ACE1, a copper-dependent transcription factor, activates expression of the yeast copper, zinc superoxide dismutase gene

(gene expression oxygen toxicity metal regulation)

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ABSTRACT Copper, zinc superoxide dismutase (SOD1 gene product) (superoxide:superoxide oxidoreductase, EC 1.15.1.1) is a copper-containing enzyme that functions to prevent oxygen toxicity. In the yeast Saccharomyces cerevisiae, copper levels exert some control over the level of SOD1 expression. We show that the ACE1 transcriptional activator protein, which is responsible for the induction of yeast metallothionein (CUP1) in response to copper, also controls the SOD1 response to copper. A single binding site for ACE1 is present in the SOD1 promoter region, as demonstrated by DNase I protection and methylation interference experiments, and is highly homologous to a high-affinity ACE1 binding site in the CUP1 promoter. The functional importance of this DNA–protein interaction is demonstrated by the facts that (i) copper induction of SOD1 mRNA does not occur in a strain lacking ACE1 and (ii) it does not occur in a strain containing a genetically engineered SOD1 promoter that lacks a functional ACE1 binding site.

Superoxide dismutase (SOD; superoxide:superoxide oxidoreductase, EC 1.15.1.1) catalyzes the disproportionation of superoxide ion to give dioxygen and hydrogen peroxide (2O2− + 2H+ → O2 + H2O2) (1, 2). This enzyme is widely believed to be one of the agents whose presence in the cell protects organisms against the toxic effects of oxygen or its metabolites, particularly O2− or its more reactive by-product, hydroxyl radical. This belief is based on the high level of catalytic activity of the SOD enzymes, their almost ubiquitous presence in aerobic organisms, and the oxygen sensitivity of sod− strains of bacteria and yeast (1–5). The copper, zinc SOD (CuZnSOD), which is the subject of this paper, is present in the cytosol of eukaryotes, while an unrelated Mn-containing SOD is present in the mitochondria (1). The SOD1 gene codes for CuZnSOD‡ (5, 6).

In the earliest studies of Saccharomyces cerevisiae CuZnSOD, it was noted that copper ion levels in the growth medium influenced the levels of CuZnSOD activity measured in extracts, although a more dramatic inducer was dioxygen (7). Other studies have confirmed these observations (8, 9) and have shown that copper induction of CuZnSOD activity in yeast occurs independently of the presence of dioxygen (10). However, it was not clear, based on these studies, whether the induction was transcriptional, translational, or posttranslational (i.e., via insertion of copper into existing apoenzyme). Greco et al. (11) extended our knowledge considerably when they showed induction of SOD1 mRNA steady-state levels by copper in yeast, suggesting that copper either stimulated transcription initiation or increased mRNA stability.

A well-characterized, copper-dependent regulatory system has already been described in S. cerevisiae (12). Yeast metallothionein, the product of the CUP1 gene, acts to prevent copper toxicity by binding free copper ion in the cell and may play a more subtle role in metal homeostasis as well. The expression of the CUP1 gene is dramatically induced by copper ion, through the action of the copper-binding regulatory protein ACE1 (13–17). ACE1 protein in the presence of copper binds four sites in the CUP1 upstream activation sequence (UASCuP1) and greatly activates transcription; in the absence of copper, no binding occurs and transcription is minimal. A strain harboring a defective ACE1 allele fails to increase SOD1 mRNA levels in response to copper (18), suggesting that ACE1 plays a direct role in SOD1 transcription.

Because of long-standing interest in the relationship of copper metabolism and CuZnSOD (19), we wondered if the mechanism of SOD1 induction by copper ion was related to that of copper metallothionein. We therefore tested whether ACE1 was directly involved in regulation of SOD1 mRNA synthesis and, if so, by what mechanism. We present data showing that the SOD1 promoter contains a single ACE1 binding site and that it functions in vivo to regulate SOD1 expression in a copper-dependent fashion.

