Multiple Protein Domains Contribute to the Action of the Copper Chaperone for Superoxide Dismutase*

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The copper chaperone for superoxide dismutase (SOD1) inserts the catalytic metal cofactor into SOD1 by an unknown mechanism. We demonstrate here that this process involves the cooperation of three distinct regions of the copper chaperone for SOD1 (CCS): an amino-terminal Domain I homologous to the Atx1p metallochaperone, a central portion (Domain II) homologous to SOD1, and a short carboxyl-terminal peptide unique to CCS molecules (Domain III). These regions fold into distinct polypeptide domains as revealed through proteolysis protection studies. The biological roles of the yeast CCS domains were examined in yeast cells. Surprisingly, Domain I was found to be necessary only under conditions of strict copper limitation. Domain I and Atx1p were not interchangeable in vivo, underscoring the specificity of the corresponding metallochaperones. A putative copper site in Domain II was found to be irrelevant to yeast CCS activity, but SOD1 activation invariably required a CXC in Domain III that binds copper. Copper binding to purified yeast CCS induced allosteric conformational changes in Domain III and also enhanced homodimer formation of the polypeptide. Our results are consistent with a model whereby Domain I recruits cellular copper, Domain II facilitates target recognition, and Domain III, perhaps in concert with Domain I, mediates copper insertion into apo-SOD1.

Recently, a new class of eukaryotic proteins that act in the intracellular trafficking of copper ions has been identified. These copper chaperones have been characterized in both yeast and humans (1) and are typically defined as soluble copper receptor proteins that function to deliver copper ions to specific intracellular targets (2). Whereas the copper enzymes that serve as targets for these molecules exhibit a high affinity for copper in vitro, the copper chaperone functions are critical in vivo because free intracellular copper concentrations are quite restricted (3).

One of the first genes identified in copper trafficking, yeast ATX1, encodes a protein that binds copper and docks with a specific partner protein (2, 4–6). This partner for Atx1p is the P-type copper-transporting ATPase in the secretory pathway, yeast Ccc2p (7), or in the case of human ATX1 (HAH1 or ATOX1), the human Wilson and Menkes gene products (8, 9). The metal delivered in this pathway is incorporated into copper-requiring enzymes destined for the cell surface or extracellular milieu. Cox17p represents another well conserved copper protein that acts in a distinct pathway to help deliver copper to the mitochondria, where the metal is ultimately incorporated into cytochrome oxidase (10–13). More recently, we identified a third eukaryotic copper chaperone that targets the cytosolic copper- and zinc-requiring superoxide dismutase (SOD1). 1 This copper chaperone for SOD1 (CCS) was originally discovered as the yeast LYS7 gene product, and a functional homologue has been identified in humans (14–17). CCS is specific for its target and is particularly crucial for activating SOD1 in vivo, where intracellular free copper is limiting (3). Yeast CCS (yCCS) protein has been purified to homogeneity and been shown to be necessary and sufficient for direct copper insertion into SOD1 (3); however, the mechanism by which CCS recognizes SOD1 as its target and inserts the metal into the enzyme is unknown.

To begin to understand the nature of CCS specificity and its mechanism of action, we have conducted a structure/function analysis on the yeast protein both in vivo and in vitro. We find that this copper chaperone consists of three structurally distinct domains that carry out independent functions in activating SOD1 with copper: (i) an amino-terminal Atx1p-like region that appears critical for capturing copper under metal starvation conditions, (ii) a central SOD1-like region that may facilitate target recognition, and (iii) a small, conserved COOH-terminal region that has the potential to bind copper and is indispensable for CCS function under all conditions. Our results are consistent with an allosteric model in which the tertiary structure of the CCS protein, in particular the COOH-terminal domain, undergoes metal-induced conformational changes that ultimately facilitate direct insertion of copper into the active site of SOD1.

