Introduction to Metals in Biology 2018: Copper homeostasis and utilization in redox enzymes

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This 11th Thematic Metals in Biology Thematic Series deals with copper, a transition metal with a prominent role in biochemistry. Copper is a very versatile element, and both deficiencies and excesses can be problematic. The five Minireviews in this series deal with several aspects of copper homeostasis in microorganisms and mammals and the role of this metal in two enzymes, copper-only superoxide dismutase and cytochrome *c* oxidase.

This 11th Thematic Series in Metals in Biology (1–10) deals with copper, an essential metal in mammals. As in the case of iron, the subject of the last Metals in Biology series (10), copper is a transition metal that is essential but can also be toxic, depending upon the concentration (11). Copper has unique properties and is specifically needed in a number of enzymes, including dopamine β-hydroxylase and other redox enzymes (12–14). Two important enzymes considered here are superoxide dismutase and cytochrome *c* oxidase (15, 16). High concentrations of labile copper are problematic in terms of oxygen toxicity, as in the case of free iron (11), but emerging data reveal contributions of dynamic copper pools to physiological signaling (17).

We have had Minireviews on aspects of copper biochemistry before (18–23) but not a complete Thematic Series. For this Metals in Biology series, we selected several areas of current interest in research with copper.

We begin the series with methanotrophic bacteria and methanobactins in the Minireview by Kenney and Rosenzweig (24). One of the needs for copper in these bacteria is for the copper prosthetic group of particulate methane monooxygenase, a major factor in the methane "economy" on the planet. Some methanotrophs use the copper-binding metallophore methanobactin. Methanobactins are peptidic natural products, produced by ribosomal synthesis and then post-translationally modified. These methanobactins have very high affinity for Cu^{I} and also bind Cu^{II} reductively. The machinery to biosynthesize methanobactins is encoded by operons, and several novel enzymes are involved. Methanobactins are exported via a MATE family multidrug efflux pump, followed by re-internalization by a TonB-dependent transporter. Release of copper from methanobactins and the copper-dependent regulation of the pathways involved in methanobactin synthesis, processing, and transport remain unresolved. Methanobactins are also under investigation as therapeutics for diseases of copper metabolism. An open area of research involves the finding that methanobactin operons are also found in non-methanotrophs, where their function has not been established, but is likely also related to copper homeostasis.

The next Minireview in our Thematic Series (25) picks up with bacteria and methanobactin. While trying to find methanobactin within a methanotroph, Dennison's group discovered a new family of copper storage proteins, the Csp group. Csp1 has 13 cysteines, none of which are in the form of disulfides, and can bind up to 13 Cu^{I} ions. All of these are found along the core of its four-helix bundle monomer in an unprecedented arrangement. *Methylosinus trichosporium* OB3b Csp1 and Csp3 are homologous; both are tetramers, but Csp3 can bind up to 20 Cu^{I} atoms per monomer. They have similar average Cu^{I} affinities but differ considerably in Cu^{I} removal rates. Csp3 is cytosolic, and Csp1 is exported. Copper-regulated Csp1 is isolated from *M. trichosporium* OB3b with copper bound, and gene deletion has a detrimental effect on methane oxidation by the particulate methane monooxygenase. Thus, Csp1 acts as a store of copper for this enzyme. The exact functions of the more widespread and common cytosolic Csp3 proteins are yet to be delineated, but *in vitro* data and emerging *in vivo* evidence are all consistent with a role in copper storage while preventing toxicity. Possible links of the Csp proteins to pathogenicity are also discussed in this Minireview.

The third Minireview, by Ackerman and Chang (26), extends the discussion of copper homeostasis to mammals. The authors' laboratory has been involved in developing new probes for tracking copper in mammalian cells and tissues (27). Mammalian copper homeostasis involves proteins such as the transporter CTR1, chaperones (Atox1), and the storage protein metallothionein. New evidence indicates that not all copper is tightly sequestered and that kinetically accessible pools of copper exist that are not tied up in enzyme active sites and can be mobilized following stimuli. Imaging has shown copper translocation upon neural stimulation. In addition to the effects of copper signaling in neurotransmission and in learning and memory, copper has also been shown to influence circadian rhythm. Copper interacts directly with prion, amyloid precursor, and huntingtin proteins, as well as superoxide dismutase, and may have roles in neurodegenerative diseases. In addition,

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mammalian immune systems utilize copper to prevent microbial growth. A copper exporter (ATP7A) in macrophages moves to the phagosomal membrane in response to infectious stimuli, and manipulating labile copper within immune cells may be a therapeutic possibility. Copper has also been studied in cancer, as a regulator of tumor cell proliferation, with the involvement of mitogen-activated protein kinase (MAPK), BRAF, V600E, MEK1/2, and ERK1/2 proteins. Finally, dysregulation of lipid metabolism is a symptom of copper deficiency or overload, and copper has been identified as an allosteric regulator of phosphodiesterase 3, which controls lipolysis in adipocytes. Cys-768 is distant from the active site but binds copper, and this allosterically regulates enzymatic activity.