MATERIALS AND METHODS

Yeast and Bacterial Strains. The following yeast strains were used in these studies: DTY7 (MATα his6 leu2-3, -112 ura-3-52 CUP1−CUP1), DTY9 (DTY7 with ace1-ΔZ25) (20), DTY96 (DTY7 with leu2-3, -112::YlpSL::LEU2) (this work), DTY97 (DTY7 with leu2-3, -112::YlpSLΔ4::LEU2) (this work). CUP1k3 indicates three copies of the CUP1 gene and promoter are present (17). Strains DTY96 and DTY97 were constructed by integration of the YlpSL and YlpSLΔ5 plasmids, respectively, at the leu2 locus in DTY7. Each plasmid was cleaved once with BsrEII within the LEU2 gene and introduced by transformation (21). Correct integration was verified by Southern blotting (22). Yeast strains were grown in rich (YPD) or synthetic complete (SC) medium lacking specified supplements (21). For copper induction studies, growth was in either SC medium prepared with ultrapure glucose and Aristar NaH2PO4 (BDH) to minimize copper contamination or synthetic medium minus copper planned as described (11). Acid rinsed plastic labware was used to minimize metal contamination. Copper sulfate (50 or 100 μM) was added to logarithmic-phase cultures and growth was continued 30 or 45 min, after which cells were harvested, washed, and frozen for RNA preparation.

Plasmids and DNA Manipulations. All DNA manipulations were carried out by standard techniques (22, 23). Plasmid pGSOD1.1 was constructed by inserting the 1.3-kilobase (kb) SOD1 Ssp I DNA fragment, containing the SOD1 upstream region and structural gene, into the Sma I site of plasmid.

Abbreviation: SOD, superoxide dismutase.

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‡The use of SOD1 for the CuZnSOD gene is in agreement with nomenclature used for humans and other eukaryotes.

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pGEM3zf(−) (Promega). Plasmid pBSKS(−)SOD1.1-Nco I has the 1.1-kb SODI fragment, with an Neo I restriction endonuclease site introduced at the translation initiation codon by site-directed mutagenesis, inserted into pBSKS(−) (Sequence, Urbana) and was kindly provided by Janet Graden. Plasmid pSODp was constructed by insertion of a 280-base-pair HindIII/Au I DNA fragment spanning the SODI promoter region into the HindIII/Smal I sites of pBSK(−). The SODI−lacZ (β-galactosidase fusion plasmids) were constructed as follows: The SODI promoter fragment in pBSKS(−)SOD1.1-Nco I was cleaved with Neo I, rendered blunt with the Klenow fragment of DNA polymerase I, cleaved with Xba I, and inserted into the Xba I/EcoRIV sites of pBSKSL (+). For YplSL, the Xba I/HindIII SODI promoter fragment was isolated and inserted into the yeast integrating lacZ fusion vector Ylp367 (24) to create an in-frame SODI−lacZ fusion. YlpSLΔA was analogous, except that site-specific mutagenesis (25) was used to change the seven G residues boxed in Fig. 3 to A residues, and the A at position −189 to a C, before insertion into Ylp367. Plasmid pEMBLYe24:ACE1 was constructed by insertion of the 1.3-kb ACEI DNA fragment (26) into pEMBLYe24 (27).

Expression of ACEI Protein in Escherichia coli. ACEI protein was expressed in E. coli using the bacteriophage T7 RNA polymerase system (28). A unique Nad I restriction endonuclease site was created in the ACEI translation initiation codon by oligonucleotide-directed mutagenesis (25). The 1.3-kb Nad I/Bgl II DNA restriction fragment encompassing the modified ACEI coding region was inserted into the Nad I/BamHI sites of PET-3b to create pPSla. E. coli strain BL21(DE3)/pLysE was used to express ACEI from pPSla, as described (28). Induced cultures (200 ml) were harvested, washed in H2O at 4°C, and suspended in 1 ml of cold extraction buffer (40 mM Tris-HCl, pH 8.0/10 mM MgCl2/1 mM diithiothreitol/1 mM phenylmethylsulfonyl fluoride/200 mM KCl). The cell mixture was frozen, thawed, sonicated briefly, and spun at 16,000 × g for 30 min at 4°C. Extracts contained 2 mg of soluble protein per ml, 10% of which was ACEI, as determined by the dye binding assay and SDS/PAGE (22).