EXPERIMENTAL PROCEDURES

Yeast Strains and Media—The yeast strains SY2850 (lys7Δ::LEU2 (18)), SL215 (ats1Δ::LEU2 (4)), and KS107 (sod1Δ::TRP1 (19)) have

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1 The abbreviations used are: SOD, superoxide dismutase; CCS, copper chaperone for superoxide dismutase; yCCS, yeast CCS; SD, synthetic dextrose; HPLC, high pressure liquid chromatography; ESI MS, electrospray ionization mass spectrophotometry; HA, hemagglutinin.
been previously described. The lys7Δ; LEU2 atx1Δ::HIS3 strain, PS113, was constructed by deleting the ATX1 gene of SY2590. Stocks of strains were maintained on enriched YPD medium (20) in anaerobic culture jars (BBL GasPak). All yeast transformations were conducted by the method of electroporation (21). Spot tests for complementation of atx1Δ strains for trypsin proteolysis, 10 μg of cell protein was applied to a precast 14% polyacrylamide gel (Novex) and subjected to SDS gel electrophoresis followed by Western blot using either an anti-hemagglutinin (HA) antibody (Babco) followed by a secondary anti-goat IgG (for detection of yCCS-HA fusions) or an anti-yCCS antibody as described (3). For analysis of SOD activity, 20 μg of the same extracts was subjected to electrophoresis on a non-denaturating 12% precast gel (Novex), followed by nitro blue tetrazolium staining for SOD activity as described (14).

Biochemical Analyses—Cell lysates were prepared for immunoblot studies and for analysis of SOD1 activity by glass bead homogenization as described (22) from cells grown overnight to confluency in selecting synthetic dextrose medium. For immunoblot analysis, 10 μg of cell protein was digested with 2 μg of trypsin at 14 °C for 30 min in 50 mM Tris, pH 7.8, with 200 mM NaCl and 1 mM each dithiothreitol, histidine, and glutathione (as competing copper ligands) was slowly charged with 150 μM Cu(II)(CH3CN)PF6 and 150 μM ZnSO4. After incubation in an anaerobic chamber at 14 °C for 20 h, the protein was exchanged repeatedly with the same buffer and competing ligands in an Amicon ultrafiltration cell. The competing ligands were removed from the protein with repeated exchanges of 50 mM Tris, pH 8.0, and 10 mM dithiothreitol only. Copper loading of the synthetic yCCS-diII (Ala216–Lys249) peptide (100 μM) was carried out under similar conditions, except that 20 mM Mes, pH 6.0, was used as buffer, and copper binding was achieved with 200 μM Cu(II)(CH3CN)PF6. For assessment of metal binding capacities, protein concentrations were determined by Bradford method, using IgG standards with appropriate correction factors applied. Bradford correction factors were determined by the Bradford method, using IgG standards.

For limited trypsin proteolysis of yCCS, a solution containing 800 μg of apo-yCCS was proteolyzed with 1 μg of trypsin for 30 min at 25 °C, after which phenylmethylsulfonyl fluoride was added to terminate the reaction. The digest fragments were separated by reverse-phase HPLC through a Vydac 214TP54 column with a H2O/CH3CN/0.1% trifluoroacetic acid gradient. Isolated fragments were dried under vacuum and resuspended in H2O with 1% formic acid for ESI MS analysis.

RESULTS

Structurally Distinct Domains of yCCS—The amino acid sequence of Saccharomyces cerevisiae Lys7 (referred to herein as “yCCS”) is similar to two yeast proteins, Atx1p and Cu,Zn-SOD (Fig. 1A). The NH2-terminal 70 amino acids are homologous to Atx1p, and the central region exhibits significant homology with yeast Cu,Zn-SOD. The most COOH-terminal 30 amino acids of yCCS are not homologous to available sequences in the data bases, although this segment is highly conserved among CCS molecules from diverse species (e.g. the yeast and human CCS molecules share nearly 50% identity over this region (14)). The limited homologies noted with the three regions of yCCS led us to test a multidomain model for this metallochaperone.

The existence of independent structural domains was established by limited trypsin digests on purified apo-yCCS (Fig. 1B). Separation of peptide fragments by reverse-phase HPLC and analysis of molecular mass by ESI MS revealed two stable units. The larger of these units consisted of the NH2-terminal region, referred to as Domain I; a central region, referred to as Domain II; and the SOD1 and Atx1p homologous regions, referred to as Domain III. These results establish that the CCS protein consists of at least three structurally distinct domains: the SOD1-like sequence (Fig. 1A, B), the remainder of the protein, suggesting that this peptide segment was much more rapidly digested by trypsin than was the remainder of the protein, suggesting that this peptide segment has relatively little structure in the absence of copper. Similar results with trypsin proteolysis were observed with human CCS (data not shown). These results establish that the CCS protein consists of at least three structurally distinct domains: an Atx1p-like NH2-terminal region, referred to as Domain I; a central SOD1-like region, referred to as Domain II, and at the extreme COOH terminus, a short region denoted as Domain III. The correlations between these proteolytically defined domains and the SOD1 and Atx1p homologous regions raise the
possibility that each domain serves an independent physiological function.

