperone and the metal accepting ATPase (17). *In vitro* assays using purified proteins showed that the Cu^+-chaperone (Cu^+•AfCopZ) complex was responsible for metal transfer to the ATPase TM-MBSs. In *A. fulgidus*, the chaperone can not be substituted in this role by the homologous cytoplasmic N-terminal metal binding domains (N-MBD) usually present in Cu^+-ATPases, although it seems to be possible in yeast (42). The interaction between the Cu^+-delivering protein and the ATPase is likely determined by a specific geometry in both interfaces that assists the positioning of the metal in the proximity of the ATPase metal accepting sites. The atomic resolution structure of *Legionella pneumophila* CopA supports this model (14). In this structure, the first and second TMs form a platform where the chaperone would likely dock to transfer the metal (Fig. 3A). This interaction is determined by electrostatic forces, in which the negatively charged face of the chaperone would interact with the positively charged docking "platform" (Fig. 3B). This would orient the chaperone-bound Cu^+ towards three conserved amino acids (a Met, an Asp, and a Glu) located at the cytoplasmic entrance of the metal transmembrane path. The electropositive exposed surface of the platform also precludes a postulated interaction of the cytoplasmic N-MBDs present in these ATPases, delivering Cu^+ to TM-MBSs. It has been shown that N-MBDs have the same electrostatic charge as the ATPase platform, and of opposite charge to the chaperone (43). In fact, the electrostatic complementation of N-MBDs and Cu^+-chaperones contributes to their interactions and subsequent Cu^+ exchange.

Testing this model, we calculated the polar binding energies involved in the docking of Cu^+•AfCopZ-AfCopA (complex 1) and Cu^+•AfN-MBD-AfCopA (complex 2) in the platform region of the ATPase. This approach estimates the stability of the complexes in a salt (0.15 M)

aqueous solution (44) (see legend of Fig. 3 for details). The energy values are obtained by solving the Poisson-Boltzmann equation through seminumeric/semianalytical methods. The polar binding energy of complex 1 was -11.11 kcal/mol, while that for complex 2 was +26.12 kcal/mol. This suggests a lack of stability of a hypothetical intra-molecular complex 2 and supports previous data indicating a regulatory role for N-MBDs rather than one delivering Cu^+ to TM-MBSs (17,45,46). More importantly, this analysis points out the likely favorable interaction of the Cu^+-chaperone and the ATPase. Interestingly, the interaction requires the metal bound chaperone, as is indicated by the unsuccessful docking of the apo-chaperone with the ATPase. This is in agreement with biochemical data showing that AfCopZ does not compete with Cu^+•AfCopZ inhibiting the Cu^+ transfer to the ATPase (17). This results in an unidirectional metal movement that yields a stoichiometric Cu^+ transfer to TM-MBSs (Cu^+•CopZ + CopA→CopZ + Cu^+•CopA) (Fig. 2) (17,46). However, the chaperone metal sites can potentially bind other metals (47), which in turn might enable docking with the ATPase and subsequent metal delivery. Then, a second layer of specificity provided by the metal coordination at the TM-MBSs becomes relevant.

Biochemical studies have shown the trigonal coordination of Cu^+ at the TM-MBSs (48,49). As mentioned, this unique geometry is distinctly associated with transport sites. When the activation of Cu^+-ATPases by various ions in the absence of chaperones is tested *in vitro*, the ATPases apparently accepts only Cu^+ and similar ions (Ag^+ and Au^+) but not others like Zn^{2+}, Cu^{2+}, Co^{2+}, or Ni^{2+} (26). Thus, independent of the chaperone, the ATPase TM-MBS selects the transported metals. Studies of *Escherichia coli* Zn^{2+}-ATPase ZntA might better support these ideas. ZntA TM-MBS binds transported substrates (Zn^{2+}, Cd^{2+} and Pb^{2+}) as well as non-transported divalent heavy metals (Cu^{2+}, Co^{2+} and Ni^{2+}) with similar affinity (20,30). Although Cu^{2+}, Co^{2+}, and Ni^{2+} tightly bind the enzyme, these metals cannot induce the required enzyme conformation that enables the catalytic hydrolysis of ATP. This would suggest that the geometry of coordination and metal-ligand bond distances play an important role in the activation of P_{IB}-ATPases. As a corollary, metal coordination geometry, rather than binding affinity, is the determinant of transport specificity. Similar phenomena are observed in metal regulatory proteins (2,50).

