right panel in the figure). It was once hoped that quantum mechanics would lead to an unconditionally secure protocol for DDM, as it does for QKD, but this hope was dashed 2 years ago (*8*), when it was shown that one prerequisite for DDM, called bit commitment, cannot be made unconditionally secure against quantum attacks. Conditionally secure DDM, based on classical bit commitments that are merely infeasibly hard, not impossible, for a quantum computer to break, remains a possibility.

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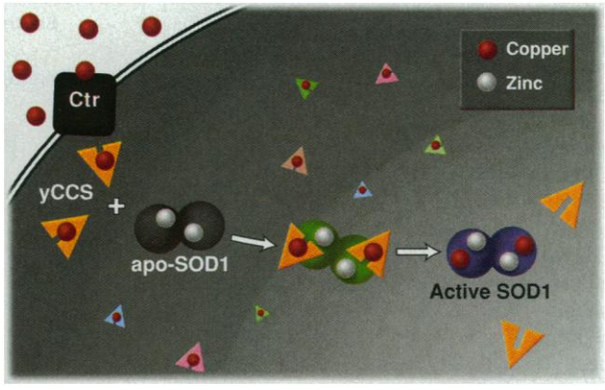
PERSPECTIVES: BIOCHEMISTRY

Free Copper Ions in the Cell?

Stephen J. Lippard

Transition metal ions are essential for life (*1*). Cells regulate the traffic of transition metal ions (such as copper and iron), maintaining the amount necessary for biological function while avoiding excess levels that are toxic (*2*). Among the factors required to achieve such metal ion homeostasis are the metallochaperones, proteins that, like chaperones in ordinary life, guide and protect transition metal ions within the cell, delivering them safely to the appropriate protein receptors (*3*). One such metallochaperone is yCCS, a yeast protein encoded by the *LYS7* gene. This copper chaperone and its homologs in mice and humans deliver copper to the antioxidant enzyme copper-zinc superoxide dismutase (SOD1) and colocalize with SOD1 in vivo (*4*–*6*). SOD1 is mutated in people with an inherited form of familial amyotrophic lateral sclerosis, a fatal neurological disorder also known as Lou Gehrig's disease, that may be caused by aberrant effects of copper facilitated by improperly folded forms of the enzyme (*7*). Colocalization of CCS and SOD1 in mammalian tissue of the central nervous system is particularly intriguing and may yield clues for developing therapeutic strategies to treat this disease (*6*). On page 805 of this issue, Rae *et al.* show that yCCS directly inserts copper into SOD1 and is active at very low copper concentrations (*8*). In the course of their investigation, the authors made the remarkable discovery that the upper limit of so-called "free" pools of copper was far less than a single atom per cell. It had been commonly believed that metal ions were in equilibrium with metalloproteins. The implications of this finding are profound, especially if applicable to other physiologically important transition metals.



Copper and its metallochaperone. Copper trafficking in yeast begins with transport of copper ions across the plasma membrane by proteins such as Ctr (*9*). By an unknown mechanism, copper is loaded into a metallochaperone, yCCS, which delivers the metal ions to an inactive form of the enzyme SOD1. yCCS docks onto apo-SOD1 (the inactive form depicted as having bound zinc) and copper is transferred to the active sites, forming active SOD1. Once copper is loaded into its binding sites, yCCS molecules lacking copper are available for additional rounds of metal ion delivery to the enzyme. Although pools of free copper are available outside the cell, none are available within under normal growth conditions. (Metallochaperones that deliver copper to target proteins other than SOD1 are drawn in different colors and sizes. Proteins such as metallothionein that buffer total copper concentrations in the cell are not shown.)

A study of how SOD1 acquires copper in vivo resulted in the discovery of yCCS (*4*). Yeast is an excellent model system to investigate the trafficking of transition metal ions in eukaryotes. The Ctr family of membrane proteins facilitates the transport of copper ions across the yeast plasma membrane, and yCCS and two other copper metallochaperones assure its delivery to specialized compartments or enzymes in the cell (*9*) (see the figure).

Rae *et al.* considered three possible ways in which yCCS could mediate transfer of copper to apo-SOD1, an inactive form of SOD1, and so activate the enzyme: (i) yCCS could fold and stabilize the apoprotein into a conformation required to bind copper, (ii) it could itself modulate intracellular concentrations of copper, or (iii) it could directly insert copper into the enzyme. They now show that the third possibility is the correct one (*8*). They established through in vitro studies that, although several low molecular weight complexes of Cu(I) and Cu(II) could add copper to SOD1, only yCCS was capable of doing so in the presence of exceptionally strong copper-chelating agents that reduced available copper ions to very low concentrations. In a second line of evidence, they found no SOD1 activity in strains of yeast that lacked the *LYS7* gene and thus were deficient in yCCS (even though the yeast had normal concentrations of copper ions). In a further experiment, the authors looked at the ability of copper in the growth medium to activate SOD1 in yeast deficient in both yCCS and metallothionein (a protein that serves to buffer cellular copper ion concentrations). The levels of cellular copper increased tenfold and SOD1 activity was observed. Much larger (toxic) amounts of extracellular copper were required to activate SOD1 in the presence of metallothionein. Taken together, these results provide an indirect but persuasive case that CCS functions in vivo as a metallochaperone, specifically transferring copper to its target enzyme (see the figure).