The ATX1-like Domain I of yCCS—To examine the function of the three CCS domains in vivo, specific truncation mutants of yCCS were expressed in yeast cells. Polypeptides spanning Domains I and II (yCCS-dI/dII), Domains II and III (yCCS-dII/dIII), and isolated Domain I (yCCS-dI) accumulated to levels similar to that of native yCCS when expressed in a lys7 null strain (Fig. 2A).

*lys7* strains are typically lysine auxotrophs when grown in air because copper-SOD1 is essential for aerobic lysine biosynthesis (24, 25). Expression of full-length yCCS complemented this defect, whereas no complementation was observed with yCCS-dI/dII lacking domain III (Fig. 2B). Surprisingly, removal of the Atx1p-like Domain I did not eliminate yCCS activity, as yCCS-dII/dIII complemented the *lys7* defect (Fig. 2B). However, this activity is weak compared with intact yCCS, demonstrating that Domain I is required for maximal yCCS activity. To test whether Domain I can act in trans with the downstream segments of yCCS, we co-expressed yCCS-dII and yCCS-dIII as separate molecules. As seen in Fig. 2C, Domain I effectively enhanced the complementation obtained with yCCS-dIII alone, suggesting that Domain I may physically interact with the carboxyl-terminal portion of yCCS to activate SOD1.

Considering the homology between yCCS Domain I and the Atx1p metallochaperone, we addressed whether these protein domains were interchangeable. Domain I can cooperate with yCCS-dII/dIII in trans; therefore, it was possible that the weak activity observed with yCCS-dII/dIII involved endogenous Atx1p. However, complementation by yCCS-dII/dIII was identical in *ATX1*-expressing and *atx1* strains (Fig. 2C), suggesting that Atx1p could not mimic the action of yCCS Domain I. To address this issue further, we constructed a chimeric gene in which Domain I of yCCS was replaced by Atx1p. This ATX1-CCS-dII/dIII fusion protein was stably expressed in a lys7 null strain (Fig. 2A), and complemented the lysine auxotrophy of this cell; however, the activity obtained was not substantially different from that seen with yCCS-dII/dIII alone (Fig. 3A). Hence, Atx1p cannot substitute for yCCS Domain I. To test whether the opposite was true, isolated Domain I was expressed in an *atx1* strain. These cells are defective for delivering copper to Fet3p copper oxidase (4) and, as such, fail to grow on medium supplemented with the iron chelator, ferrozine (Fig. 3B). This defect is complemented by expression of...
Domain Organization of the CCS Metallochaperone

ATX1 but not by isolated yCCS Domain I. Thus, Domain I cannot substitute for Atx1p in delivering copper to Ccc2p and Fet3p in the secretory pathway. It is noteworthy that the ATX1-CCS-dII/dIII chimera did complement the atx1 mutant defect (Fig. 3B), indicating that this fusion copper chaperone molecule can deliver copper to both SOD1 and to Ccc2p. Presumably, specificity for Ccc2p is mediated by the amino-terminal Atx1p portion, whereas activation of SOD1 involves the carboxyl-terminal yCCS-dII/dIII region of the chimera molecule.

The weak activation of SOD1 observed with isolated yCCS-dII/dIII prompted us to examine the requirement for Domain I under varying copper conditions. When the growth medium was treated with the Cu(I)-specific chelator, bathocuproine sulfonate, yCCS-dII/dIII lost all complementation activity, whereas intact yCCS retained full activity under these copper starvation conditions (Fig. 4A). In comparison, treatment of cells with additional copper dramatically enhanced the activity obtained with yCCS-dII/dIII. Normally, the level of SOD1 activation by yCCS-dII/dIII is too weak to be detected by an in situ gel assay for SOD1 (Fig. 4B), which has a limit of detection of 2 ng of active SOD1 (3). Yet when cells are grown in the presence of somewhat elevated, but non-toxic, copper concentrations (10 μM), yCCS-dII/dIII exhibited nearly wild type activation of SOD1 as monitored by the gel assay (Fig. 4B). In comparison, this copper treatment failed to rescue the defect of yCCS-dII/dIII lacking Domain III (Fig. 4B). Hence, the Atx1p-like Domain I of yCCS is only necessary under strict copper limitation conditions. These observations are consistent with a role for the Atx1p-like NH2-terminal domain in the recruitment of copper to the yCCS molecule and a role for the remainder of the protein in target recognition and direct transfer of copper to SOD1.

