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On Allosteric Modulation of P-Type Cu^+-ATPases

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Abstract

P-type ATPases perform active transport of various compounds across biological membranes and are crucial for ion homeostasis and the asymmetric composition of lipid bilayers. Although their functional cycle share principles of phosphoenzyme intermediates, P-type ATPases also show subclass-specific sequence motifs and structural elements that are linked to transport specificity and mechanistic modulation. Here we provide an overview of the Cu^+-transporting ATPases (of subclass P_{IB}) and compare them to the well-studied sarco(endo)plasmic reticulum Ca^{2+}-ATPase (of subclass P_{IIA}). Cu^+ ions in the cell are delivered by soluble chaperones to Cu^+-ATPases, which expose a putative "docking platform" at the intracellular interface. Cu^+-ATPases also contain heavy-metal binding domains providing a basis for allosteric control of pump activity. Database analysis of Cu^+ ligating residues questions a two-site model of intramembranous Cu^+ binding, and we suggest an alternative role for the proposed second site in copper translocation and proton exchange. The class-specific features demonstrate that topological diversity in P-type ATPases may tune a general energy coupling scheme to the translocation of compounds with remarkably different properties.

Introduction

In 1957, Jens Christian Skou discovered the Na^+,K^+-ATPase.[1] Later, it became evident that this crucial membrane transporter represented a large superfamily,[2,3] referred to as P-type ATPases.[4] These proteins may be divided into five subclasses (I–V) according to their transport specificity,[3] ranging from protons and abundant metal ions (e.g., Na^+, K^+ and Ca^{2+}) to less abundant heavy metals (e.g., Cu^+ and Zn^{2+}) and phospholipids. Underscoring their significance, all organisms except for a few archaea and parasitic bacteria encode a selection of P-type ATPases.[5,6]

Albers *et al*. and Post and Sen outlined the transport mechanism for Na^+,K^+-ATPases,[7,8] and de Meis and Vianna outlined that for Ca^{2+}-ATPases,[9] pointing to a functional cycle associated with so-called E1 and E2 states and phosphoenzyme intermediates that are observed for all P-type ATPases. The E1/E2 cycle follows the "alternating access" transport mechanism for membrane transporters (Fig. 1a)[10] that has been depicted in detail for the Ca^{2+}-ATPase.[11] The E1 state exchanges ions at intramembranous sites exposed to the intracellular environment. High-affinity binding of the extruded ion stimulates ATP-driven auto-phosphorylation of a conserved aspartate residue leading to the E1P state with ions occluded in the transmembrane (TM) transport pathway. A conformational change leads to the E2P state associated with ADP release and opening of an extracellular ion pathway with low affinity for the extruded ion. Binding of counter-transported ions to the membranous sites stimulates re-occlusion coupled to auto-dephosphorylation, and phosphate release yields the E2 state. ATP binding then stimulates the E2 to E1 shift, initiating a new reaction cycle.[11,12]

For the sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA; P-type ATPase subclass IIA), several structures of E1/E2 cycle intermediates have been characterized by X-ray crystallography.[11–18] With additional structures of Na^+,K^+-ATPase (subclass IIC),[19] H^+-ATPase (IIIA)[20] and Cu^+-ATPase