Metal release from the protein occurs upon the major conformational change. Here, P_{IB}-ATPases also highlight a common feature of metal transporters: a slower transport rate when compared to closely related alkali/alkali earth-transporting ATPases. This is especially evident in those Cu^+-ATPases of the FixI/CopA2 subgroup (18,34). These ATPases present the slowest transport rates, most probably to couple metal transport with the export of metal accepting apoproteins. In multicellular organisms, a similar mechanism seems to be in place (16).

The resistance-nodulation-cell division (RND) metal transporters.

The members of the resistance-nodulation-cell division (RND) superfamily are tripartite transporters widespread in Gram-negative bacteria (6). This superfamily contains seven sub-families with different substrate specificities. These include antimicrobial agents, organic solvents, and heavy metals, among other molecules. In all cases the substrate appears to be transported from the periplasm to the extracellular space. The systems span the periplasmic space with a cytoplasmic membrane protein (RND), an outer membrane porin (OMP), and a periplasmic membrane fusion protein (MFP) bridging the inner and outer membrane components. A H^+ antiport is used to satisfy the energetic requirements of the substrate efflux (51).

The best-characterized heavy metal transporter RND is the *E. coli* Cu^+/Ag^+ efflux CusCFBA system (52-55). The corresponding operon encodes the three characteristic proteins of these systems: the RND (CusA), the OMP (CusC) and the MFP (CusB). These proteins are arranged in a multimeric form with trigonal symmetry: a CusA trimer contacts a CusB hexamer which interacts with a CusC trimer (Fig. 1B) (55,56). In the most likely model, metal transport is initiated by binding of periplasmic Cu^+ to the N-terminal domain of CusB (57), where it is again coordinated in planar trigonal geometry by three

Met (55). The sequence of this site corresponds to the transported metal substrate. For instance, in the MFP protein of the Zn^{2+} transporting ZneABC, the metal is coordinated by two His and a Glu (58). In this case, an additional ligand should be involved to achieve the tetradentate Zn^{2+} coordination common to Zn^{2+} transporters and metalloproteins (23,59) (Raimunda, Stemmler and Argüello unpublished results). The fate of the metal after binding CusB has not been established. It has been proposed that metal binding to CusB causes a conformational change that might position the metal closer to the metal binding site of CusA in the plasma membrane (58,60). This site, constituted by three Met, is in a periplasmic cleft formed by two large loops between TM 1-2 and TM 7-8 (54) (Fig. 1B). Following this model, the subsequent step involving the transfer from CusA to other components of the system has not been established.

Since free Cu^+ is toxic, the metal has to be provided by a periplasmic chaperone to CusB. To this purpose , the CusCFBA operon also encodes for a periplasmic metal binding protein, CusF. In a role analogous to the cytoplasmic CopZ, CusF appears as a periplasmic Cu^+ chaperone that delivers the metal to CusABC (57). Cu^+ coordination in EcCusF is achieved by a trigonal coordination by two Met and a His (52). The presence of Met in the CusF binding site solves the likely oxidation of periplasmic -SH groups if Cys were part in this site. *In vitro*, CusF transfers metal directly to metal binding sites in the N-terminal domain of CusB (61).

Evidently, a central element of the transport mechanism of RND systems is the transfer of metals between different protein components. For instance, Cu^+ transfer from CusF to CusB is considered part of the metal transport path. However, CusF exchanges Cu^+ with CusB with K_{eq}~1 (60). Thus, Cu^+ transfer is far from unidirectional. If this is part of the transport pathway; then, a largely irreversible step should occur later on the translocation process. Furthermore, metal occupancy of CusB rather than the level of Cu^+•CusF would control the transport rate. Alternatively, it could be postulated that Cu^+ binding to CusB might have a regulatory effect and that CusF might be able to transfer metal directly to CusC, as cytoplasmic chaperones deliver Cu^+ to the TM-MBS of Cu^+-ATPases. Supporting the later model, in some cases CusF and CusB are fused in a single protein, as it seems to be the case for SilB, the MFP subunit of SilABC, a RND system in *Cupriavidus metallidurans* CH4 (62). In this protein, the MFP has an extended C-terminal metallochaperone domain closely related to CusF. Some organisms even appear to lack CusB (*Legionella longbeachae*, several *Pseudomonas* and *Shigella* species, *Xanthomonas campestris*, etc.) while maintaining the other elements of the system (Hernández, Argüello and Valderrama, unpublished results). This would suggest that CusB is not an essential component of the system.