Domains II and III of yCCS—The surprising activity observed with isolated yCCS-dII/dIII suggested that this region may contain a functional copper-binding site that is involved in copper transfer to SOD1. We have recently demonstrated that copper can be directly transferred to SOD1 in vitro from yCCS loaded with a single Cu(I) ion (3). When treated with an excess of metal ion in the presence of stringent competitors, yCCS can tightly bind an additional copper ion and retain activity. Although no binding of Zn(II) was observed under these conditions, yCCS appears to have more than one high affinity copper site; one possible site was noted in the SOD1-like Domain II. As is the case with SOD1, Domain II of yCCS contains four histidines (Fig. 1A). Although the spacing between these His side chains at positions 130, 134, 151, and 198 does not completely correspond to the highly conserved pattern in SOD1 and hCCS, it is possible that the three-dimensional fold of yCCS could accommodate a functional copper transfer site. Each histidine in Domain II was mutated to alanine. As seen in Fig. 4C, wild type levels of SOD1 activation were obtained with each
corresponding mutant of full-length yCCS. Furthermore, the same histidine substitutions in isolated yCCS-dII/dIII did not impair activity of this truncated yCCS (not shown). Thus the potential copper site of Domain II is not necessary for copper activation of SOD1.

The most highly conserved region among CCS molecules is Domain III (Fig. 5A). As described above (Figs. 2B and 4B), this domain is indispensable for CCS activity in vivo. Domain III contains an invariant CXC motif that is found in all members of the CCS family, including that of mammals, plants, fungi, and insects (Fig. 5A). To assess the role of this motif in metallochaperone function, Cys to Ser mutations were introduced singly and in combination at positions 229 and 231 in yCCS. The mutant derivatives were stably expressed in lys7 null yeast (Fig. 5B). These mutations had a dramatic effect on yCCS activity. All three Cys to Ser derivatives failed to activate SOD1 to levels that could be detected by the in situ gel assay (Fig. 5B). In the more sensitive assay of lysine-independent growth, variant C231S was completely inactive, whereas yCCS C229S exhibited weak activity that was estimated to be less than 10% of wild type yCCS (Fig. 5B). Therefore, the CXC motif in Domain III of CCS is critical for copper chaperoning activity. Although the role of these cysteines in activation of SOD1 is not known, one possibility is that they directly participate in copper transfer.

To test whether Domain III is capable of binding copper, a peptide corresponding to residues Ala216–Lys249 of CCS was synthesized and treated with Cu(I) under anaerobic conditions similar to those used to load Atx1p with metal (2). The Cu(I)-peptide complex was exposed to several buffer exchanges against metal-binding competitor molecules to remove metal from low affinity sites on the peptide. Removal of unbound metal and competitors revealed a copper peptide stoichiometry of 0.53:1, demonstrating that Domain III indeed has the capacity to bind Cu(I), most likely forming a copper-bridged peptide dimer under this condition. Taken together, these observations are consistent with a role of the COOH-terminal domain of CCS in copper-handling steps during activation of SOD1.

**Copper-mediated Changes in yCCS Conformation**—The cooperation of multiple CCS domains may proceed through copper-mediated changes in protein structure. Two biochemical assays were employed to probe the effects of Cu(I) binding on yCCS conformation. First, analytical gel filtration revealed that apo-yCCS migrates as a monomer (molecular mass, 29 ± 2 kDa) under physiological buffer conditions at all protein concentrations examined (100–750 μM) (Fig. 6). In comparison, the copper-loaded form of yCCS exists as a mixture of monomers and dimers (molecular mass, 54 ± 4 kDa) in the 10–100 μM protein concentration range. In both the one-copper and two-copper loaded states, the dimeric form of yCCS was slightly more abundant than the monomer (Fig. 6). These results are consistent with a model in which copper induces a conformational change in yCCS that significantly increases the monomer-dimer equilibrium constant.