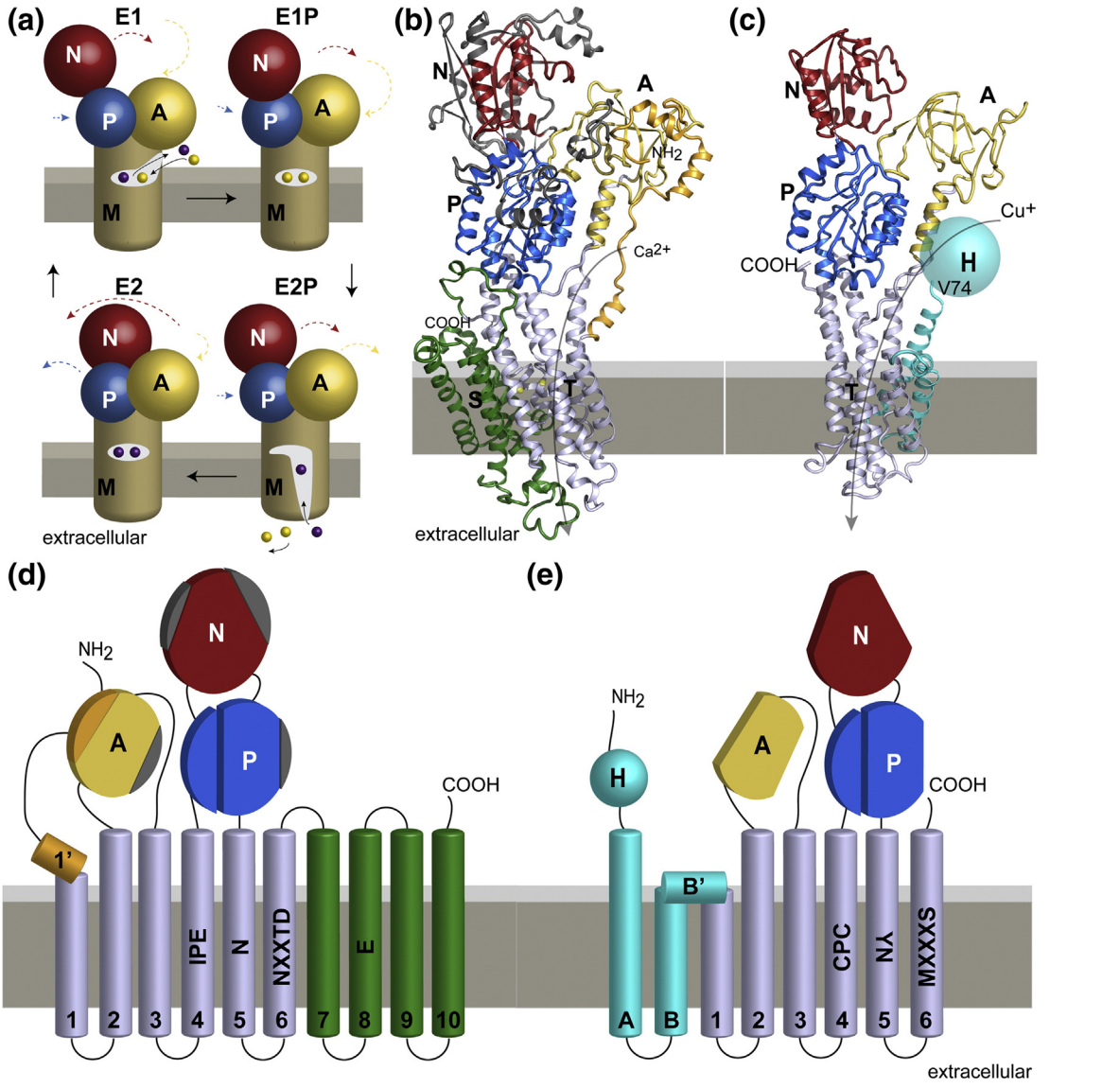

Fig. 1. Ca^{2+}-ATPases and Cu^{+}-ATPases. Ca^{2+}- and Cu^{+}-ATPases share a P-type ATPase core consisting of domains for nucleotide binding (N, red), phosphorylation (P, blue) and dephosphorylation/actuation (A, yellow), as well as a membrane-spanning domain (M, gold), which may be subdivided into the conserved unit (M1–M6, light blue) and class-specific segments (class II, dark green; class IB, cyan). (a) The Post–Albers cycle allows alternating access for transported compounds to the intra and extracellular sides through conformational changes of soluble and membrane-spanning domains (arrows). The E1 states have high affinity for the exported ion (yellow) and become occluded and phosphorylated (E1P), while counterions (purple) associate in the E2P state coupled to dephosphorylation and re-occlusion (E2.P_i and E2), prior to a return to the next transport cycle. (b) Structure of SERCA [Protein Data Bank (PDB) ID 3N5K]. (c) Structure of LpCopA with an approximate position of the class-specific HMBD (H, sketched as a cyan sphere; PDB ID 3RFU). (d) Cartoon representation of SERCA highlighting the distinct ion recognition motifs of the M-domain, the class-specific TM helices (dark green) and domain insertions (dark gray) and the M1/A-domain linker (orange). (e) Cartoon representation of LpCopA with the class-specific HMBD (H) and membrane-spanning segments MA as well as MB/MB′ and the conserved motifs in the M-domain of Cu^{+}-ATPases.

(IB),[21] it has become evident that the P-type ATPase core is highly conserved. The core structure includes the intracellular domains for nucleotide binding (N-domain), phosphorylation (P-domain) and dephosphorylation/actuation (A-domain) linked to a TM domain (M-domain) encompassing six topologically conserved membrane-spanning segments (M1–M6) that determine transport specificity and display many of the subclass-specific sequence motifs (Fig. 1b–e).[22,23] These four core domains are tightly coupled in their function and primarily responsible for the vectorial transport activity.[19]