Although metal RND efflux systems seem to be primarily responsible for detoxification of periplasmic metals (57), it has been suggested that the system would also transport cytosolic metal across the plasma membrane (63). In this model, Cu^+ would follow a Met "shuttle" in CusA. Experiments testing the transport into reconstituted liposomes show that CusA transports Ag^+ in favor of a large gradient (0.5 mM Ag^+ in the cytoplasmic side) in a pH dependent fashion. However, in spite of the large gradient, transport is quickly inhibited (20 sec). The mechanism of this transport, how Cu^+ is transferred to CusC, as well as the role of a cytoplasmic Cu^+ efflux system in addition to the ubiquitous Cu^+-ATPase, are not clear.

The Ctr family of eukaryote Cu^+ transporters

The Ctr family of transporters are found exclusively in eukaryotes, where they enable the flux of Cu^+ into the cytoplasm either facilitating its incorporation from the extracellular space or mobilizing the vacuolar stores (64). Their importance is highlighted, for instance, by the embryonic lethal phenotype resulting from the *CTR1* gene knock out in mice (65). Ctr proteins are homotrimers. Monomers are 140-400 amino acid proteins with three TMs (TM1, 2, and 3) and frequently present an extracellular N-terminal Met rich motif (MXXM/MXM) (12). TM2 contains a conserved MXXXM motif that faces a path at the center of the trimer (Fig. 1C) (25). The final structure appears as a "channel" or "pore" with a conical side narrower at the extracellular/luminal side of the protein. Consistent with this channel-

like structure, it has been proposed that Cu^+ uptake through Ctr transporters is driven by a passive, membrane potential-dependent, mechanism (12). However, this model still needs to be supported by strong experimental evidence, as well as it has to take into account the role of metal accepting-chaperones.

The functional Ctr transporter complex can stably bind two Cu^+ per trimer (25). One of these sites is within the Ctr "pore" and constituted by a Met from each monomer; thus, providing a trigonal planar coordination as observed in Cu^+ sites of Cu^+-ATPases and RND transporters. The second site has not been identified, but likely candidates are the N-terminal Met rich region or the HCH motive at the C-terminus (25). The functional roles of the N-terminal region, amino acids in the transmembrane region, and C-terminal HCH motives have not been well defined. Structural data suggests that Met in TM2 should play an important role in the Cu^+ transport across the membrane, providing a mechanism of selectivity through an appropriate geometry of coordination (25). However, mutation of transmembrane Met located in TM2 does not abolish metal flux although it decreases the rate of transport (66). On the other hand, the Met-rich N-terminal region in some cases seems to be essential for metal transport (66), where it could play a role in binding extracellular Cu^+. Based on relatively limited experimental evidence, two models have been proposed for the transport mechanism: a "channel-like" model where Cu^+ would interact weakly with ligands facing the inner face of the pore (66), and another in which Cu^+ would be translocated passing through several binding sites conformed by "essential" residues accommodated by hierarchical affinities in the Cu^+ pathway (25,67). Several findings, such as differential trypsin digestion in presence of Cu^+ (68), the C-termini interaction likely coupled to Cu^+ transport activity (67) and molecular dynamic simulations (69) point to a Ctr metal ion transport mechanism involving structural conformational changes.

As cells strive to prevent the presence of free Cu^+, part of a Ctr transport mechanism is the metal delivery to specific Cu^+ chaperones that would carry the ion to appropriate targets. *In vitro* studies have shown that the C-terminal domain of *Saccharomyces cerevisiae* Ctr1 would interact with the corresponding Cu^+-chaperone Atox1 (70). This is a relevant finding to elucidate how the secretory pathway may acquire Cu^+. However, it does not address whether Ctr1 is directly involved in the metallation of other cytosolic Cu^+-chaperones like CCS, or if it supplies Cu^+ to cellular labile metal complexing pools such us glutathione.

Cation Diffusion Facilitators

Cation diffusion facilitator (CDF) transporters are ubiquitous membrane proteins responsible for cytosolic efflux of divalent cations coupled to nte influx of H^+ or Na^+ (71). In eukaryotes, they are localized in the plasma membrane and in organelles (vacuole, endoplasmic reticulum, Golgi, etc.) (72-74), where they participate in metal detoxification, metalloprotein assembly, and in packaging of secretory vesicles (75-77). The functional forms of the transporters are homo dimers. The topology of the subunit, 6 TMs and a cytoplasmic hydrophylic C-terminal domain, is well conserved among all family members (Fig. 1D). In addition, all eukaryotic and some bacterial CDFs present a His-rich cytosolic region between TM4 and TM5 (78,79).