To further evaluate whether metal binding induces conformational changes in yCCS, the apo- and copper forms of the metallochaperone were subjected to a proteolysis time course. As seen in Fig. 7, copper binding to yCCS resulted in a significantly slower rate of chymotrypsin cleavage at Domain III. Estimated half-lives of Domain III with full-length yCCS under these conditions were 15 min for apo-yCCS versus 50 min for Cu-yCCS (Fig. 7). Thus, the binding of one copper to yCCS attenuates chymotrypsin digestion at sites distant from the proposed copper-binding sites. Together with the gel filtration data of Fig. 6, these observations indicate that copper binding induces allosteric conformational changes in yCCS, and one important consequence of these changes is an alteration at the dimerization interface.

**DISCUSSION**

The in vivo insertion of copper into SOD1 is shown here to be a complex allosteric process that involves the concerted actions of three distinct domains of the metallochaperone, CCS. At the amino terminus of CCS, an Atx1p-like Domain I appears responsible for capturing copper under conditions of metal starvation, whereas a central SOD1-like region is proposed to serve in target recognition. Copper insertion into SOD1 then requires a small copper-binding peptide at the COOH terminus of CCS. Although our studies do not directly test a role for the metallochaperone in loading of zinc into SOD1, our experiments with purified protein indicate that Zn(II) binding to yCCS is weak. Hence, CCS may be specifically designed for the copper transfer process.

The NH2-terminal domain of CCS was originally suspected to be the key player in copper activation of SOD1. This domain harbors the same high affinity MXXXC copper-binding site that, in the case of Atx1p, directly transfers the metal from the metallochaperone to its target (2). Yet with CCS, the Atx1p-like Domain I is only required under conditions of strict copper limitation. We have recently demonstrated that the intracellular level of free copper available to SOD1 is in the attomolar range (less than one free atom per yeast cell), despite the micromolar quantities that are typically accumulated by the cell (3). The Atx1p-like Domain I in CCS ensures that the
metallochaperone recruits the metal regardless of copper availability. It is noteworthy that the mere presence of MXCXXC in Domain I is not sufficient, since Atx1p fails to substitute for Domain I in promoting CCS activity under low copper conditions. Our finding that Atx1p and yCCS Domain I are not interchangeable in vivo demonstrates that sequences unique to these polypeptides must facilitate recognition of, and copper transfer to, the cognate targets.

FIG. 5. Domain III of yCCS. A, an alignment of the COOH-terminal regions of various homologues to yCCS. Amino acid sequences that are identical or similar across unrelated species are boxed. The conserved Cys are marked with asterisks. Arrows mark predicted positions of chymotrypsin cleavage. Numbers indicate amino acid numbering of wild type yCCS. Sequences were derived as follows: yeast (18); human (14); mouse (32); rat (EST accession no. AA96543); Drosophila (EST accession no. AFO83312); tomato (cDNA accession no. AAD12307; A. Nersissian and J. S. Valentine, unpublished data); Arabidopsis (EST accession no. AFO83312). B, expression of yCCS Domain III mutants in lys7 null yeast and metallochaperone activity was monitored by Western blot (top left), SOD activity (bottom left), and complementation of aerobic lysine auxotrophy as in Fig. 4.

FIG. 6. Gel filtration analyses of yCCS oligomerization. Protein samples (100 μM) were subjected to gel filtration chromatography as described under “Experimental Procedures.” Cu1-yCCS (solid line) eluted as two peaks, whereas apo-yCCS gave a single peak (dashed line). The inset shows the standard curve generated under the experimental conditions with molecular mass standards (bovine serum albumin, ovalbumin, carbonic anhydrase, cytochrome c, aprotinin, and vitamin B-12; molecular masses given “Experimental Procedures”) indicated by the open circles. Calculated results of yCCS molecular masses are shown by the solid circles. Kav = [Ve - Vb]/(Vo - Vb), where Ve is elution volume of protein, Vo is void volume of column, and Vb is bed volume of column.

