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Minireview

Copper pumping ATPases: common concepts in bacteria and man

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Abstract

Recently, four genes encoding putative copper pumping ATPases have been cloned from widely different sources: two genes from *Enterococcus hirae* that are involved in copper metabolism and two human genes that are defective in the copper-related Wilson and Menkes disease. The predicted gene products are P-type ATPases. They exhibit extensive sequence similarity and appear to be members of a new class of ATP driven copper pumps involved in the regulation of cellular copper.

Key words: Copper; ATPase; Transport; Menkes disease; Wilson disease; *Enterococcus hirae*

1. Copper is a toxic but essential element

Copper functions as cofactor in various redox enzymes such as lysyl oxidase, cytochrome *c* oxidase, superoxide dismutase, dopamine β-hydroxylase, and tyrosinase. Copper is also a component of bacterial azurins and plastocyanins. At the same time, copper is very toxic to both eukaryotic and prokaryotic cells. Copper ions can bind to proteins and nucleic acids and can cause the oxidation of lipids and proteins. The formation of deleterious free radicals is also enhanced by copper ions. Indeed, this toxicity is put to use in disease prevention in vegetable cultures. For cell viability, regulation of intracellular copper activity is thus crucially important and mechanisms must exist for the homeostasis of copper.

Recent studies of copper resistance in the Gram-positive bacterium *Enterococcus hirae* has led to the discovery of two putative copper transporting ATPases. Interestingly, these enzymes exhibit extensive sequence identity to two human ATPases that are defective in the copper-related Menkes and Wilson disease. Copper homeostasis has also been extensively studied in other bacteria, notably *Escherichia coli* and *Pseudomonas syringae*. However, there is at present no evidence for ATP driven copper transport in these organisms and they will not be considered here.

2. Genes of copper metabolism in *Enterococcus hirae*

In *Enterococcus hirae*, an operon involved in copper homeostasis has recently been identified [1,2]. It contains at least five genes in the order: *cop*X, Y, Z, A and B. CopX, Y and Z are polar proteins and probably involved in the regulation of the operon (A. Odermatt, unpublished observations). *cop*A and *cop*B encode P-type ATPases of 727 and 745 amino acids, respectively [3]. In the current working model, CopA serves in the uptake of copper and CopB in its extrusion. While wild-type *E. hirae* can tolerate up to 6 mM $CuSO_4$ in the growth media, cells disrupted in *cop*B, or in *cop*A and *cop*B, lose their high level copper resistance; in contrast, disruption of *cop*A alone has no significant effect on the copper tolerance. However, *cop*A-disrupted cells cease to grow after two to three generations when heavy metal ions in the media are complexed with 8-hydroxyquinoline, indicating a role of CopA in import.

Silver is known to replace copper in some processes [4]. When wild-type *E. hirae* cells are loaded with radioactive Ag^+, it is actively extruded when energy is supplied. Mutants lacking CopA can still extrude silver, but cells deficient in CopB can not (A. Odermatt, unpublished observations). These findings support the notion that CopB serves in the extrusion of heavy metal ions from the cytoplasm. That monovalent silver ions are a substrate would suggest that CopB is a pump for monovalent rather than divalent heavy metal ions.

The expression of the *cop* operon is regulated by the ambient copper concentration. Enhanced expression is observed with increasing copper concentrations in the media, reaching a maximum at 2 mM $CuSO_4$. Induction is also observed in response to 5 μM Ag^+ or 5 μM Cd^{2+}, but no effect was seen with Ca^{2+}, Cr^{3+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Sr^{2+}, Ba^{2+}, La^{3+}, Au^{3+}, Hg^{2+}, Pb^{2+}, Bi^{3+}. Surprisingly, full induction was also apparent if 100 μM of the heavy metal ion chelators *o*-phenanthroline or 8-hydroxyquinoline was added. The induction effect of these