Significant understanding of the mechanism of CDFs emerged from the biochemical characterization and structural studies of the *E. coli* Zn^{2+} transporter YiiP (15,23,79). YiiP is a homo dimer of two 33 KDa subunits in a two-fold symmetry (Fig. 1D) (15,79). It presents several high affinity Zn^{2+}/Cd^{2+} binding sites with seemly different coordination geometry (28,80). These are located in the transmembrane region (site I), the membrane-cytosol interface not fully conserved in all CDF (site II) and the C-terminal domain (site III) (15,80).

Site A binds the transported metal (15,23). This site defines the selectivity of YiiP towards Zn^{2+}/Cd^{2+} over Fe^{2+}, Mn^{2+}, Ni^{2+} and Co^{2+}. The site is constituted by two Asp in TM2, a His, and an Asp in TM5, binding the metal with tetrahedral coordination. Mutation of these residues prevents metal transport (23,81,82). Kinetic evidence suggests that once Zn^{2+} is bound to site I, it is quickly extruded ensuring an unidirectional, largely irreversible, transport mechanism (28).

This analysis also highlights the relatively low transport rate of metal transporters (2.6 s^{-1} for Zn^{2+} transport by *E. coli* ZitB) (28).

Although it has been speculated that the conserved C-terminal domain (site III) might act as a metallochaperone (15) there is no experimental evidence for this hypothesis. Site III appears to be involved in YiiP dimerization and consequent activation (80). Zn^{2+} is bound tetrahedrically by amino acids from each monomer (Fig. 1D). Metal binding in this site contributes to the stabilization of the interaction between the C-terminal domains of each monomer. Then, this appears as a regulatory mechanism by which the functional transporter is assembled when excess substrate is present.

Future directions

In the last few years, the first high-resolution structures of representative members of some of the main metal transporter families have been obtained. Further progress is expected in this direction with the structural characterization of other metal transporter families such as ZIP or Nramp, as well as further refinements on already determined structures in all their conformational stages. This will help establish the structural and functional determinants that lead to distinct metal transport mechanisms and transport specificity required by the cell to handle fundamental, but highly toxic, transition metal ions. However, to validate the accuracy of novel models similar advances in biochemical and biophysical studies will be required. Because of their importance in metalloprotein assembly and consequently in the overall cell physiology, the determination of the precise interaction mechanism of metal transporter and metal-delivering and metal-accepting chaperones is one of the areas where significant developments are likely.

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FOOTNOTES

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The abbreviations used are: A-domain, Actuator domain; ATP-BD, ATP binding domain; MBD, metal binding domain; TM, transmembrane segment; TM-MBS, transmembrane metal binding site.

FIGURE LEGENDS

Figure 1. Structures and metal binding sites of metal transporters. (A) Structure of the Cu^+-ATPase CopA (3RFU) with amino acids forming the two transmembrane Cu^+ binding sites indicated in red. (B) CusABC model assembled with Swiss PDB viewer using the CusAB (3NE5) and the CusC (3PIK) structures. Amplified section indicates in red the Cu^+ binding sites in a CusA monomer. (C) Side and apical view of modeled Ctr transporters (provided by Dr. Vincent Unger, Northwestern University). Extracytoplasmic and transmembrane methionine rich Cu^+ binding sites are indicated in red. Darker helices correspond to those involved in transmembrane metal binding. (D) Structure of CDF transporter YiiP (2QFI). Red amino acids indicate Zn^{2+} binding sites. Dotted areas indicate the three metal binding sites in each YiiP monomer.

Figure 2. Catalytic cycle of a Cu^+-ATPase. Cu^+ binding to two TM-MBSs is required for catalytic phosphorylation by ATP ($E1P(Cu^+)_2$). Note the irreversibility of Cu^+ transfer from the chaperone (CopZ) to the transport site and that full occupancy is only reached in presence of ATP. Metal is released after a conformational change (to E2P) leading TM-MBSs to open to the vesicular/extracellular medium. E2→E1 transition is accelerated by ATP (or ADP) acting in a modulatory mode (low affinity). See (46) for more details.

Figure 3. Cu^+-chaperone-Cu^+-ATPase interaction. (A) Docking was modeled using ClusPro (83). *A. fulgidus* CopA, in green, was modeled after *Legionella pneumophila* CopA (3RFU), while the model of the C- terminal domain of *A. fulgidus* CopZ, in ochre, was built using *Enterococcus hirae* CopZ (1CPZ) as template. CopA "platform" for interaction with CopZ is indicated in blue. (B) Surface charges in the predicted docking of CopZ with CopA. Positive and negative charge densities are indicated in blue and red, respectively.