FIG. 7. Copper-induced conformational changes in yCCS as monitored by time-resolved proteolysis. Purified yCCS protein (400 μg) was incubated with chymotrypsin at a 200:1 w/w ratio at 14 °C, pH 8.0, for the indicated time periods prior to analysis by 15% SDS-polyacrylamide gel electrophoresis and Coomassie Blue staining. A, apo-yCCS; B, Cu(I)-yCCS containing 1.1 mol of copper/mol of protein. MW, broad range molecular mass standards; d-I,II, 5 μg of a purified yCCS spanning Domains I and II (M1–A216). Sites of chymotrypsin proteolysis in both apo- and Cu-yCCS were determined by ESI MS of HPLC-purified fragments to be after Trp222 and Trp237.
rather than in direct transfer of copper. A possible set of copper-binding histidine side chains was recognized in Domain II, yet all of these residues were found to be irrelevant to metallochaperone activity in vivo. Since copper loading of SOD1 by yCCS has been shown to involve direct transfer from one protein to the other without release of the free metal (3), formation of a specifically docked yCCS-SOD1 complex is a likely prelude to the copper transfer step. Based on its homology to SOD1, Domain II may facilitate this docking by heterodimer formation with SOD1. We show here that yCCS homodimer formation in vitro is stimulated upon copper binding to yCCS. Yet given the copy number of intracellular yCCS (3) and estimates of the dimerization constants, we suspect that the amount of yCCS homodimer in the cell is relatively low. It is therefore conceivable that copper loading of yCCS in vivo facilitates heterodimer formation with SOD1. Gitlin and co-workers (15) recently obtained evidence for in vivo docking between mammalian SOD1 and CCS, where hCCS regions corresponding to yCCS Domains II and III were seen by immunoprecipitation studies to interact with SOD1. The interactions between metallochaperone and target are expected to be transient. Steady state SOD1 exists as a homodimer, and because holo-SOD1 is present in many fold excess over CCS (3, 26), the metallochaperone must be released from SOD1 after metal transfer in order to carry out subsequent copper transfer cycles. An attractive idea is that metallochaperone release from SOD1 is facilitated by metal-induced allosteric changes involving Domains I and III.

The carboxyl-terminal Domain III of CCS emerges from these studies with a central role in CCS function. This domain is indispensable for in vivo activation of SOD1 even under conditions of excess copper. Domain III appears unique to CCS molecules and represents the most highly conserved region by comparison of fungal, mammalian, insect, and plant polypeptides. We propose a model in which a copper-binding CXC domain in Domain III cooperates with the amino-terminal domain of CCS in directing insertion of copper into SOD1. As evidence for interaction between the amino and carboxyl regions of the metallochaperone, Domain I was found to act in trans with a yCCS molecule spanning Domains II and III.

Perhaps the most intriguing insight that arises from these studies is the complexity of the mechanism by which copper is inserted into SOD1 in vivo. In comparison to our results shown here for CCS, the delivery of copper by the Atx1p metallochaperone involves a single metallochaperone domain and a lone copper site (2). The basis for these differential requirements can be best explained in terms of the respective protein targets for the two metallochaperones. In the case of Atx1p, copper is delivered to Atx1p homologous metal-binding domains present on the Ccc2p copper transporter target (2, 5, 6). This homology should obviate the requirement for a separate metallochaperone domain involved in target recognition. The Ccc2p target for Atx1p harbors precisely the same surface-exposed MXXXC copper site employed by the metallochaperone (5), and copper transfer can readily proceed through an associative exchange mechanism involving dual cysteines on the two molecules (2).

Thus the Atx1p MXXXC copper site serves a 2-fold purpose in initially capturing copper and in direct metal transfer. By comparison, the MXXXC copper site on CCS Domain I may not be amenable to direct insertion of the metal into SOD1. The copper site in SOD1 is buried within the enzyme, and only a few square angstroms are solvent-exposed. This copper in SOD1 is coordinated to three or four histidines, depending on the oxidation state of the metal (27–29). Based on studies with Atx1p, the MXXXC copper site is predicted to transfer the metal to solvent-exposed sulfur ligands (2, 5). We therefore propose a model in which copper in CCS can undergo direct intramolecular ligand exchange between cysteines of Domain I and Domain III. The insertion of copper into SOD1 then poses two major challenges for the metallochaperone: the change in coordination environment upon metal transfer and insertion of the metal at a position deep within the enzyme. The COOH-terminal copper-binding peptide of CCS may be uniquely designed to accommodate this process.

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Note Added in Proof—Crystallographic characterization of yCCS also reveals distinct folds for Domains I and II of the apo-form of this protein and is consistent with the functional roles described here (Lamb, A. L., Wernimont, A. K., Pufahl, R. A., Culotta, V. C., and Rosenzweig, A. C. (1999) Nat. Struct. 6, in press).

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