DNase I Footprinting and Methylation-Interference Assays. These analyses were carried out as described (22). The SODI promoter fragment from plasmid pSODp was uniquely end-labeled with [α-32P]dCTP and the Klenow fragment of E. coli DNA polymerase I on the noncoding strand at the Xba I site or on the coding strand at the Spe I site. For methylation interference assays, 100,000 cpm of each end-labeled probe was partially methylated with dimethyl sulfate and used in preparative mobility-shift assays with ACEI extract. Both free probe and the protein-DNA complex were eluted from the gel, and modified DNA was cleaved with piperidine. Chemical DNA sequencing reactions with 3′-end-labeled SODI promoter fragments were cofractionated with DNase I cleavage samples on 8% polyacrylamide/urea gels. The gels were dried and exposed to Kodak XAR-5 film at −80°C with intensifying screens (22).

Primer-Extension Assays. RNA was prepared by hot extraction as described (29). Quality of the RNA was verified by agarose gel electrophoresis (23). Specific mRNA was measured by primer-extension reactions. Deoxyoligonucleotides complementary to the mRNA of interest were labeled with phosphoramidite kinase and [α-32P]ATP (Amersham) (23). For annealing, 0.2 pmol of labeled primer and 10–20 µg of total RNA in a vol of 20 µl were heated to 100°C for 5 min, followed by 1 h at 45°C. Ten microliters of reaction mixture containing (per sample) 3 µl of 10× RT buffer (0.5 M Tris-HCl, pH 8.3/0.4 M KCl/0.06 M MgCl2/10 mM diithiothreitol), 6 µl of dNTP mixture (2.5 mM in each), and 1 µl (0.75 unit) of diluted avian myeloblastosis virus reverse transcriptase (Pharmacia) was added and incubation was continued for 1 h at 45°C. The reaction was stopped with 3 µl of 0.25 M EDTA, followed by precipitation with 200 µl of ethanol. Samples were washed twice with ethanol, dried, resuspended in formamide, and fractionated on a 6% polyacrylamide/urea gel. The SODI transcription start site was determined by comparing primer-extension products with the products of a sequencing reaction (Sequence, United States Biochemical) using the same labeled primer and SODI template DNA.

RESULTS

Copper Induces SODI mRNA in Yeast with Functional ACEI. We began by determining whether Cu-dependent regulation of SODI occurred in our system. Yeast strains DTY7 (ACEI wild type) and DTY39 (ace1-Δ225) were grown in copper-free medium to midlogarithmic phase and treated for 45 min with 50 µM CuSO4. Total RNA was prepared and specific SODI mRNA levels were analyzed by primer extension. Results of a typical experiment are shown in Fig. 1. Densitometric scanning of the autoradiogram showed a 2.5-fold copper-induced increase in SODI mRNA in the wild-type strain. In the ace-Δ225 strain, the basal level was reduced to 60% of the wild type. In this experiment, a 1.5-fold induction by copper occurred in the ace-Δ225 strain, but this was not reproducibly observed. In a strain that overexpressed the ACEI protein, induction was not increased. In the same experiment, CUP1 message was dramatically induced by CuSO4 in the wild type but not in the ace-Δ225 mutant strain (data not shown). This experiment and similar ones allowed us to map the specific transcriptional start sites. There are two approximately equal major starts at bases +1 and +2 (a C and an A located 44 and 43 bases upstream of the ATG translation initiation codon), and two minor starts at +10 and −5 (both A residues). The putative TATA box is located at −64 to −54, and the ACEI site is at −206 to −177. (All numbering is relative to the transcription start at +1.)