Our last two Minireviews deal with copper in important enzyme systems, superoxide dismutase (SOD)[2] and cytochrome *c* oxidase (COX) (15, 16). Superoxide dismutase was originally discovered by McCord and Fridovich in 1969 as a Cu/Zn enzyme (28) but there are versions known to use other metals (*e.g.* iron, manganese, nickel). Very recently, a new class of "copper-only" SODs has been identified in mycobacterium, fungi, and fungus-like oomycetes, as discussed in the fourth Minireview by Robinett, Peterson, and Culotta (15). The eukaryotic copper-only SODs are strictly extracellular; they lack both the zinc group and the electrostatic loop for substrate guidance that are hallmarks of Cu/Zn-SODs. Nevertheless, the copper-only SODs still catalyze the disproportionation of superoxide with diffusion-controlled efficiency. Copper-only SOD sequences are not just SOD enzymes but also occur in animals as repeated protein domains in large molecules called Cu-SOD–repeat proteins (CSRPs).

The last Minireview in the series, by Jett and Leary (16), discusses some aspects of the assembly of COX, a protein with two copper sites composed of three copper atoms (because of the mononuclear Cu_B site and the binuclear Cu_A site), essential for life. The assembly of this multimeric protein, coded for by both nuclear and mitochondrial genomes, involves several proteins. In terms of function, the Cu_A site accepts electrons from cytochrome *c*, and subsequent electron transfer to the heme *a* (iron) and then the heme a_3 (iron)–Cu_B metal centers of COX1 are involved in the reduction of molecular oxygen to water. The individual structural subunits of COX are matured and assembled in modules, specific to COX1, COX2, and COX3. Holoenzyme assembly requires a large number of COX assembly factors. Deficiencies of any of these result in death or serious diseases in humans. As an example of the complexity of the systems, the proteins COA6, SCO1, and SCO2 form a metallochaperone module that interacts with the COX20–COX2 complex just to add copper to the Cu_A site, with the Cu^I coming from a labile pool housed in the mitochondrial matrix (29, 30). This process involves four stages, including export of the N-terminal tail of COX2 into the intermembrane space association of COX20 with COX2 during membrane insertion (for stabilization), COX18 release from the COX20–COX2 complex accompanied by recruitment of the SCO1/SCO2/COA6 metallochaperone module, and finally reduction of two cysteinyl sulfurs of COX2 with SCO1 inserting two Cu^I ions into the Cu_A site (16).

A good review not only answers questions but also identifies new ones to be addressed. In this Thematic Series, there are many things left to know, and I will touch on only some of the questions I had after reading. How are the precursor peptides processed to generate methanobactins (24)? New knowledge about the enzymes involved in other peptide post-translational modifications is becoming available (31, 32). Why are methanobactin-like genes found in non-methanotrophs? What are the functions of bacterial cytosolic copper storage proteins (Csp3s) (25)? Why do bacteria store copper in the cytosol when they are not thought to possess copper enzymes in this cellular compartment? Is there more to learn about copper and virulence in mammalian hosts? What are the origins of some of the copper signals seen in mammals (26)? Do changes in redox state trigger mobilization of copper signals? Can new imaging technologies be harnessed to provide more effective disease therapies? How much do copper issues contribute to diseases such as neurodegeneration, infection, obesity, and cancer? With regard to SODs, what are the Cu-SOD–repeat proteins doing in animals without lungs (15)? Have all of the accessory proteins for cytochrome oxidase assembly been identified (16)? Are the sequential order and timing of events in cytochrome oxidase assembly critical? What is the role of redox states—both copper and thiols—in cytochrome oxidase assembly? How are pools of "available" copper regulated and used in prokaryotes (24, 25) and eukaryotes (16, 26)? Perhaps we can revisit progress toward these and other questions in a future Metals in Biology Thematic Series.

The authors and I hope that you enjoy reading this Thematic Series. We have begun to plan the next Metals in Biology, the theme of which will be determined soon.

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[2] The abbreviations used are: SOD, superoxide dismutase; COX, cytochrome *c* oxidase.

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