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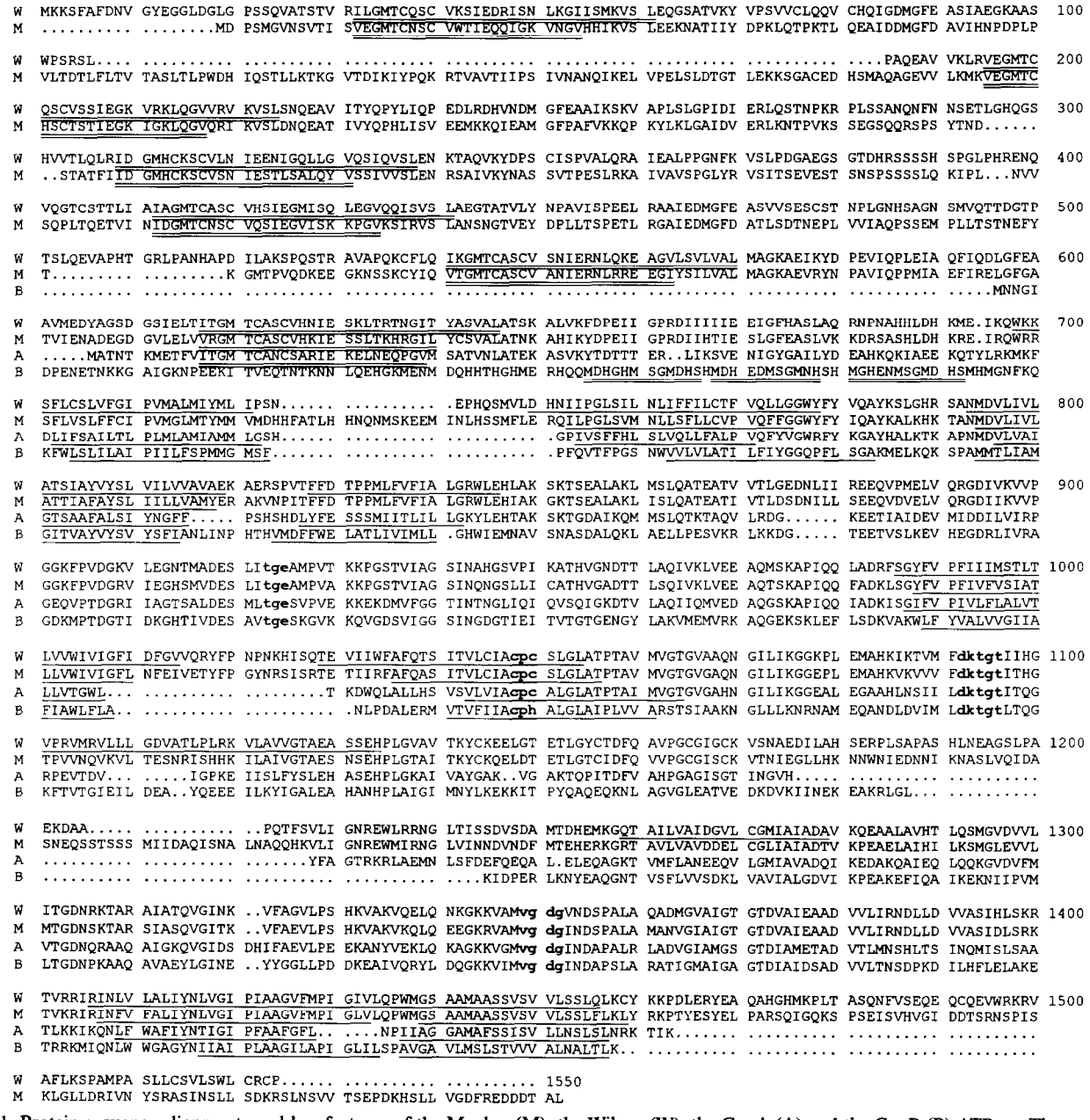