ACEI Binds a Specific Sequence in the SODI Promoter. The dependence of SODI on a wild-type ACEI gene for copper-inducible expression suggests that ACEI might directly interact with the SODI promoter. To test this possibility, we used E. coli extract containing ACEI protein (ACEI extract) and extract from cells harboring pET3b (control extract) in mobility-shift assays using the SODI promoter fragment from −306 to −28. These experiments demonstrated that a single, copper-dependent DNA–protein complex was formed by using ACEI extract, but not with control extract in the presence of copper. The specificity of the complex was demonstrated by the observation that an oligonucleotide

![Fig. 1. SODI mRNA induction by copper in wild-type and ace1Δ strains. Cultures of DTY7 (ACEI) and DTY39 (ace1-Δ225) were grown in synthetic medium without copper to midlogarithmic phase. Parallel cultures were untreated (−) or treated (+) with 50 µM copper sulfate for 45 min as indicated. Specific SODI mRNA was measured by primer extension. Arrows indicate positions of major extension products.](image-url)
corresponding to two ACE1 binding sites in the CUP1 promoter (−139 to −108), but not an unrelated oligonucleotide, could compete for the formation of this complex. Higher levels of ACE1 in the binding reaction mixture did not give rise to additional complexes (data not shown; but see Fig. 4A for similar data).

DNase I footprinting experiments were performed to localize the ACE1 binding site within the SOD1 promoter fragment. The data in Fig. 2A demonstrate that a single region on the noncoding strand of the SOD1 promoter fragment is protected from DNase I cleavage when either 5 or 10 μg of copper-activated ACE1 extract was present in the binding reaction mixture. The addition of 10 μg of ACE1 extract resulted in the most extensive protection of this region from DNase I cleavage, extending from −178 to −202 on this strand. Similarly, the data in Fig. 2B demonstrate protection of a single region of the SOD1 coding strand, spanning from −183 to −206 when 10 μg of extract was used in the binding reaction. Shorter electrophoresis times did not reveal other ACE1 binding sites (data not shown).

Methylation-interference analyses were used to characterize the ACE1 binding site in the SOD1 promoter more precisely and to identify guanine residues that are in close association with bound ACE1 protein. The data in Fig. 2C show that when guanine residues −183, −185, −194, or −197 on the noncoding strand, or −187, −195, or −199 on the coding strand of the SOD1 promoter are methylated, ACE1 protein binding is inhibited. Although the inhibition was not complete for residues −187 and −199 on the coding strand, this effect was reproducible and suggests that ACE1 may not associate as closely with these residues. For each strand, the residues shown to be important for ACE1 binding were contained within the region of the SOD1 promoter protected from DNase I cleavage by binding of copper-activated ACE1 (Fig. 3).

Comparison of the ACE1 binding sequence in the SOD1 promoter to the four ACE1 binding sites in the CUP1 promoter (13–15) indicated strong homology, most extensively with the ACE1 binding site located between −128 and −142 on the coding strand of CUP1 (Fig. 3). A stretch of 15 nucleotides in the ACE1 binding site in SOD1, between −183 and −197 on the noncoding strand, displays 80% identity with the ACE1 binding site in CUP1, with strict conservation of four guanosine residues important for ACE1 binding at each site. Furthermore, the ACE1 binding site in the SOD1 promoter has conserved residues located at −184, −189, −190, −191, −193, −195, and −196, each of which was demonstrated to be critical for copper inducibility of CUP1 transcription in vivo (16). These properties suggest that the ACE1 binding site in the SOD1 promoter is functionally important for copper-activated expression of SOD1 in vivo.