FIGURE 1

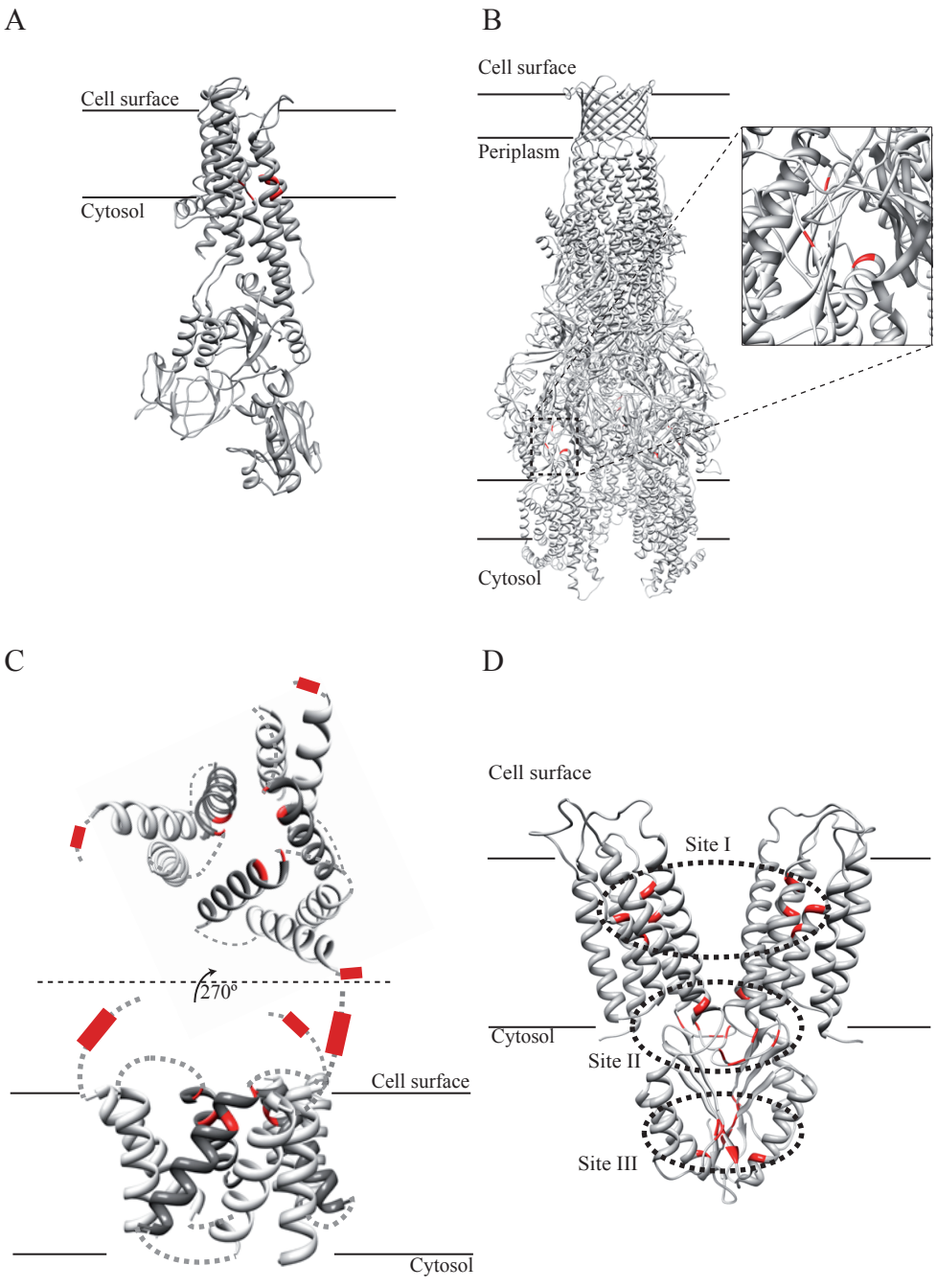

FIGURE 2

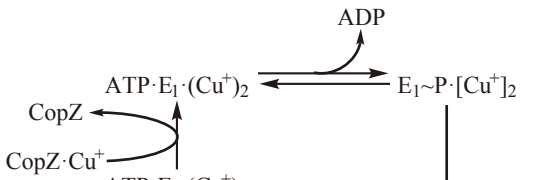

FIGURE 3