Fig. 1. Protein sequence alignments and key features of the Menkes (M), the Wilson (W), the CopA (A) and the CopB (B) ATPase. The sequences were aligned with the program Pileup of the Genetics Computer Group [20]. The following features common to all P-type ATPases are indicated in **bold small type**: TGE is part of the 'Phosphatase' domain, DKTGT is the site of aspartyl phosphate formation, and VGDG is predicted to form a Mg^{2+}-mediated salt bridge to γ-phosphate of ATP in the 'Aspartyl kinase' domain. These assignments are based on site-directed mutagenesis and the analysis of several other P-type ATPases [21–23]. The heavy metal ion binding sites described in the text are double underlined and putative membrane spans as predicted for W by Bull et al. [10], for M by Vulpe et al. [7] and for A and B by Odermatt et al. [2] are underlined. The conserved CPC (H), located in the most conserved region, is also indicated in *bold small type*. The numbering of the amino acids is only relative due to the introduction of gaps in the sequences. The sequences have the following accession numbers: U03464, L06133 and L13292.

agents was abolished if equimolar concentrations of Cu^{2+} were added simultaneously. It thus appears that either low or high concentrations of ambient copper lead to induction of CopA and CopB [2].

The two putative copper ATPases of *E. hirae* exhibit extensive sequence identity to two recently discovered human ATPases that are also believed to be copper pumps. Considering the evolutionary distance from bacteria to man, the observed sequence similarities are outstanding. These four enzymes are probably members of a new class of copper ATPases and their features will be compared.

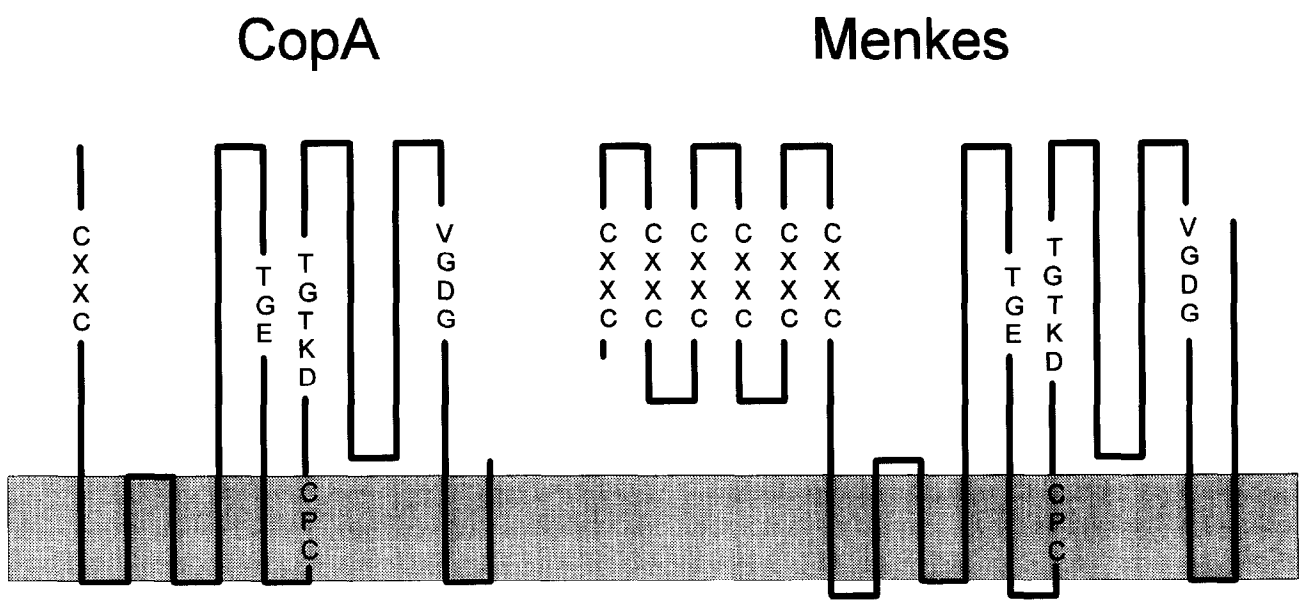

Fig. 2. Folding model for CopA and Menkes ATPase. The bulk of the proteins protrude on the cytoplasmic face of the membrane. CXXC indicates putative canonical copper binding sites in the 'Copper binding' domain. Other features are described in the legend of Fig. 1 and in the text.

