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Another pearl in the "copper-transport" necklace

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Life depends on metals, and copper is one of the key transition metal ions that humans and other organisms need for survival. Many fundamental reactions in our body, such as creating energy from oxygen during respiration, involve copper-dependent enzymes. But free copper is toxic; its redox activity can cause damage to biomolecules and thus there is no free copper in living organisms (1). Instead, copper is carefully transported around the body, always bound to smaller biomolecules or proteins. When I and my group recently counted how many proteins there are in a human cell that bind to copper, the result was 54, which corresponds to almost 1% of all proteins (2). To note, 54 is not a fixed number, it will likely be changed in the future as more copper-dependent proteins become discovered. Almost 30% of these 54 proteins function in cellular copper transport. That is, their primary function is to move copper ions from one place to another in cells, ultimately delivering the metal to a specific copper-dependent enzyme (1). The rest of the 54 proteins are either copper-dependent enzymes or copper-binding proteins with unknown function. For these roughly 40 proteins, how they get the copper (i.e., the copper-transport pathway) is only established for a handful of well-known copper-dependent enzymes (such as ceruloplasmin, superoxide dismutase, and lysyl oxidase). Clearly, there is a lot more to unravel in the field of "copper in biology." Such work is of uttermost importance because it is becoming more and more evident that copper dyshomeostasis is part of or can even initiate diseases such as cancer and neurodegeneration (3,4). In respect to the many unknowns, the new discovery made by Uhlemann et al., reported on in this issue of *Biophysical Journal* (5), is not surprising. However, the results are unexpected because they concern one of the copper-transport proteins for which we have collected a lot of data already: ATP7A, or the Menke's disease protein.

Copper ions enter cells via the copper importer Ctr1. Ctr1 then hands the copper over to three cytoplasmic transport systems. The copper chaperone CCS deliver copper to superoxide dismutase, whereas other chaperones bring copper to cytochrome c oxidase in mitochondria. In the third, general pathway, also called the secretory pathway, the copper chaperone Atox1 delivers copper to the P_{1B}-type ATPases, ATP7A and ATP7B, in the Golgi network (Fig. 1). ATP7A and ATP7B are homologous (but with some distinct features) multidomain, membrane-spanning proteins that use ATP to move the copper into the Golgi lumen (6), where proteins in the secretory pathway, such as ceruloplasmin and lysyl oxidase, get loaded with copper before moving to their final destinations. In contrast to their bacterial and yeast counterparts, which only have one or two, the ATP7A and ATP7B proteins contain an N-terminal cytoplasmic tail with six metal-binding domains, like a set of pearls on a necklace string. Each such domain has a folded structure and copper-binding site like that of Atox1. It is believed that Atox1 docks with one of these metal-binding domains, forming a transient or long-lived complex bridged by the copper and then the copper is moved along the chain of metal-binding domains before it reaches the membrane-spanning channel in the protein core. Why the human proteins have as many as six domains remains puzzling, but there have been many studies in vitro trying to elucidate how the different domains contribute to metal transport as well as regulation via domain-domain interactions (7,8). The discovery of a seventh pearl in the ATP7A chain, as described in (5), makes this puzzle even more complicated.

In their study (5), biophysical data are presented that reveal an additional domain in the linker region between metal-binding domains 1 and 2 in ATP7A. This part of the protein was previously thought to be unstructured but, using NMR and computations, is now shown to contain a metastable folded structure. This peptide stretch does not harbor a copper-binding site, but the authors speculate that phosphorylation of a particular residue

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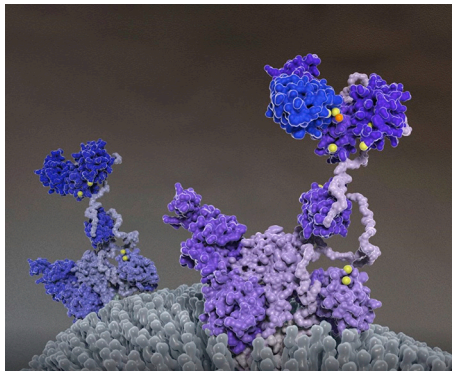

FIGURE 1 Illustration of two ATP7A(ATP7B) proteins sitting in the Golgi membrane with protruding cytoplasmic domains. Here, Atox1 is shown delivering copper (*orange*) to a metal-binding domain in the ATP7A(ATP7B) nearest in view. Copper coordinates to sulfurs (*yellow*) in the active sites of the metal-binding domains and hand over is thought to involve a three-coordinated copper ion (that bridges cysteine sulfurs from two proteins). For more details, see the video at https://youtu.be/rSUXyFjC4QE, from which this snapshot is taken.

regulates the structural stability. They propose that, somehow, copper binding to metal-binding domains nearby regulates phosphorylation of the metastable part. In this way, there may be a link between copper binding, phosphorylation, and domain stability. This is a thought-provoking regulatory hypothesis. As with all scientific studies, there are more experiments desired. I wonder if this metastable domain is present in ATP7A homologs in other mammals? It appears so from sequence analysis, but do those peptides form metastable structures in a test-tube? What is the biological significance of phosphorylation-regulated copper transport? Does the folded-unfolded status of the metastable domain affect Atox1 interaction with and metal delivery to nearby metal-binding domains? I am sure the authors of the study have already asked similar questions and are working on answers right now.

Copper-dependent activities and thus copper transport are central to life, but this discovery also relates more generally to how we view biological function. Historically, we all believed that proteins had to be folded to have a function. But in recent decades we have discovered many unstructured proteins, so-called intrinsically disordered proteins (IDPs), that play functional roles (9). For example, emerging data suggest that liquid-liquid phase separation is a new physical principle for cellular organization, in which IDPs play key roles (10). The metastable domain reported here in ATP7A (5) is reminiscent of zinc finger peptides, which fold upon zinc binding, and unstructured proteins, which fold upon binding to their target proteins. A metastable unit that can easily switch between folded and unfolded states in response to a specific signal is another class of proteins, in addition to well-folded proteins and IDPs, that nature uses to tune biological activity. I suspect we will discover additional metastable domains when we begin to explore in more detail the many large, multidomain proteins that exist in the human proteome. Our experimental toolbox is highly sophisticated today, so we most certainly will uncover more mysterious pearls in our proteins.

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