The finding that the insertion of copper into apo-SOD in vivo requires CCS is interesting because the equilibrium constant for the dissociation of Cu(II) from the enzyme is in the femtomolar range (*10*). From this thermodynamic information and a measure of the number of SOD molecules in the cell, the investigators estimated an upper

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limit of 10^{-18} M for the free concentration of copper in the cell (*8*). Assuming a cell volume of 10^{-14} liters, such a concentration corresponds to 10^{-33} mol or 10^{-9} atoms, leading to the conclusion that cellular pools of free copper are virtually nonexistent.

There is considerable interest in factors that control the concentrations of transition metal ions other than copper in the cell. Proteins have been discovered that facilitate the transport of manganese, iron, and zinc as well as copper across the yeast plasma membrane (*2*), and a bacterial metallochaperone that delivers nickel to the enzyme urease has been reported (*11*). Reliable estimates of the free concentrations of these other transition metal ions in cells are not yet available. The acquisition of such information would be greatly facilitated by the development of fluorescent intracellular metal ion sensors, such as those already available for calcium (*12*). Even if free pools of transition metal ions in cells do not exist, the possibility that they might occur in diseased or otherwise altered states of the cell could be determined if such sensors were at hand. Dysregulated transition metal ion concentrations in cells have been associated with several human diseases, including pediatric neurological disorders (manganese), hereditary hemochromatosis, Parkinson's disease, Friedreich's ataxia (iron), Menkes' syndrome, and Wilson's disease (copper) (*2*).

Although the new findings reveal that yCCS loads SOD1 with copper, several questions still need to be addressed. From what source does this metallochaperone receive its copper? What is the structure of yCCS and, in particular, what are the ligands that bind the copper atom(s)? What are the molecular hand-off mechanisms for transferring copper to and from the protein? Are there common strategies evolved by nature that might apply to other copper trafficking proteins in yeast, such as Atx1 and Cox17 (*9*), and to chaperones for the other transition metal ions? And, recalling that SOD1 contains zinc as well as copper, is there a metallochaperone that also delivers zinc to the enzyme? Designing experiments to answer these questions will ensure that this discipline of bioinorganic chemistry will remain a hotbed of research activity for a long time to come.

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PERSPECTIVES: IMMUNOLOGY

A New Look at MHC and Autoimmune Disease

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A strong genetic association exists between certain autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis, and insulin-dependent diabetes mellitus (IDDM), and the expression of certain kinds (haplotypes) of major histocompatibility complexes (MHCs) (genes encoding cell-surface molecules that display peptides for immune recognition) (*1*). The current explanation for this association proposes that disease-associated MHC molecules efficiently bind autoantigens involved in the pathophysiology of the disease. This results in a peripheral T cell–mediated immune response to the autoantigen and autoimmune sequelae.

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Recent results in an animal model of autoimmune diabetes, the nonobese diabetic (NOD) mouse, however, suggest a new hypothesis to explain the role of MHC in autoimmunity. This hypothesis proposes that the genetic association between MHC and autoimmune disease results from "altered" thymic selection in which high-affinity self-reactive (potentially autoreactive) T cells escape selection in the thymus as a result of the poor self peptide–binding properties of the disease-associated MHC class II molecules (*2*). This model offers an explanation for the unusual requirement of homozygous MHC class II expression in human IDDM and IDDM in NOD mice.

MHC molecules bind peptides for presentation to antigen-specific T cell receptors (TCRs) on T lymphocytes. The TCRs recognize MHC-peptide complexes on the surface of antigen-presenting cells (APCs). There are two major roles for MHC class II gene products: (i) selection of the T cell repertoire in the thymus, and (ii) presentation of foreign antigens in the periphery. By processing and presenting self peptides bound to MHC molecules to developing thymocytes, thymic APCs first select the potential peripheral T cell repertoire (positive selection) and then purge this positively selected repertoire of T cells that react too strongly to self peptide–MHC complexes (negative selection). Less than 1% of precursor T cells entering the thymus survive this selection (*3*). Subsequently, by presenting foreign peptides to the peripheral T cells that have survived thymic selection, peripheral APCs—expressing the same MHC molecules as their thymic counterparts—initiate protective immune responses. This "dual role" of MHC class II molecules has contributed to our difficulty in deciphering their role in autoimmune disease, because the response (or lack of response) of the peripheral T cell population to any antigen is determined both by MHC-mediated thymic selection events and by the capacity of the MHC molecules to bind and present foreign antigen to peripheral T cells. It is generally believed that some autoimmune diseases are caused by "pathologic" T cells, which "inappropriately" recognize and respond to self peptide–MHC complexes, possibly after activation by foreign peptides that closely resemble self peptides (molecular mimics). But it has been difficult to determine whether the MHC association with autoimmune disease lies in the thymus or periphery, or both. Another complexity is that autoimmune diseases are multigenic. Thus, the MHC acts in conjunction with many other genes, none of which are necessary or sufficient, to produce autoimmunity.

Studies in the NOD model of autoimmune diabetes have begun to solve the puzzle. NOD mice spontaneously develop an autoimmune syndrome in which autoreactive $CD4^+$ T cells infiltrate multiple organs, including the pancreas. Autoreactive T cells in NOD mice recognize multiple pancreatic and nonpancreatic self peptides and can transfer at least three distinct autoimmune syndromes to naïve recipients (*4–7*). Self-tolerance of NOD mice can be "broken" by immunization with self peptides (*8*). The immunized NOD mice develop

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