3. Human genes of copper metabolism

The inherited Menkes and Wilson disease both cause a disturbance of the copper metabolism. In the X-linked Menkes disease, copper is normal in the liver, but accumulates in intestinal mucosa, kidney, and connective tissue due to a defect in export. This results in a deficiency in copper-dependent enzymes that is eventually lethal. The candidate Menkes gene has been cloned [6–8]; it encodes a P-type ATPase of 1500 amino acids that was proposed to be a copper-transporting ATPase. Its has been shown to be expressed in heart, brain, placenta, lung, muscle, kidney and pancreas, but not in the liver.

In the autosomal Wilson disease, copper secretion into the bile is reduced, with a concomitant toxic accumulation of copper in the liver and eventually also other tissues. The Wilson disease gene encodes a P-type ATPase of 1411 amino acids [9–11]. In contrast to the Menkes gene product, this ATPase is most strongly expressed in liver and kidney.

4. Structural features of the putative copper ATPases

Fig. 1 shows an alignment of the four ATPases, Menkes and Wilson of humans, and CopA and CopB of *E. hirae*. The two human enzymes are approximately twice as large as the bacterial ones. This is due to extra sequences that are predominantly located in the polar N-terminal domain. The Wilson sequence shares 59% identity with the Menkes sequence, and both share around 43 and 33% identity with CopA and CopB, respectively.

The four ATPases exhibit the typical features that are conserved in all known P-type ATPases (Fig. 1). However, there are a number of unique features that set these enzymes apart from other P-type ATPases. The Menkes, Wilson and CopA proteins contain, in their polar N-terminal region, conserved domains containing the invariant motif GMXCXXC. While this motive is repeated six times in the Menkes and Wilson gene products, it is only present once in CopA and absent in CopB. This motif is also found in mercuric reductases that reduce Hg^{2+} to Hg^0 [12], in a periplasmic mercury binding protein [13], and in the cadmium-transporting ATPase of *Staphylococcus aureus* [14]. This suggests that the conserved GMXCXXC is (part of) a general heavy metal ion binding site.

CopB contains three copies of a different putative metal binding element with the consensus sequence MXHXXMSGMXHS (Fig. 1). Closely similar repeats are present in a *Pseudomonas syringae* protein that was demonstrated to be a periplasmic copper binding protein [15]. This would suggests that the N-terminal region of the CopB ATPase constitutes a copper binding domain.

The putative ion transduction regions of the four ATPases under discussion here contain a proline that is located in a hydrophobic domain. While this proline residue is conserved in all P-type ATPases, it is flanked by cysteines only in some enzymes, notably the Cd^{2+}-ATPases [16]. Interestingly, three P-type ATPase of unknown function that have recently been cloned also contain an intramembraneous CPC that may indicate a role of these proteins in heavy metal ion translocation [17–19]. The startling similarity between the Menkes and Wilson gene products and the evolutionary very distant

CopA protein points to high evolutionary constraints in these enzymes, most likely associated with the transduction of copper ions.

Based on hydropathy profiles, transmembraneous helices were propose for the four ATPases (Fig. 1). For CopA, CopB and later also for the Menkes ATPase (J. Gitschier, personal communication), eight transmembranous helices have been postulated, while ten membrane spans were proposed for the Wilson ATPase [10]. Fig. 2 shows folding models for the bacterial and the human copper ATPases based on our interpretation of the data.

5. Conclusion

Taken together, it appears that the four genes described here encode ion-motive ATPases that effect translocation of copper and possibly other metal ions across the cell membrane or membranes of a cellular compartment. This proposal rests on the following evidence: (i) these enzymes are P-type transport ATPases based on sequence similarity, (ii) these enzymes show N-terminal and intramembraneous features observed in known heavy metal ion binding proteins, (iii) the CopA and CopB ATPases are inducible by either high or low ambient copper concentrations, (iv) defective Menkes or Wilson genes result in defects in copper metabolism, (v) null-mutations of CopB leads to copper sensitive cells.

The presence of similar enzymes in such diverse species as man and *E. hirae* suggests that ATP-driven copper transport is a mechanism of copper homeostasis that has been well conserved in evolution. Copper-transporting ATPases represent a novel mechanism for the control of intracellular copper and future work will have to address the question of the localization and function of these copper ATPases.

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