Copper-Activated SOD1 Expression Requires a Functional ACE1 Binding Site. The identification of an ACE1 binding site in the SOD1 promoter coupled with the absence of induction in the aceI-Δ225 strain, suggested that a direct interaction between ACE1 protein and the SOD1 promoter mediates copper-inducible transcription in vivo. However, in experiments of the type shown in Fig. 1, levels of a presumably uninduced mRNA (Fig. 2, lanes 17 and 20) were sometimes observed to drop after copper addition, particularly in the synthetic copper-free medium. This observation raised concerns about the possibility that copper toxicity in the aceI-Δ225 strain (which is highly copper sensitive due to its inability to induce CUP1) rather than lack of specific induction, might be rendering SOD1 nonresponsive to copper stimulation. To circumvent this problem, we constructed a mutant derivative of the SOD1 promoter in which all seven guanine residues shown to be in close association with ACE1 (Fig. 3) were changed to adenine residues by oligonucleotide-directed mutagenesis. The mutant SOD1 promoter fragment, denoted SOD1-ΔA, was used in mobility-shift assays to assess whether this altered sequence could support the copper-activated binding of ACE1 in vitro. The data in Fig. 4A demonstrate that the wild-type

Fig. 2. ACE1 binds to a specific site in the SOD1 promoter. (A and B) DNase I footprinting analysis of the ACE1–SOD1 interaction. The wild-type SOD1 promoter fragment from −306 to −28 was end-labeled on the noncoding strand (A) and coding strand (B) and used in DNase I footprinting reactions with ACE1 extract and copper. The levels of ACE1 extract used (μg) and the omission (−) or addition (+) of 20 μM copper sulfate in the binding reaction is indicated. The bar on the right indicates the extent of protection from DNase I cleavage on each strand using 10 μg of ACE1 extract. (C) Methylation-interference analysis of the ACE1–SOD1 interaction. Guanine residues on the noncoding strand (NC) and coding strand (C) are indicated, which, when methylated, inhibit the binding of ACE1 protein to the SOD1 promoter fragment. F, free probe; B, ACE1–SOD1 complex.

Fig. 3. Sequence of the ACE1 binding site in the SOD1 promoter and comparison to UASCuP1. (A) Summary of the ACE1 binding region of the SOD1 promoter. Brackets indicate the extent of the SOD1 promoter protected by ACE1 from DNase I cleavage. Boxed residues are guanines, which, when methylated, interfere with copper-activated ACE1 binding in vitro. Numbering is relative to the start site for SOD1 transcription (located 44 bases upstream of the ATG translation start). (B) Comparison of the ACE1 binding site sequence on the SOD1 noncoding strand with an ACE1 binding site sequence on the coding strand of UASCuP1. Dots indicate identity and boxed guanine residues are those shown to be in close association with bound ACE for SOD1 (this work) and CUP1 (15).
SOD1 promoter fragment bound ACE1 in a copper-activated, dose-dependent manner. However, even at the highest concentration of ACE1 extract (2 μg), the SOD1-ΔA promoter fragment failed to bind ACE1.

To test whether the ability to bind ACE1 in vitro correlated with ACE1-mediated activation in vivo, we constructed SOD1-lacZ and SOD1-ΔA-lacZ promoter fusions in yeast integrating vectors to create plasmids YlpSL and YlpSL-ΔA, respectively. These plasmids, having the SOD1 promoter or its nonbinding derivative controlling expression of the E. coli β-galactosidase gene, were integrated in single copy at the leu2 locus in strain DTY7, using wild-type SOD1 promoter and DTY79 (altered SOD1-ΔA fusion promoter). These strains have wild-type ACE1 levels and, therefore, copper resistance, as well as normal dioxygen tolerance. Figure 4B shows primer-extension assays performed on RNA extracted from copper-induced and control cultures of DTY7 and DTY79 using an oligonucleotide primer that specifically hybridizes to the coding strand of the lacZ gene. The pattern of transcription starts is identical to that of the native SOD1 gene, indicating that the promoter was functioning normally. The presence of 100 μM copper induced a 5- to 10-fold increase in expression from the wild-type promoter, while no increase was seen in the promoter that lacks a functional ACE1 binding site. Again, the basal level of transcription decreased in the absence of ACE1 binding. ADH1 mRNA levels remained constant in all samples, and CUP1 was induced dramatically in both strains (data not shown), as expected. It should be noted that qualitatively similar data were obtained when β-galactosidase activity from control and copper-treated DTY76 and DTY79 cultures was measured (data not shown). Because this experiment was carried out in a copper-resistant strain with a wild-type ACE1 gene, the lack of induction of the YlpSL-ΔA fusion cannot be attributed to cell death due to metal poisoning and therefore demonstrates that ACE1 acts as a transcriptional regulator of CuZnSOD by binding in the presence of copper to the SOD1 promoter region.

**DISCUSSION**

Our results demonstrate that a binding site for the copper-activated yeast transcription factor ACE1 exists in the promoter region of the yeast CuZnSOD gene (SOD1). The site was characterized by DNase I protection and methylation interference assays (Fig. 2). It shows strong homology with the highest-affinity binding site for ACE1 in the CUP1 promoter (13, 15), including conservation of the seven G residues shown by methylation interference to be important for ACE1 binding in both promoter sequences (Fig. 3) (13–15).

The in vitro function of this promoter element was further demonstrated by determination of specific mRNA levels in two systems. First, expression from the wild-type SOD1 promoter was measured in DTY7 (ACE1 wild type) and DTY79 (ace1-Δ225, lacking ACE1 protein) strains using primer extension with an SOD1-specific oligonucleotide (Fig. 1). Copper-stimulated SOD1 expression was observed in DTY7 but not in DTY79. However, the stimulation was a fewfold at best, rather than the 20- to 50-fold ACE1-dependent stimulation seen at the CUP1 promoter (17). Carri et al. (18) reported 30–60% copper-induced increases in steady-state SOD1 mRNA, which were reduced to 5–10% increases in ace1 strains, while Greco et al. (11) reported a 230% increase in wild-type yeast. Our results agree more closely with the latter, which is probably explained by the difference in medium, in particular the lower initial levels of copper. Because of the copper sensitivity of the ace1-Δ255 strain, we considered it formally possible that the loss of copper stimulation of SOD1 transcription in this strain was attributable to cell death rather than to lack of the ACE1 gene product. Therefore, we devised a method to study the SOD1 promoter–ACE1 interaction in a wild-type ACE1 strain. First, a mutant SOD1 promoter (SOD1-ΔA) was constructed and shown to have no ACE1 binding activity in vitro (Fig. 4A). Fusion genes were then constructed with the two promoter regions and the E. coli lacZ gene and integrated in single copy into DTY7, our wild-type ACE1 parental strain. Expression from the fusion gene was measured by primer extension using a lacZ specific primer and found to be copper stimulated only in the strain with the wild-type SOD1 promoter, indicating that ACE1 binding is required for this induction (Fig. 4B). The copper induction effect seen in the fusion construct was more dramatic and clear cut than that seen in the SOD1 gene itself (compare the first two lanes of Figs. 1 and 4B) for reasons that are not yet clear. The SOD1 promoter fragment used for the construction may be too small to carry all the regulatory apparatus for the SOD1 gene. The absence of the SOD1 coding region or the presence of the lacZ coding region may influence transcription levels or mRNA stability. Or, the higher level of induction may be due to local chromatin effects of the lacZ locus on the fusions integrated there. In both the SOD1 gene and the fusion constructs, basal levels are also affected by lack of ACE1 binding, dropping to 50–60% that of wild type. This is not too surprising in view of the fact that ACE1 is at least partially responsible for basal CUP1 expression (20). The slight copper induction of SOD1 that we sometimes observed in the ace1-Δ225 strain was not observed in the gene fusion system. We postulate that the induction may be indirect or due to
stress-related signals, which have been shown to also affect 
*CuPi* expression in the absence of ACE1 (30). In addition, 
the possible involvement of other factors cannot be com-
pletely ruled out.

Even with the increased copper effect in the fusion con-
struct, however, the *SOD1* promoter does not begin to 
approach the level of copper inducibility observed in the 
*CuPi* promoter. There are several possible explana-
tions for this. One is that the *SOD1* promoter has only a single ACE1 
binding site, while the *CuPi* promoter contains four. The 
single nonhyd nature of this site precludes the possibility of 
cooperative binding or activity of ACE1 in the *SOD1* pro-
motor. Genetic studies suggest that each of the ACE1 binding 
sites in the *CuPi* promoter contributes to the magnitude of 
metal-inducible transcription (31). The observation that 
ACE1 binds to the *CuPi* promoter as monomers sequentially 
(15) makes cooperative binding of ACE1 seem unlikely.

However, the possibility of cooperative activity remains 
open in the *CuPi* promoter and is a likely explanation of the 
differences seen between the two promoters. Second, ACE1 
sites in the two promoters are oriented in opposite direc-
tions. Again there is precedent in the *CuPi* gene. Previous studies 
demonstrated that a synthetic ACE1 binding site activated a 
**heterologous promoter** better when placed in the authentic 
orientation than in reversed orientation (31). Third, there may 
be a positional effect, since the ACE1 binding site in SOD1 
is rather far from the transcriptional site: 183 on the SOD1 
coding strand, compared to 113 on the *CuPi* coding strand 
(14). Fourth, it may be that bound ACE1 in the *CuPi* 
promoter works synergistically with ancillary *CuPi* tran-
scription factors, such as heat shock transcription factor (31) 
or others, that may not act on SOD1 transcription. The 
possibility that SOD1 induction is limited by titration of the 
ACE1 factor is eliminated by the observation that increased 
induction is not observed in cells overproducing ACE1 (data 
not shown). A teleological explanation for the low induc-
ibility may be rooted in the fact that too much SOD may also be 
harmful in conditions of oxidative stress (32), which would 
be exacerbated by extra free copper.

We come now to the question of functional significance. 
Why should CuZnSOD gene expression be regulated by 
copper levels? The most conservative explanation is that it is 
simply an energy-saving device—i.e., a cell should not waste 
resources synthesizing a protein that requires copper for 
activity if there is no copper available. In addition, since 
apoenzyme CuZnSOD has a high affinity for copper (19), 
excess apoenzyme SOD under conditions of low copper 
could compete for the limited copper needed for other 
enzymes. A second possibility is that copper and dioxygen 
induction are somehow linked by an as yet unknown mech-
nism. Copper is a redox metal that is known to catalayze 
reactions of dioxygen resulting in formation of reactive 
oxygen radicals and in oxidative damage to vulnerable cell 
components. Therefore, copper toxicity and dioxygen toxic-
ity could, in theory, be intimately related. In this regard it 
should be noted, however, that copper induction of CuZn-
SOD has been shown to occur anaerobically (10, 11). Our 
future studies on the mechanism of induction of CuZnSOD by 
dioxygen may help us to evaluate this hypothesis. A third 
possibility is that CuZnSOD, by virtue of being an abundant, 
stable, high-affinity metal binding protein, is somehow in-
volved in metal metabolism (33, 34). In this case, induction 
by the metal involved would be logical, although CuPi1 with 
its more flexible induction would seem more suited for such 
a function. In this context, it is interesting to note that in *E.
coli* (which contains no CuZnSOD), the genes for both FeSOD and MnSOD are regulated in part by the iron-
responsive *fur* repressor protein (35, 36). Our demonstra-
tion of a second gene regulated by ACE1 makes it tempting to 
speculate that ACE1, CuPi1, and SOD1 represent compo-
nents of a similar copper-based regulon in yeast. Further 
experimentation is required to test such a hypothesis.

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